WHO FOOD ADDITIVES SERIES: 61

Prepared by the Seventieth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

TYLOSIN page 183-216

Toxicological evaluation of certain veterinary drug residues in food

WHO Food Additives Series No. 72,

The summaries and evaluations contained in this book are, in most cases, based on unpublished proprietary data submitted for the purpose of the JECFA assessment. A registration authority should not grant a registration on the basis of an evaluation unless it has first received authorization for such use from the owner who submitted the data for JECFA review or has received the data on which the summaries are based, either from the owner of the data or from a second party that has obtained permission from the owner of the data for this purpose.

World Health Organization, Geneva, 2009

Food and Agriculture Organization of the United Nations

World Health Organization

TYLOSIN

First draft prepared by

Dr Gladwin Roberts,[1] Dr Carl Cerniglia[2] and Dr João Palermo Neto[3]

[1] Consultant, Preston, Victoria, Australia
[2] National Center for Toxicological Research, Food and Drug Administration, Department of Health and Human Services, Jefferson, AR, United States of America (USA)
[3] Department of Pathology, Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil

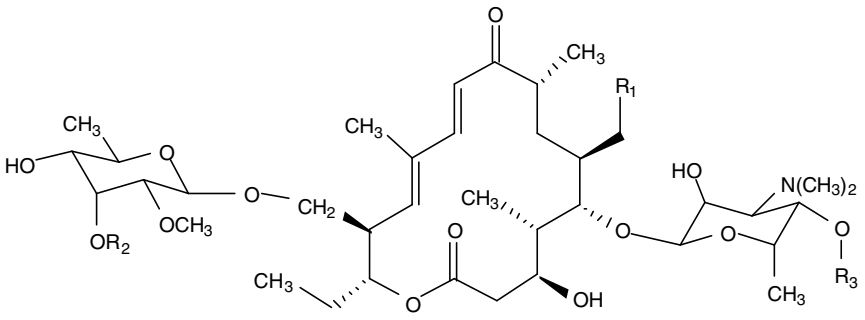
Explanation 183
Biological data 185
Biochemical aspects 185
Absorption, distribution, biotransformation and excretion 185
Toxicological studies 189
Acute toxicity 189
Short-term studies of toxicity 191
Long-term studies of toxicity and carcinogenicity 195
Genotoxicity 196
Reproductive and developmental toxicity 197
Special studies 199
Observations in humans 207
Comments 207
Biochemical data 207
Toxicological data 208
Microbiological data 209
Evaluation 211
References 211

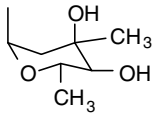
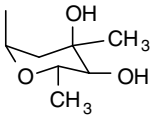
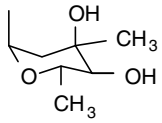
1. EXPLANATION

Tylosin is a macrolide antibiotic produced by fermentation from a strain of the soil microorganism, *Streptomyces fradiae.* The compound is active against Gram-positive bacteria, mycoplasma and certain Gram-negative bacteria. Like other macrolide antibiotics, tylosin inhibits protein synthesis by inhibiting aminoacyl-transfer ribonucleic acid (tRNA) and peptidyl-tRNA binding to the ribosomes.

Tylosin consists of one major factor, tylosin A. Three minor factors—desmycosin (tylosin B), macrocin (tylosin C) and relomycin (tylosin D)—may also be present in varying amounts, depending upon the manufacturing source (Figure 1). Most of the microbiological activity resides with tylosin A. Tylosin B, C and D and dihydrodesmycosin (a metabolite) have approximately 83%, 75%, 35% and 31% of the activity of tylosin A, respectively.

Figure 1. Structures of tylosin factors A, B (desmycosin), C (macrocin) and D (relomycin)



	Tylosin A	Tylosin B (desmycosin)	Tylosin C (macrocin)	Tylosin D (relomycin)
R_1	-CHO	-CHO	-CHO	$-CH_2OH$
R_2	$-CH_3$	$-CH_3$	-H	$-CH_3$
R_3		-H		

Tylosin and its phosphate and tartrate salts are used in pigs, cattle and poultry for the treatment of infections caused by organisms sensitive to tylosin. They may be administered to calves, orally in the milk replacer, at a dose of 40 mg/kg body weight (bw) and to cattle, by intramuscular injection, at a dose of 4–10 mg/kg bw. In pigs, tylosin is used for the prevention and control of diseases such as swine dysentery and enzootic pneumonia. It may be administered in the drinking-water at a dose of 25 mg/kg bw, in the feed at a dose of 3–7 mg/kg bw or by intramuscular injection at a dose of 2–10 mg/kg bw. Tylosin is administered to poultry in the drinking-water at a dose equivalent to 75 mg/kg bw. It is used primarily in the treatment of chronic respiratory disease complex in chickens and infectious sinusitis in turkeys caused by *Mycoplasma gallisepticum*. Tylosin has also been approved for emergency use in the control of American foulbrood of honey bees.

Tylosin was evaluated at the twelfth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1968 (Annex 1, reference *17*). 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 acceptable daily intake (ADI) was established.

The drug was subsequently evaluated at the thirty-eighth meeting of the Committee in 1991 (Annex 1, reference *97*). Because of deficiencies in the toxicological and microbiological data, the Committee was not able to establish an

ADI. Before reviewing the compound again, the Committee wished to have 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 body weight gain in these rats.
4. Studies from which a no-observed-effect level (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.

At the sixty-sixth meeting of the Committee in 2006 (Annex 1, reference *181*), none of the requested information was provided. A review of the available toxicological data in the published scientific literature indicated that publicly available data were insufficient to allow a toxicological and residue evaluation of tylosin to be performed. Therefore, tylosin could not be evaluated at the sixty-sixth meeting.

Tylosin was included on the agenda and call for data for the current meeting of the Committee as a result of a request from the 17th Session of the Codex Committee on Residues of Veterinary Drugs in Foods (Codex Alimentarius Commission, 2007).

New data on microbiological effects and detailed information on the reproductive and developmental toxicity studies reported at the thirty-eighth meeting were made available for the present meeting. This report consolidates the available information for tylosin relevant to the establishment of an ADI and maximum residue limits (MRLs).

The Committee considered the results of studies on pharmacokinetics, pharmacology, acute and short-term toxicity, genotoxicity, fertility and developmental toxicity, microbiological safety and studies in humans. The genotoxicity and microbiological studies and some acute toxicity studies were carried out according to appropriate standards. The majority of the other studies were performed prior to the establishment of standards for study protocol and conduct. Several were available in summary form only and thus could not be independently assessed.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution, biotransformation and excretion

Unless otherwise mentioned, tylosin activity was measured using a microbiological assay against *Sarcina lutea*.

Pharmacokinetic data for tylosin in plasma of rats, rabbits, dogs and cats are summarized in Table 1.

Table 1. Summary of plasma kinetics in rats, rabbits, dogs and cats

Species	Test substance, dose and route	C_{max} (µg/ml)	T_{max} (h)	References
Rats, intact	Tylosin base; 50 mg/kg bw; oral	<LOQ–1.0	1	Anderson (1960); Anderson et al. (1966a)
	Tylosin tartrate; 50 mg/kg bw; oral	0.11–1.35	2	
Rabbits, intact	Tylosin hydrochloride; 10 mg/kg bw; intramuscular	0.26–0.88	1.5	Anderson (1960); Anderson et al. (1966a)
	Tylosin tartrate; 10 mg/kg bw; intramuscular	0.96–1.4	1	
	Tylosin tartrate; 25 mg/kg bw; intramuscular	4.7–7.2	1	
Dogs, intact	Tylosin base; 25 mg/kg bw; oral	1.4–2.7	2	Anderson (1960); Anderson et al. (1966a)
	Tylosin base; 100 mg/kg bw; oral	2.7–4.6	4	
Dogs, duodenal fistula	Tylosin base; 25 mg/kg bw; intraduodenal	1.98	0.5	Anderson (1960); Anderson et al. (1966a)
Dogs, intact	Tylosin hydrochloride; 10 mg/kg bw; intravenous	6.8	0.25	Anderson (1960); Anderson et al. (1966a)
Dogs, intact	Tylosin; 11 mg/kg bw; intramuscular	2.5	1	Van Duyn et al. (undated)
Dogs, intact	Tylosin; 11 mg/kg bw; intramuscular	1.8	2	Van Duyn & Kline (undated)
Cats, intact	Tylosin; 11 mg/kg bw; intramuscular	3.0	2	

C_{max}, maximum concentration in plasma; LOQ, limit of quantification; T_{max}, time to reach the C_{max}.

(a) Rats

Rats were given oral doses of 50 mg tylosin/kg bw as the base or tartrate. After 15 and 30 min and 1, 2, 4, 5, 7 and 24 h, groups of five rats were killed, and

the serum was assayed for tylosin activity. A small amount of activity was present 1–2 h after treatment (≤1.35 µg/ml), but no clear pattern was established because of high individual variability. Serum levels decreased to below the limit of quantification (LOQ = 0.1 µg/ml) within 5 h (Anderson, 1960; Anderson et al., 1966a).

Four anaesthetized rats were given an intraperitoneal injection of 100 mg tylosin/kg bw, and bile was collected for 2 h. The bile:serum ratio for tylosin activity ranged from 143 to 266 (Anderson, 1960; Anderson et al., 1966a).

Four rats received oral doses of 10 mg unlabelled tylosin/kg bw per day for 3 days, followed by similar doses of [^{14}C]tylosin for 5 days. The animals were killed 4 h after the last dose. Tissue levels of radioactivity were 0.23 mg equivalents/kg in liver, 0.18 mg equivalents/kg in kidney and 0.08 mg equivalents/kg in fat. Approximately 99% of the radiolabel was excreted in faeces, and 1% in urine. The proportion of radioactivity in faeces that was extractable was 93%. The major components of the extractable residue in rat faeces were relomycin (10%), tylosin A (6%) and dihydrodesmycosin (4%). The remaining more polar metabolites were not identified. The metabolic fate of tylosin was shown to be qualitatively similar in rats and pigs (Sieck et al., 1978).

Fischer 344 rats were given oral doses of 10 mg [^{14}C]tylosin/kg bw per day for 4 days. Urine and faeces were collected daily. Approximately 95% of the excreted radioactivity was found in faeces. The rats were euthanized 4 h after the last dose, when the liver had a mean radioactivity concentration of 0.09 mg equivalents/kg. Fractionation of radioactivity indicated the presence of multiple metabolites in liver, including tylosin A, relomycin, dihydrodesmycosin and cysteinyl-tylosin A, although evidence for the latter was not conclusive. The faeces contained relomycin (24%) and dihydrodesmycosin (11%) as the major components. Minor components in the faeces included tylosin A, macrocin, the seco acid of tylosin A, the seco acid of relomycin and desmethyl dihydrodesmycosin. The seco acids are products of hydrolysis of the lactone in the macrolide ring (Kennington & Donoho, 1994).

(b) Rabbits

Three rabbits were given an intramuscular dose of 10 mg tylosin/kg bw as the hydrochloride. Blood was collected at 1.5, 4, 7 and 24 h after treatment and assayed for tylosin activity. The highest level of activity (≤0.88 µg/ml) was found in the samples taken at 1.5 h. A further four and two rabbits were given an intramuscular injection of 10 or 25 mg tylosin/kg bw as the tartrate, respectively. Blood was taken at 1, 2, 4, 6 and 24 h after treatment and assayed for tylosin activity. Peak serum levels of activity were found at 1 h after treatment (≤1.4 and ≤7.2 µg/ml). In all animals, activity had declined to less than the limit of detection (LOD = 0.5 µg/ml) by 6–7 h (Anderson, 1960; Anderson et al., 1966a).

(c) Dogs

Tylosin activity was determined in the serum of two dogs given daily doses of 25 or 100 mg tylosin base/kg bw by capsule for 1, 15 or 29 days. Blood samples

were taken at 0, 1, 2, 3, 4, 5, 6 and 7 h after administration. Peak serum activity occurred at 2–4 h (≤2.7 and ≤4.6 µg/ml) and was less than proportional to dose (Anderson, 1960; Anderson et al., 1966a).

In a group of four dogs with a duodenal fistula, intraduodenal application of 25 mg tylosin base/kg bw gave rise to higher serum levels of activity than after oral dosing. Urinary recovery amounted to 7.2% of the dose in 5 h after intraduodenal application, but only 2% of the oral dose (Anderson et al., 1966a).

In a 2-year toxicity study in dogs, tylosin activity in blood was determined in groups of eight animals after 8, 148, 622 and 723 doses of 1, 10 or 100 mg tylosin base/kg bw per day given in capsules. After 8 days, activity was detected in the serum at all dose levels, but was less than dose proportional (≤2.15, ≤2.15 and ≤9.5 µg/ml, respectively). At later times, none of the dogs given the lowest dose contained tylosin in blood above the LOD of 0.15 µg/ml. Dogs given 10 mg/kg bw per day showed the presence of tylosin activity in the blood at levels of <LOD–1.9 µg/ml. At the 100 mg/kg bw per day dose, blood levels at 2 h were <LOD–35 µg/ml. In an extension of the study, dogs given 200 or 400 mg/kg bw per day had serum tylosin activity of 8–29 µg/ml 2 h after dosing. Activity levels were no higher and in fact appeared to be lower as the study progressed, indicating that there was no accumulation (Anderson, 1960; Anderson et al., 1966a).

Four dogs received an intravenous injection of 10 mg tylosin/kg bw as the hydrochloride. Blood was collected at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5 and 6 h after treatment and assayed for tylosin activity. The highest activity in serum (6.8 µg/ml) was detected at 0.25 h and decreased with a half-life of 48 min. Urinary recovery amounted to 15.7% in the first 2 h and 18.8% over a 6-h period (Anderson, 1960; Anderson et al., 1966a).

A single intramuscular injection of Tylocine Injection at a dose of 11 mg tylosin/kg bw was given to five Beagle dogs. Blood samples were collected at 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h after injection, and the serum was assayed for tylosin activity. The mean tylosin activity in the blood reached a peak of 2.5 µg/ml at 1 h; by 10 h after the injection, the concentration of tylosin in the blood had declined to the LOD of 0.1 µg/ml (Van Duyn et al., undated).

Six Beagle dogs (three male and three female) were given two intramuscular injections of Tylocine Injection at a dose of 11 mg tylosin/kg bw 12 h apart. Samples of blood were collected and assayed for tylosin activity at 2-h intervals up to 24 h after the first injection. Tylosin reached its maximum concentration (1.9 and 1.7 µg/ml) in the blood at approximately 2 h after each injection and had disappeared again after approximately 12 h (Van Duyn & Kline, undated).

One anaesthetized dog was given an intravenous injection of 10 mg tylosin/kg bw as the hydrochloride. The bile:serum ratio for tylosin activity ranged from 1233 to 3778. Approximately 13.7% of the dose was recovered in the bile in 5 h, and 25.2% was recovered in the urine (Anderson, 1960; Anderson et al., 1966a).

(d) Cats

Six cats (three males and three females) were given two intramuscular injections of Tylocine Injection at a dose of 11 mg tylosin/kg bw 12 h apart. Samples of blood were collected and assayed for tylosin activity at 2-h intervals up to 24 h after the first injection. Tylosin reached its maximum concentration (3.0 µg/ml) in the blood at approximately 2 h after each injection and had disappeared again after approximately 12 h (Van Duyn & Kline, undated).

2.2 Toxicological studies

2.2.1 Acute toxicity

In the majority of the studies, the sex of the rodent used was not specified, and the tests employed an observation period of 7 days following dose administration. This observation period is less than the 14 days currently required by regulatory guidelines. Most of these results were provided in summary form only. They are summarized in Table 2.

Table 2. Summary of acute toxicity studies with tylosin

Tylosin form	Species	No. of animals per dose (M & F)	Route of administration	Dose range (mg/kg bw)	LD_{50} (mg/kg bw)	References
Phosphate	Mouse	10	Oral	4000–6200	>6200	Anderson & Worth (1961)
Phosphate	Mouse	10	Intraperitoneal	400–800	492	Anderson & Worth (1961)
Phosphate	Mouse	10	Subcutaneous	620–1250	784	Anderson & Worth (1961)
Phosphate	Mouse	10	Intravenous	275–500	386	Anderson & Worth (1961)
Phosphate	Rat	10	Oral	4000–6200	>6200	Anderson & Worth (1961)
Phosphate	Rat	10	Intraperitoneal	700–3650	1001	Anderson & Worth (1961)
Phosphate	Rat	10	Subcutaneous	2750–5000	4083	Anderson & Worth (1961)
Base	Mouse	5–10	Oral	2500–3650	>3650	Anderson et al. (1966a)
Base	Mouse	10	Subcutaneous	1250–2500	>2500	Anderson et al. (1966a)

Table 2 (contd)

Tylosin form	Species	No. of animals per dose (M & F)	Route of administration	Dose range (mg/kg bw)	LD_{50} (mg/kg bw)	References
Base	Mouse	10	Intraperitoneal	400–800	594	Anderson et al. (1966a)
Base	Rat	10	Oral	5000	>5000	Anderson et al. (1966a)
Base	Dog	2	Oral	10–800	>800	Anderson et al. (1966a)
Tylosin hydrochloride	Mouse	5	Intravenous	400–800	582	Anderson et al. (1966a)
Lactate	Mouse	5	Intravenous	400–620	589	Anderson et al. (1966a)
Tartrate	Mouse	10	Oral	4000–6200	>6200	Anderson et al. (1966a)
Tartrate	Mouse	5–10	Oral	2500–5000	>5000	Morton (1988)
Tartrate	Mouse	5	Oral	4500–5600	>5600	Morton (1988)
Tartrate	Mouse	10	Subcutaneous	1000–2000	1354	Anderson et al. (1966a)
Tartrate	Mouse	5–10	Subcutaneous	1000–2000	1439	Morton (1988)
Tartrate	Mouse	10	Intravenous	450–700	589	Anderson et al. (1966a)
Tartrate	Mouse	5	Intravenous	400–800	668	Morton (1988)
Tartrate	Mouse	10	Intravenous	400–500	435	Morton (1988)
Tartrate	Mouse	10	Intravenous	400–620	436	Morton (1988)
Tartrate	Mouse	10	Intravenous	225–500	321	Quarles (1983)
Tartrate	Rat	10	Oral	4000–6200	>6200	Anderson et al. (1966a)
Tartrate	Rat	10	Subcutaneous	1000–3000	>3000	Anderson et al. (1966a)
Tartrate	Rat	10	Intravenous	450–900	695	Anderson et al. (1966a)
Tartrate	Rat	10	Intraperitoneal	620–2500	>2500	Anderson et al. (1966a)

F, female; LD_{50}, median lethal dose; M, male.

Intraperitoneal treatment of mice with tylosin base caused vocalizing and fighting, suggesting irritation or pain. Death of mice was preceded by depression and prostration. High doses of tylosin base resulted in salivation, vomiting and defecation in dogs (Anderson et al., 1966a).

The intravenous toxicities of tylosin tartrate, desmycosin (tylosin B) and macrocin (tylosin C) were compared in the female ICR mouse. This study utilized 10 females per dose, and the animals were observed for 14 days following administration. Signs of toxicity included tremors, clonic convulsions and leg weakness with each compound. In addition, poor grooming was seen with tylosin, and hypoactivity and ataxia occurred with desmycosin. Body weights were unaffected. The median lethal doses (LD_{50}s) were 321, 193 and 189 mg/kg bw for tylosin, desmycosin and macrocin, respectively (Quarles, 1983).

The acute toxicity of desmycosin was determined for the mouse. The LD_{50}s for the oral, subcutaneous and intraperitoneal routes of administration of desmycosin were >5000, 1593 and 483 mg/kg bw, respectively. Values for the oral, subcutaneous and intravenous routes of administration of the tartrate salt of desmycosin were >5000, 1706 and 323 mg/kg bw, respectively (Morton, 1988).

Smitherman cross cockerels, 10 per group, were administered single doses of tylosin phosphate either orally or by subcutaneous injection. The LD_{50} was 3765 mg/kg bw orally and 501 mg/kg bw subcutaneously (Richards & Berkman, undated).

Bobwhite quail (*Colinus virginianus*), five per sex per group, were given a single oral dose of 0, 1000 or 2000 mg tylosin base/kg bw. There were no deaths, but transient diarrhoea occurred in the treated birds (Kehr et al., 1978a).

2.2.2 Short-term studies of toxicity

(a) Rats

Groups of five female Harlan rats were treated subcutaneously with tylosin base suspended in 5% acacia at 10, 25, 50 or 100 mg/kg bw per day for 28 days. Food intake and body weight gains were similar between groups, and terminal blood cell counts were within normal limits. Gross and microscopic examination of the heart, lungs, kidney, liver, spleen, ovary, uterus, stomach and intestines and salivary, thymus, pancreas, adrenal and thyroid glands revealed no abnormalities. This study had no control group, and the report was provided in summary form only (Anderson et al., 1966a,b).

Tylosin tartrate was administered by the subcutaneous route at doses of 0, 100, 250, 500 or 1000 mg/kg bw per day to groups of six male and six female Harlan rats for 28 days. Diarrhoea occurred during the 1st week of treatment in the groups receiving 250, 500 and 1000 mg/kg bw per day. Occasional soft faeces were noted at all treatment levels. There were no significant differences in body weight gain, relative organ weights or haematological parameters and no treatment-related gross or microscopic abnormalities in heart, lungs, liver, spleen, kidney, stomach, intestines, skeletal muscle, gonads, salivary gland, pancreas, adrenal, thymus, thyroid or mesenteric lymph nodes. This study was provided in summary form only (Anderson et al., 1966a,b).

Groups of six male (29 days old) and six female (28 days old) Wistar rats were given gavage doses of 0, 0.005, 0.2, 10 or 200 mg tylosin base/kg bw per day for 6 weeks. Diarrhoea was noted in most rats receiving 200 mg/kg bw per day. Body weight gain and feed intake, the day of vaginal opening and preputial separation were unaffected. At study termination, slight changes included increased platelet volume, decreased white blood cells and decreased monocytes at all doses. In females, serum levels of alanine aminotransferase (ALT) and total bilirubin were increased at the highest dose, whereas lactate dehydrogenase, follicle stimulating hormone and prolactin were decreased at doses of 0.2–200 mg/kg bw per day. In males, ALT was decreased and testosterone increased at 10 and 200 mg/kg bw per day, and prolactin and luteinizing hormone were decreased at doses of 0.2–200 mg/kg bw per day. Thyroid hormones were unaltered. Immunoglobulins G and M were decreased at 0.2 mg/kg bw per day and above. Enlarged caeca were observed at doses of 0.2–200 mg/kg bw per day, but organ weights were not meaningfully altered. Infiltration of basophilic materials or eosinophilic fluids in renal tubular epithelial cells and reduced sperm counts were found in tylosin-treated groups, but were not dose related. Sperm motility was increased at 200 mg/kg bw per day. Ribonucleic acid (RNA) isolated from pituitary glands was used to analyse gene expression. There was a dose-related increased induction of genes related to cell proliferation and adhesion in females and to metabolism, regulation of cell cycle and neuronal development in males (Jeong et al., 2007).

Groups of 15 male and 15 female Wistar rats were administered tylosin base in the diet for 1 year at levels of 0, 1000, 5000 and 10 000 mg/kg in the feed. These rats were the F_1 offspring from the fertility study (see section 2.2.5) in which parents were fed test diets for approximately 10 weeks prior to mating and thereafter. The highest level was chosen because it had been tolerated without any effect in the previous three-generation rat study. Based on food intake, the doses were equal to 0, 68–76, 345–391 and 684–842 mg/kg bw per day for weeks 1–13 and 0, 39–64, 192–283 and 391–586 mg/kg bw per day for weeks 14–52. Treated rats appeared moderately hyperirritable and hyperactive from 7 to 12 months on test, but there was no mortality attributable to treatment. Toxicologically significant changes were not observed for food consumption, body weight gain, blood chemistry parameters or organ weights. Significantly increased numbers of lymphocytes, significantly decreased numbers of neutrophils and significantly increased urine pH were observed in females given 5000 and 10 000 mg/kg diet. Microscopic examination revealed a slight increase in pituitary tumours in females of all treated groups: 1, 3, 4 and 3 adenomas and 0, 0, 1 and 0 carcinomas at 0, 1000, 5000 and 10 000 mg/kg diet, respectively. Since the tumour incidences were not dose related, the no-observed-adverse-effect level (NOAEL) was considered to be 1000 mg/kg diet, equal to 39 mg/kg bw per day (Broddle et al., 1978a).

(b) Dogs

Two female dogs were given oral doses of 25 or 100 mg tylosin base/kg bw per day in capsules for 30 days. Haematological parameters were within normal ranges. Bone marrow was normal, and ratios of myeloid to erythroid precursors

in bone marrow (M/E ratios) were within expected ranges. Haematuria and albuminuria occurred in both dogs. Gross and microscopic examinations of heart, lung, liver, pancreas, spleen, gastrointestinal tract, kidney, bladder, adrenal, thyroid and bone marrow were normal, except for the bladder, which showed mild cystitis in both animals. This study had no control group, and the report was provided in summary form only (Anderson et al., 1966a,b).

One male and one female dog were given oral doses of 25 mg tylosin base/kg bw per day in capsules twice daily for 25 days. Haematological parameters and bone marrow were normal, and the M/E ratios were within expected limits. Although a slight amount of albumin was found in the urine of the male dog, urine from the female was albumin-free. All tissues appeared normal on gross and microscopic examination. This study had no control group, and the report was provided in summary form only (Anderson et al., 1966a,b).

In a 2-year study, Beagle dogs and mixed-breed dogs were divided into groups of eight animals (both sexes) and given oral doses of 0, 1, 10 or 100 mg tylosin base/kg bw per day by capsule. After this study had progressed for 153 days, it was expanded by adding further groups of two male and two female mongrel dogs given doses of 200 or 400 mg tylosin base/kg bw per day for the remainder of the study period. Occasional diarrhoea and vomiting occurred in dogs given 10–400 mg/kg bw per day. Transient elevated bromosulfophthalein retention times were recorded in two dogs at 100 mg/kg bw per day and one dog at 400 mg/kg bw per day. Haematology, urinalysis, bone marrow sections, body weights and organ weights were unaffected, and no changes were noted in the faecal microbiological flora. At necropsy, mild pyelonephritis was found in one dog given 200 mg/kg bw per day, and bilateral nephrosis, mild chronic pyelonephritis and mild chronic cystitis were seen in one dog given 400 mg/kg bw per day. Since diarrhoea and vomiting are common observations in untreated dogs, the NOAEL was 100 mg/kg bw per day (Anderson et al., 1966a).

(c) Quail

Bobwhite quail chicks (5–10 per group) were fed diets containing 0, 1250, 2500 or 5000 mg tylosin base/kg for 5 days, followed by 3 days of basal diet. There were no treatment-related deaths or overt signs of toxicity. Food consumption and body weight gains were unaffected (Kehr et al., 1978b).

(d) Ducks

Groups of 10 mallard ducklings (*Anas platyrhynchos*) were fed diets containing 0, 1250, 2500 or 5000 mg tylosin base/kg for 5 days, followed by 3 days of basal diet. There were no overt signs of toxicity and no deaths. Food consumption and weight gains were depressed during the treatment period in all tylosin-fed ducklings, presumably due to rejection of food. All birds showed normal or accelerated weight gains during the withdrawal period (Kehr et al., 1978c).

(e) Chickens

Replicate groups of 10 1-day-old unsexed White Rock chickens were fed tylosin tartrate at levels of 0, 220, 550, 1100 or 3300 g equivalent base activity per tonne of feed. The dietary mixtures were prepared in Lilly Broiler ration for the first 9 weeks of treatment and subsequently in Lilly Pullet Grower ration. The chickens were on treatment for a total period of 18 weeks. At week 8, five chickens from each replicate of each treatment group were sacrificed, and the remaining birds were killed terminally. Body weight gain and feed conversion, haematology, organ weights and histopathology were similar between groups (Berkman & Van Duyn, undated).

Seventy-five male and 75 female 1-week-old Cobb broiler chickens were allocated into three equal groups that received 0, 0.5 or 1.5 g tylosin tartrate/l in drinking-water for 8 days. All birds remained in good health throughout the study, and no treatment-related effects on body weight gains or feed and water consumption were noted (Cameron, 1990a).

(f) Turkeys

Seventy-five male and 75 female 11-day-old “Big 6” type turkey poults were allocated into three equal groups that received 0, 0.5 or 1.5 g tylosin tartrate/l in drinking-water for 5 days. All birds remained in good health throughout the study, and no treatment-related effects on body weight gains or feed consumption were noted. There was a slight dose-related reduction in water consumption, but all group values remained within normal limits (Cameron, 1990b).

(g) Pigs

Nine male and nine female weaned pigs, approximately 8 weeks of age, were allocated into three equal treatment groups that received 0, 0.25 or 0.75 g tylosin tartrate/l in drinking-water for 10 days. All animals remained in good health throughout the study, and no treatment-related effects on body weight gains or food and water consumption were noted (Cameron, 1990c).

(h) Cattle

Nine male and nine female calves were allocated into three equal treatment groups that received 0, 1 or 3 g tylosin tartrate per calf in milk replacer feeds for 14 days. All treated animals remained in good health throughout the study. Hard, dry faeces were noted during the 2nd week of the test period in both groups treated with tylosin, but no other treatment-related clinical abnormalities were observed. There were no treatment-related effects on body weight gain or milk feed consumption (Cameron, 1990d).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Rats

Harlan rats (three animals per sex) were given diets containing 0, 1000, 3000 or 10 000 mg tylosin base/kg for 17 months. There were no effects on body weight, and haematological parameters were within normal limits. Ovary size and weights were depressed, and uterus thickening and weights were increased in 1/3, 3/3, 2/3 and 2/3 rats in the control, 1000, 3000 and 10 000 mg/kg diet groups, respectively. Squamous metaplasia in the uterus was observed in two females given 10 000 mg/kg diet. These effects were considered to be age related rather than treatment related. This study was provided in summary form only (Anderson et al., 1966a,b).

Groups of about 25 Harlan rats per sex per dose were given 0, 10, 100 or 1000 mg tylosin base/kg in the diet for 2 years. Survival at 2 years was 30%, 41%, 70% and 51% with increasing dose. There were no effects on body weight, and haematological parameters were within normal limits. There were no differences in organ weights, nor were any gross or histopathological findings attributable to tylosin treatment. This study was provided in summary form only (Anderson et al., 1966a,b).

Groups of 10 male and 10 female rats were fed diets containing 0, 20 000, 50 000, 100 000 or 200 000 mg tylosin base/kg diet for 2 years. Body weight gain and food intake were reduced at the two highest doses. All high-dose rats died within 12 months and exhibited high incidences of malnutrition and atrophy/necrosis of lymphoid organs. This study was provided in summary form only (Anderson et al., 1966a,b).

Harlan albino rats were given 0, 100 or 10 000 mg tylosin base/kg admixed in the diet for 2 years. Each group consisted of approximately 30 animals per sex. After the 16th week of feeding, 10 females and 5 males from the control and high dose groups were mated within groups. Pregnant females were allowed to litter and complete lactation and, following a 1-week rest period, were remated with a different male from the same group. This process was continued for a minimum of six pregnancies. The effects on reproductive indices are reported in section 2.2.5. Survival was enhanced in males and females of the 10 000 mg/kg diet group (57% versus 29%). Food intake and body weight gain were similar between groups, and haematology, urinalysis and organ weights were unaffected. Slightly increased fatty change in the liver was observed in both sexes given 10 000 mg/kg diet. The NOAEL was 100 mg/kg diet, equivalent to 5 mg/kg bw per day (Harris, 1961).

Weanling Wistar rats, the F_1 offspring from the fertility study (see section 2.2.5), were used in replicate 2-year chronic studies. Each study consisted of a control group of 60 rats per sex and treated groups of 40 per sex per dose given 1000, 5000 and 10 000 mg tylosin base/kg in the diet. Based on food intake, the mean doses were equal to 106, 517 and 1080 mg/kg bw per day during the 1st week of study and 39, 192 and 402 mg/kg bw per day during the last week of study. The findings were similar in both replicates. Physical signs of toxicity, haematology, blood chemistry, urinalysis and organ weights were not affected. Survival during the

final 3–6 months was slightly higher (5–10%) in tylosin-treated male rats, but was not dose related. Food consumption was increased in males and females given 5000 and 10 000 mg/kg diet, and body weight gains were generally increased in males given the highest dose. The incidence of bacterial pneumonia was decreased in a dose-related manner. The incidences of benign pituitary adenomas in male rats are outlined in Table 3. The incidences in one middle dose group and one high dose group were above the historical control range for this laboratory (1.7–23.3%). Tylosin had no effect on the incidence of malignant tumours in males or females (Gries, 1980a).

Table 3. Incidences of benign pituitary adenomas in male rats (Gries, 1980a)

	Incidence of pituitary adenomas at following dietary doses			
	0 mg/kg	1000 mg/kg	5000 mg/kg	10 000 mg/kg
Replicate 1	1/60	3/40	10/40[a]	8/40
Replicate 2	5/60	6/40	8/40	12/40[a]

[a] Incidence was higher than historical control range.

Further information was provided that purported to demonstrate a high correlation between benign pituitary tumours in male rats and food consumption/ body weight. These studies documented comparative findings from experiments in which four strains of rat were placed on restricted diets or were fed ad libitum and from another 10 separate chronic studies in Wistar rats conducted in the above laboratory. The increase in pituitary adenomas was considered not to be due to tylosin per se but secondary to the increased food consumption and survival associated with tylosin ingestion (Ross et al., 1970; Gries, 1980b; Gries & Young, 1982). Pituitary tumours are commonly observed in ageing rats, and the apparent increase in some treated groups was considered to be associated with the increased survival in treated males. The NOAEL was 10 000 mg/kg diet, equal to 402 mg/kg bw per day, the highest dose tested.

2.2.4 Genotoxicity

Tylosin was evaluated for potential genotoxicity in a battery of in vitro and in vivo tests. The results are summarized in Table 4. The tests included 1) an in vitro assay for the induction of gene mutations in L5178Y mouse lymphoma cells, 2) an in vitro assay for the induction of gene mutations in $HGPRT^+$ Chinese hamster ovary cells, 3) an in vitro chromosomal damage assay in Chinese hamster ovary cells and 4) an in vivo assay for cytogenetic damage in mouse bone marrow.

A total of three experiments were conducted with mouse lymphoma cells in the absence of metabolic activation. The mutation frequency was increased by only 2.7- to 3.8-fold at 1000 µg/ml and by 2.7- to 2.8-fold at 850 µg/ml. These concentrations were cytotoxic, and the mean per cent survival was 13% and 25% at 1000 and 850 µg/ml, respectively. Owing to decreased survival of cells, the positive mutagenicity in the mouse lymphoma assay was considered to represent

Table 4. Results of tests for genotoxicity with tylosin

End-point	Test object	Concentration/ dose[a]; tylosin form	Result	References
Forward mutation in vitro[b]	L5178Y mouse lymphoma cells	10–1000 µg/ml −S9, 10–750 µg/ml +S9[c]; tylosin (salt unknown)	Positive[d]	Michaelis (1990)
Forward mutation in vitro[b]	Chinese hamster ovary cells	100–1500 µg/ml ±S9[c]; tylosin (salt unknown)	Negative	Garriott et al. (1991a)
Chromosomal damage in vitro[e]	Chinese hamster ovary cells	500–1000 µg/ml −S9, 250–750 µg/ml +S9[c]; tylosin (salt unknown)	Negative	Kindig (1990)
Chromosomal damage in vivo[f]	Micronucleus formation in bone marrow from ICR mice	Two daily oral doses of 1250, 2500 or 5000 mg/kg bw; tylosin base	Negative	Gries et al. (1990)

[a] Concentrations/doses are expressed as tylosin base.
[b] Positive controls were ethylmethane sulfonate in the absence of S9 and 3-methylcholanthrene in the presence of S9.
[c] 9000 × *g* supernatant of rat liver used for metabolic activation.
[d] The mutation frequency was increased at 850 and 1000 µg/ml −S9.
[e] Positive controls were mitomycin C in the absence of S9 and cyclophosphamide in the presence of S9.
[f] Positive control was cyclophosphamide.

an unreliable result. The sponsor advised that the majority of mutant colonies associated with tylosin treatment were small in nature (Garriott et al., 1991b), suggesting that tylosin produces a clastogenic effect rather than a mutagenic effect (Clive et al., 1979; Moore et al., 1985). However, no satisfactory data to support this contention were provided. Owing to the markedly decreased survival of cells, the positive mutagenicity in the mouse lymphoma assay was considered to represent an unreliable result. It is concluded that tylosin presents a low potential for genetic damage.

2.2.5 Reproductive and developmental toxicity

(a) Fertility studies

(i) Mice

Groups of 7–8 male and 14–17 female ICR mice were paired and given diets containing 0, 1000 or 10 000 mg tylosin base/kg continuously for two litters in each of two generations. The commencement of treatment was variable, but in most cases it began prior to conception in the F_0 mice. Females were allowed to deliver naturally and rear their young for 4 weeks in each generation. There were no

significant effects on litter size, growth of pups, number of young weaned or fertility. The NOAEL was 10 000 mg/kg diet, equivalent to 1500 mg/kg bw per day, the highest dose tested (Tsubura et al., undated).

(ii) Rats

A rat reproduction study was conducted as part of a 2-year study in Harlan albino rats (see section 2.2.3). Groups of approximately 30 animals per sex were given 0 or 10 000 mg tylosin base/kg admixed in the diet. After the 16th week of feeding, 10 females and 5 males were mated within groups. Pregnant females were allowed to litter and complete lactation and, following a 1-week rest period, were remated with a different male from the same group. This process was continued for a minimum of six pregnancies. First litters were discarded, and 5 males and 10 females per group were selected from second litters for the subsequent F_1, F_2 and F_3 generations. Growth curves and survival of offspring and fertility and reproductive indices were similar for the control and treated groups in each succeeding generation. The NOAEL was 10 000 mg/kg diet, equivalent to 500 mg/kg bw per day, the only dose tested (Tsubura et al., undated; Harris, 1961).

Wistar rats (35 control, 25 treated per sex per dose) were fed diets containing 0, 1000, 5000 or 10 000 mg tylosin base/kg for 10 weeks prior to mating and until about 6 weeks after their offspring were distributed to 1-year chronic toxicity studies (see section 2.2.2) and 2-year carcinogenicity studies (see section 2.2.3), a total period of about 5 months. Based on food intake, the doses were equal to 0, 61–70, 311–379 and 635–795 mg/kg bw per day. There were no treatment-related clinical signs, and food intake and weight gain were similar in all groups. White blood cell counts were statistically significantly decreased in males given 10 000 mg/kg diet, but the value was within the normal range. Blood chemistry, fertility and reproductive performance of the parental generation and growth and survival of the F_1 progeny were not affected. Sera collected from parental rats after approximately 150 days on experimental diets did not contain detectable levels (LOD = 0.1 µg/ml) of tylosin. The NOAEL was 10 000 mg/kg diet, equal to 635 mg/kg bw per day, the highest dose tested (Broddle et al., 1978b; Hoyt & Higdon, 1991).

(b) Developmental studies

(i) Mice

Tylosin base was given by gavage to A/Jax or CBA strain mice (10 females per group) at doses of 0, 100, 500 or 1000 mg/kg bw per day on gestation days 7–12. Another two females (A/Jax × CBA hybrid) per group were given 0 or 1000 mg/kg bw per day on the same schedule. Four A/Jax mice per group given 0 or 500 mg/kg bw per day were allowed to deliver and rear their young for 4 weeks. The remainder were killed on gestation day 18. There were no treatment-related differences in maternal body weight gains. There were no effects on survival or external, visceral or skeletal development of fetuses. Delivered offspring were maintained until they were 9 weeks old. No adverse effects were noted in growth, survival, vaginal patency or testicular descent in the pups. Activity was normal when measured at 7 and 9 weeks, and no changes were found in sensory measurements. There were no treatment-related findings in the visceral or skeletal examinations

performed at 9 weeks. The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Tsuchikawa & Akabori, undated; Kelich & Byrd, 1991).

(ii) Rats

Groups of female Wistar rats were given diets containing 0, 1000, 10 000 or 100 000 mg tylosin base/kg. Based on food intake, the doses were equal to 0, 60.5, 725 and 4800 mg/kg bw per day. Ten control and 15 animals from each treated group were dosed on gestation days 0–20 and killed on gestation day 20. There were no treatment-related effects on survival during gestation and no treatment-related fetal abnormalities from external, visceral or skeletal examination. At 100 000 mg/kg diet, body weight was depressed in dams and fetuses, and ossification was retarded. Fifteen females from the control and the two highest dose groups were treated from gestation day 0 to postnatal day 21 and allowed to deliver and rear their litters until postnatal day 21. The body weight gain of offspring was lower at 100 000 mg/kg diet. There were no remarkable findings from external, visceral or skeletal examination of the weanlings. The NOAEL was 10 000 mg/kg diet, equal to 725 mg/kg bw per day (Terashima & Maeda, undated; Kelich & Byrd, 1991).

2.2.6 Special studies

(a) Pharmacology

The general pharmacological properties of tylosin have been evaluated in dogs (Anderson, 1960; Anderson et al., 1966a). The effects of tylosin on blood pressure, cardiac activity, intestinal mobility and respiration were determined in six anaesthetized dogs. Tylosin, as the hydrochloride, was administered intravenously in doses of 10–40 mg/kg bw.

Injection at all dose levels was followed by a decrease in mean arterial pressure. This varied from a 13–18% fall following 10 mg/kg bw to 20–40% following 40 mg/kg bw. In some instances, the depressor action was transient; in other cases, it persisted for 1 h or more. This depressor action of tylosin was similar to that reported for erythromycin. In three dogs, there was a slight increase in respiration rate. There were no changes in heart rate, as indicated by the electrocardiogram (ECG). However, in one dog, the ECG pattern showed an increase in height of the T- and S-waves following the dose of 40 mg/kg bw. Duodenal motility generally tended to increase for 10–25 min. In two dogs, there was no change following 10 mg/kg bw. In one dog, there was a relaxation of the duodenum following 20 mg/kg bw and a relaxation followed by stimulation after 40 mg/kg bw.

(b) Neurotoxicity

One female and three male cats were treated subcutaneously with 200 mg tylosin tartrate/kg bw per day for 90 days. Three cats served as untreated controls. There was a slight reduction (25–35%) in the post-rotatory nystagmus response, but hearing appeared unimpaired. When the cats were allowed to fall from a height of approximately 1 m, each landed on all four feet. In addition, there was no ataxia.

It was concluded that there was no significant indication of neurotoxicity. This study was provided in summary form only (Anderson et al., 1966a).

(c) Intracellular accumulation

The uptake and intracellular accumulation of tylosin were studied in primary swine and bovine alveolar macrophage cultures using ^{14}C-labelled material. Tylosin reached a concentration of 197 µg/ml in swine macrophages and 122 µg/ml in bovine macrophages after a 24-h incubation time in culture medium containing 20 µg/ml of antibiotic. The ratio of intracellular to extracellular concentrations for tylosin was 9.8 and 6.1 for swine and bovine alveolar macrophage cultures, respectively (Chamberland, 1993).

(d) Interaction with metabolic enzymes

Sprague-Dawley rats were given intraperitoneal injections of 500 mg tylosin tartrate/kg bw per day for 3 days and killed 24 h after the last injection. Cytochrome P450 (CYP) content in liver microsomes was similar to that in untreated animals, and formation of a tylosin metabolite–CYP complex was not detected. By comparison, a number of macrolide antibiotics, most notably troleandomycin and erythromycin, induced CYP content and formed a CYP–iron–nitrosoalkane metabolite complex, leading to inhibition of enzymatic activity. Structural factors were thought to be responsible for the differing responses (Delaforge et al., 1983).

The relative CYP3A inhibiting potency and CYP–iron–nitrosoalkane metabolite complex formation of a series of macrolide antibiotics were studied in liver microsomal fractions of goat and cattle and in a cell line expressing bovine CYP3A. Tylosin tartrate showed typical complex formation as measured by spectral analysis, but was a weak inhibitor (≤10%) of the CYP3A-catalysed hydroxylation of testosterone in microsomes and was not an inhibitor in the V79 bovine CYP3A cell line. Higher complex formation and greater inhibition were exhibited by triacetyl oleandomycin and erythromycin (Zweers-Zeilmaker et al., 1999).

(e) Skin and eye irritation

Eight dogs and eight cats received two intramuscular injections of Tylocine Injection, once in each leg, at a dose of 11 mg tylosin/kg bw. The animals were killed 7 and 14 days post-injection, when internal and external examination of the injection sites showed no evidence of irritation (Van Duyn & Kline, undated).

Tylosin was applied topically to the skin of New Zealand White rabbits at a dose of 2.0 ml Tylan 200 Injection/kg bw or 2000 mg Tylosin Concentrate or Tylan Soluble/kg bw. After 24-h exposure, the treatment sites were rinsed with warm water, and the rabbits were observed for the subsequent 14 days. No treatment-related deaths or signs of systemic toxicity were observed. Very slight dermal irritation occurred, which cleared within 48 h after treatment with Tylan 200 Injection. No dermal irritation occurred after exposure to Tylosin Concentrate. Very slight irritation, which cleared within 8 days, and slight desquamation in two animals were attributed to the treatment with Tylan Soluble (Downs & Negilski, 1985a,b; Negilski & Downs, 1985).

Tylosin was placed in one eye of New Zealand White rabbits in an amount of 0.1 ml, 52 mg or 58 mg of Tylan 200 Injection, Tylosin Concentrate or Tylan Soluble, respectively. Tylan 200 Injection caused very slight conjunctival hyperaemia, which cleared within 48 h. Tylosin Concentrate caused corneal dullness, slight corneal opacity, slight to moderate iritis and moderate conjunctivitis within 1 h post-exposure. However, all irritation cleared within 14 days. Tylan Soluble caused slight to moderate corneal opacity, marked iritis and moderate conjunctivitis within 1 h. In this study, all irritation cleared within 7 days post-exposure (Downs & Negilski, 1985a,b; Negilski & Downs, 1985).

(f) Sensitization

Ten guinea-pigs were given an intraperitoneal injection of 10 mg tylosin base. One week later, the seven surviving animals were given a second 10 mg injection of tylosin. All animals died during the following 3 weeks prior to administration of the challenge dose (Anderson et al., 1966a).

Groups of three guinea-pigs were given a single intraperitoneal injection of 2, 4 or 7 mg tylosin hydrochloride/kg bw, and eight guinea-pigs were given a single intraperitoneal injection of 10 mg/kg bw. The three, two, one and two animals (respectively) surviving after 5 weeks were challenged with an intravenous dose of 5 mg tylosin/kg bw. None exhibited overt signs, indicating the absence of a sensitization response. This study was provided in summary form only (Anderson et al., 1966a).

These studies were conducted prior to the development of more advanced methods of determining sensitization potential and were also compromised by poor survival due to general systemic toxicity unrelated to sensitization.

(g) Antigenicity

Attempts were made to produce antibodies in eight rabbits injected intradermally with a combination of 100 mg tylosin lactate, human serum albumin and Freund's complete adjuvant. Blood was collected 3 days after the sensitization phase, and the resulting sera were tested for the production of passive cutaneous anaphylaxis in guinea-pigs. There were no reactions following intravenous challenge with the antigens (Horiuchi, undated).

(h) Stimulation of hormones in vitro

Using transformed HeLa cells expressing human thyroid hormone responsive elements, tylosin tartrate, at concentrations up to 100 µmol/l, failed to exhibit any direct interaction with the receptors. However, concentrations of 1 pmol/l to 100 µmol/l inhibited triiodothyronine stimulation of receptors, with a flat dose–response (Jeong et al., 2007).

Tylosin tartrate at concentrations up to 100 µmol/l did not affect the synthesis of growth hormone in cultured rat pituitary epithelial tumour cells (ATCC CCL-82.1). The release in growth hormone stimulated by triiodothyronine was inhibited by

tylosin at concentrations from 1 pmol/l to 100 µmol/l, with a flat dose–response (Jeong et al., 2007).

(i) Microbiology

A JECFA decision tree approach that was adopted at the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and complies with Guideline 36 of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL36) (VICH, 2004) was used by the Committee to determine the need to establish a microbiological ADI for tylosin. The decision tree approach initially seeks to determine if there may be microbiologically active tylosin residues entering the human colon. If the answer is "no" to any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are to be considered: 1) disruption of the colonization barrier and 2) increase of the population(s) of resistant bacteria. At Step 4 of the decision tree process, it is possible to provide scientific justification to eliminate testing (i.e. the need for a microbiological ADI) for either one or both end-points. Step 5 is where a microbiological ADI would be determined. Should a microbiological ADI not be necessary, then the toxicological or pharmacological ADI would be used. The Committee evaluated minimum inhibitory concentration (MIC) susceptibility, faecal binding interaction and biological activity of tylosin metabolites and used the decision tree to answer the following questions in the assessment of tylosin.

Step 1: Are residues of the drug, and (or) its metabolites, microbiologically active against representatives of the human intestinal flora?

Yes. Tylosin is microbiologically active against the majority of the bacterial genera and species representative of the human intestinal flora. Most of the intestinal bacteria tested were susceptible to tylosin, with the Gram-positive anaerobes being more susceptible than the Gram-negative strains. *Bifidobacterium* spp. and *Clostridium* spp. had the lowest MIC_{50} values (0.062 µg/ml). Tylosin did not show activity against any of the strains of *Escherichia coli* (MIC_{50} >128 µg/ml).

MICs for tylosin were determined in 100 bacterial strains representative of the normal human intestinal microflora. The organisms were isolated from faeces of healthy volunteers who had not received antibiotic therapy during the 3 months prior to faecal collection and who had experienced no symptoms of diarrhoea within 4 weeks of sample collection. Ten predominant human faecal flora species were isolated, and 10 strains of each bacterial species were grown for use in the MIC tests. For each bacterial strain used in the test, the standardized inoculum was enumerated to demonstrate compliance with Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2004). The MIC range, MIC_{50} and MIC_{90} results are presented in Table 5. Tylosin activity varied considerably between bacterial groups and also within the majority of groups. A consistent absence of antibacterial activity was apparent against any of the strains of *Escherichia coli*, which had MIC values >128 µg/ml. Antibacterial activity was demonstrated among the strains from other species; the most susceptible were the Gram-positive anaerobes, *Bifidobacterium*, *Clostridium*, *Eubacterium* and *Peptostreptococcus*. The MIC_{50} of *Bifidobacterium* spp. and *Clostridium* spp. was 0.062 µg/ml (Pridmore, 2006a).

Table 5. MIC values of tylosin on human microflora

Microflora[a]	Inoculum density ($\times 10^8$ cfu/ml)	Tylosin MIC values (µg/ml)			
		MIC range	MIC_{50}	MIC_{90}	Geometric mean MIC
Bacteroides fragilis	1.5–5.8	0.5–128	1	32	2.6
Bacteroides (other species)	1.8–12	0.25–32	0.5	32	1.3
Bifidobacterium spp.	0.34–6.5	0.031–2	0.062	1	0.12
Clostridium spp.	0.21–13	0.031–0.5	0.062	0.25	0.094
Enterococcus spp.	1.3–5.6	1–4	1	4	2.0
Eubacterium spp.	0.46–2.4	0.125–1	0.25	0.5	0.31
Fusobacterium spp.	0.46–3.4	0.062–64	1	4	1.5
Lactobacillus spp.	0.23–8	0.5–8	2	8	2.0
Peptostreptococcus	0.33–5.5	0.125–0.5	0.5	0.5	0.41
Escherichia coli	2.3–59	>128	>128	>128	>128

cfu, colony-forming unit.
[a] Ten isolates per organism (total 100 isolates) were used.

Step 2: Do residues enter the human colon?

Yes. Residue studies using ^{14}C radiolabelling to detect total residues have been conducted or analytical methods to detect parent tylosin have been determined in poultry, pigs, cattle, eggs and milk. Under the most conservative assumptions, tylosin residues may be present at low levels in meat products consumed by humans. Therefore, tylosin-related residues could enter the colon of a person ingesting edible tissues or milk from tylosin-treated animals.

Step 3: Do the residues entering the human colon remain microbiologically active?

Yes. Tylosin residue will be extensively transformed to metabolites with reduced activity prior to entering the colon of the consumer; within the colon, it will become diluted and substantially bound (~36%) to faecal material. Therefore, approximately 64% of tylosin residues in the human colon are free (unbound). Since tylosin metabolites reaching the colon would have 35% of the activity of tylosin A (Teeter & Meyerhoff, 2003), then approximately 22.4% of the fraction of oral dose is available and potentially microbiologically active.

Faecal binding experiments were conducted using 12 tylosin concentrations between 0 and 3.3 µg/ml in linear arithmetic increments of 0.3 µg/ml. The reference bacterial strain was *Enterococcus faecalis*, which is susceptible to tylosin. Each drug concentration was mixed with three separate sterile faecal samples at concentrations of 0 (Mueller Hinton Broth only), 25% and 50% faeces and incubated for 0, 1, 2, 6, 8 or 12 h. The antibacterial activity of the supernatant obtained from each of the incubation times was assessed for the presence or absence of bacterial

growth before and after incubation with faeces. The results are summarized in Table 6. Binding of tylosin to faeces was not influenced by the concentration of faeces present, but it was time dependent. Within 1 h, binding was of the order of 20–28%. Maximal binding of tylosin to each concentration of faeces occurred between 1 and 8 h, and the extent of the binding varied between 28.6% and 42.9%. Practical limitations made it impossible to perform in vitro faecal binding assays using faecal concentrations that exceeded 50% weight by volume (w/v). Therefore, the 50% concentration provided the closest possible in vitro representation of the in vivo situation with regard to the binding of ingested tylosin residues to intestinal contents. On this basis, it was estimated that maximal tylosin binding of undiluted faecal material would occur within 1–8 h and would probably exceed 30% (Pridmore, 2006b).

Table 6. Determination of tylosin binding to human faeces[a]

Incubation time (h)	Broth only (no faeces)	25% w/v faeces		50% w/v faeces	
	Initial drug concentration (µg/ml) required to inhibit growth (a)	Initial drug concentration (µg/ml) required to inhibit growth (c)	% of drug bound to faeces [(c − a)/c] × 100	Initial drug concentration (µg/ml) required to inhibit growth (d)	% of drug bound to faeces [(d − a)/d] × 100
0	1.2	1.2	0.0	1.5	20.0
1	1.2	1.5	20.0	1.5	20.0
2	1.2	1.2	0.0	1.5	20.0
6	1.2	1.8	33.3	1.8	33.3
8	1.2	2.1	42.9	2.1	42.9
12	1.2	2.1	42.9	2.1	42.9

[a] Concentrations of tylosin used in this experiment were 0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7, 3.0 and 3.3 µg/ml.

Tylosin is extensively metabolized in pigs and rats, demonstrated by in vivo and in vitro radiolabelled tylosin studies. The metabolic pathway in humans is unknown. The metabolism of tylosin in rats is similar to that in pigs; however, the metabolic profiles in the two species are different. Major metabolites found were tylosin D, dihydrodesmycosin and seco acid of tylosin D (Kennington & Donoho, 1994). Tylosin D and dihydrodesmycosin have about 35% of the biological activity of tylosin A. Seco acids of tylosin D are biologically inactive (Teeter & Meyerhoff, 2003).

Step 4: Is there any scientific justification to eliminate testing for either one or both end-points of concern, i.e. disruption of the colonization barrier or resistance development?

Yes. There is potential for a disruption of the colonization barrier of the human gastrointestinal tract, as tylosin is most active against both *Bifidobacterium* spp. and *Clostridium* spp. Even though the majority of tylosin residues in the colon are bound to faeces and biologically inactive and the bioavailable concentration is close to the lowest MIC_{50} (0.062 µg/ml) for both *Bifidobacterium* spp. and *Clostridium* spp., as listed in Table 4, there is a need to determine a microbiological ADI, as there were no definitive analytical chemical assay data submitted to validate the faecal binding inactivation bioassay results. Tylosin residues are unlikely, but they may disrupt the colonization barrier of the human gastrointestinal tract. In terms of resistance development, all of the bacterial species representative of the human gastrointestinal tract had MIC values above the incurred active tylosin concentrations present in the human gastrointestinal tract. Thus, the small amount of active tylosin present in the human gastrointestinal tract would exert negligible pressure in selecting for resistance. Consequently, there is no need to consider resistance development as an end-point of concern.

Step 5: 1) Determine the no-observed-adverse-effect concentrations/levels (NOAECs/NOAELs) for the end-point(s) of concern as established in Step 4; and 2) The most appropriate NOAEC/NOAEL should be used to determine the ADI_{mic}.

The formula for calculating the upper bound of the microbiological ADI for the end-point of concern (disruption of the colonization barrier) is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, the estimated NOAEC (MIC_{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence limit (CL) of the mean MIC_{50} for the most relevant and sensitive human colonic bacterial genera for which the drug is active.

MIC_{calc} is derived as follows:

$$\text{Lower 90\% CL} = \text{Mean } MIC_{50} - \frac{\text{Std Dev}}{\sqrt{n} \times t_{0.10,df}}$$

where:

- Mean MIC_{50} is the mean of the log-transformed MIC_{50} values;
- Std Dev is the standard deviation of the log-transformed MIC_{50} values;
- n is the number of MIC_{50} values used in the calculations; and
- $t_{0.10,df}$ is the 90th percentile from a central t-distribution with degrees of freedom (df) = $n - 1$.

The tylosin ADI was derived from in vitro MIC data as described in VICH GL36 (VICH, 2004). The nine strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included, so *Escherichia coli* was excluded. Additionally,

although VICH GL36 requires the use of genera with a MIC_{50}, the Committee used the same genera but used the MIC_{90} to determine the MIC_{calc}. The justification is based on CLSI guidelines (CLSI, 2004) and the observation that some of the strains within each of the genera tested exhibited high MICs, presumably due to resistance. Given that the strains with the high MICs are more likely to fill niches vacated by susceptible strains (i.e. those with low MICs), the argument is advanced that these strains represent a more realistic scenario of what might occur in the human colon.

The MIC_{90} values for *Bacteroides fragilis*, other *Bacteroides* spp., *Bifidobacterium* spp., *Clostridium* spp., *Enterococcus* spp., *Eubacterium* spp., *Fusobacterium* spp., *Lactobacillus* spp. and *Peptostreptococcus* (Table 4) were used to obtain the following values:

- MIC_{90} = 5.44
- t = 1.397
- Lower 90% CL = 3.76
- MIC_{calc} = 1.698

Mass of colon content: A value of 220 g is based on the colon content measured from humans.

Fraction of oral dose available to microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine.

From the discussion above, the mix of metabolites reaching the colon would have 35% of the activity of tylosin A based on results in pigs as a surrogate model for the human. The faecal inactivation study indicates that at least 36% of tylosin A is bound to faeces, so 64% is available to microorganisms. Multiplying these two "availability" factors together (i.e. 0.35 × 0.64) gives 0.224. This is the fraction of an oral dose available to microorganisms.

Body weight: The body weight of an adult is assumed to be 60 kg.

The upper bound of the microbiological ADI can therefore be calculated as follows:

$$\text{Upper bound of ADI} = \frac{1.698 \times 200\ \text{g}}{0.224 \times 60\ \text{kg}}$$

$$= 25.3\ \mu\text{g/kg bw}$$

2.3 Observations in humans

Groups of 11 or 12 human volunteers were given daily oral doses of 20 mg tylosin or a placebo control for 6 months. There was no significant increase or decrease in the total number of staphylococci or lactobacilli in weekly faecal samples. The total number of streptococci was significantly increased at the end of the 6-month period. The occurrence of tylosin-resistant organisms followed a random pattern and showed no difference between groups. In addition, no overgrowth of coliform or yeast forms occurred. Of the 336 staphylococci isolates obtained from a hospital where other antibiotics were in use but tylosin was not, only 2 were resistant to tylosin lactate at 5 µg/ml. In both studies, no regular pattern was observed in cross-resistance to related and other antibiotics (Malin & Silliker, 1966).

Healthy adults (two per group) were given tylosin at oral doses of 0, 2 or 5 mg/day for 3 months. Faeces were inspected for *Escherichia coli*, enterococci and staphylococci at intervals of 1–2 weeks from 2 months prior to administration to 3 months after the start of administration. Bacterial counts were highly variable, but were not influenced by treatment with tylosin. At no time was any change in susceptibility or resistance pattern observed (Kuwabara, undated).

Of 3812 human cultures of *Staphylococcus aureus*, *Streptococcus pyogenes* and *Campylobacter* spp. isolated between May 1985 and April 1987, only 1% was found to be resistant to tylosin. There was no evidence for a significant animal source of these resistant cultures (Lacey, 1987).

Cases of occupational dermatitis following exposure to tylosin have been reported (Veien et al., 1980; Verbov, 1983). These reports suggest that, in humans, tylosin may cause irritation and/or allergic dermatitis.

3. COMMENTS

3.1 Biochemical data

Administration by the oral route in rats and dogs gave rise to peak serum concentrations within 2 h, followed by a rapid decline. Based on the findings in dogs, absorption appeared to take place in the intestine rather than in the stomach. In dogs given increasing doses, absorption was less than proportional to dose. Limited tissue distribution studies in rats with radiolabelled drug revealed higher levels in liver and kidney than in fat. The urinary recovery of oral doses in rats and dogs accounted for 1–2% of the dose, with the remainder in the faeces. Biliary excretion was not quantified, but tylosin concentrations were considerably higher in bile than in serum in both rats and dogs.

The biotransformation of tylosin was extensive in rats. Major compounds found in the liver were tylosin A, relomycin and dihydrodesmycosin. In the faeces, major compounds were relomycin and dihydrodesmycosin, and minor compounds included tylosin A, macrocin and a range of metabolites arising from hydrolysis of the lactone ring.

3.2 Toxicological data

Tylosin was relatively non-toxic in mice, rats and dogs after single oral dosing with the base and a variety of salts. The oral LD_{50} was generally >5000 mg/kg bw in rodents and >800 mg/kg bw in dogs.

Several short-term toxicity studies were performed in rats and dogs that received oral doses. Studies of up to 1 month in duration were provided in summary form only and were unsuitable for evaluation. In a 1-year rat study, there were increases in the numbers of lymphocytes and urinary pH was elevated at doses of 5000 mg/kg in the feed and above. The NOAEL in rats was 1000 mg/kg in the feed, equal to 39 mg/kg bw per day.

In a 2-year dog study, tylosin was given by capsule at doses of 0, 1, 10, 100, 200 or 400 mg/kg bw per day. Pyelonephritis was seen at 200 mg/kg bw per day, and pyelonephritis, nephrosis and cystitis were seen at 400 mg/kg bw per day. The NOAEL in dogs was 100 mg/kg bw per day.

Five long-term studies in orally dosed rats were available. Three studies were provided in summary form only and were unsuitable for evaluation. In the other studies, survival was enhanced by exposure to tylosin. In one study, fatty change in the liver was increased at a dose of 10 000 mg/kg in the feed, equivalent to 500 mg/kg bw per day. The other study revealed increased food intake at 5000 and 10 000 mg/kg in the feed and body weight gain at 10 000 mg/kg in the feed. At these same doses, the incidences of pituitary adenomas were increased in males. This type of tumour is commonly observed in ageing rats, and the apparent increase in some treated groups was considered to be associated with the increased survival in treated males. The overall NOAEL in rats was 5000 mg/kg in the feed, equal to 192 mg/kg bw per day.

Tylosin was evaluated for potential genotoxicity in an in vitro assay for the induction of gene mutations in L5178Y mouse lymphoma cells, in an in vitro assay for the induction of gene mutations in $HGPRT^+$ Chinese hamster ovary cells, in an in vitro chromosomal damage assay in Chinese hamster ovary cells and in an in vivo assay for cytogenetic damage in mouse bone marrow. Negative findings were obtained in Chinese hamster ovary cells investigated for gene mutations and chromosomal damage and in mouse bone marrow investigated for cytogenetic damage. An increase in gene mutations was detected in mouse lymphoma cells, in the absence of metabolic activation only. Owing to the markedly decreased survival of cells, the positive mutagenicity in the mouse lymphoma assay was considered to represent an unreliable result. It is concluded that tylosin presents a low potential for genetic damage.

Multigeneration reproduction studies were conducted in mice and rats. No adverse effects were found on reproductive indices or on growth and survival of offspring. The NOAEL was 10 000 mg/kg in the feed in all studies, equivalent to 1500 mg/kg bw per day in mice and 500 mg/kg bw per day in rats.

In developmental studies, fetal growth and development were unaffected at the highest administered doses of 1000 mg/kg bw per day in mice (given by gavage) and 725 mg/kg bw per day in rats (given as 10 000 mg/kg in the feed).

The most relevant study for determining a toxicological NOAEL is the 2-year study in dogs. The NOAEL was 100 mg/kg bw per day, and a safety factor of 100 was considered appropriate. Therefore, an ADI of 0–1 mg/kg bw could be established on the basis of the toxicological data.

3.3 Microbiological data

A JECFA decision tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and complies with VICH GL36 (VICH, 2004) was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for tylosin. Studies of microbiological activity against bacterial strains representative of the human colonic flora were presented. A consistent absence of antibacterial activity against any of the strains of *Escherichia coli*, which had MIC values >128 µg/ml, was apparent. Antibacterial activity was demonstrated among strains from other species. The most susceptible groups were the Gram-positive anaerobes—*Bifidobacterium*, *Clostridium*, *Eubacterium* and *Peptostreptococcus*. The MIC_{50} of both *Bifidobacterium* spp. and *Clostridium* spp. was 0.062 µg/ml.

Faecal binding experiments were conducted using tylosin concentrations between 0 and 3.3 µg/ml. The reference bacterial strain was *Enterococcus faecalis*, which is susceptible to tylosin. The binding of tylosin to faeces was not influenced by the concentration of faeces present (up to 50%), but it was time dependent. Maximal binding of tylosin to faeces at each concentration occurred between 1 and 8 h, and the extent of the binding varied between 28.6% and 42.9%. Practical limitations made it impossible to perform in vitro faecal binding assays using faecal concentrations that exceeded 50% w/v. Therefore, the 50% concentration provided the closest possible in vitro representation of the in vivo situation with regard to the binding of ingested tylosin residues to intestinal contents. On this basis, it was estimated that maximal tylosin binding of undiluted faecal material would occur within 1–8 h and would probably exceed 30%.

In studies in human volunteers given up to 20 mg tylosin orally for 6 months, there was no evidence for an increase in resistant microorganisms in the faeces or for the emergence of cross-resistance to other antibiotics.

Tylosin residues may disrupt the colonization barrier of the human gastrointestinal tract, as tylosin is most active against *Bifidobacterium* spp. and *Clostridium* spp. In terms of the development of resistance, all of the bacterial species representative of the human gastrointestinal tract had MIC values above the incurred active tylosin concentrations present in the human gastrointestinal tract. Thus, the small amount of active tylosin present in the human gastrointestinal tract would exert negligible pressure in selecting for resistance. Consequently, there is no need to consider the development of resistance as an end-point of concern.

The formula for calculating the upper bound of the microbiological ADI is as follows:

$$\text{Upper bound of ADI (µg/kg bw)} = \frac{\text{MIC}_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, calculation of the estimated NOAEC (MIC_{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence limit (CL) of the mean MIC_{50} for the most relevant and sensitive human colonic bacterial genera.

MIC_{calc} is derived as follows:

$$\text{Lower 90\% CL} = \text{Mean MIC}_{50} - \frac{\text{Std Dev}}{\sqrt{n} \times t_{0.10,df}}$$

where:

- Mean MIC_{50} is the mean of the log-transformed MIC_{50} values;
- Std Dev is the standard deviation of the log-transformed MIC_{50} values;
- n is the number of MIC_{50} values used in the calculations; and
- $t_{0.10,df}$ is the 90th percentile from a central t-distribution with degrees of freedom (df) = $n - 1$.

The tylosin ADI was derived from in vitro MIC data as described in VICH GL36 (VICH, 2004). The strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included; thus, *Escherichia coli* was excluded. Additionally, although VICH GL36 requires the use of genera with a MIC_{50}, the Committee used the same genera, but used the MIC_{90} to determine the MIC_{calc}. The justification is based on CLSI guidelines (CLSI, 2004) and the observation that some of the strains within each of the genera tested exhibited high MICs, presumably due to resistance. Given that the strains with the high MICs are more likely to fill niches vacated by susceptible strains (i.e. those with low MICs), the argument is advanced that these strains represent a more realistic scenario of what might occur in the human colon.

The MIC_{90} values for *Bacteroides fragilis*, other *Bacteroides* spp., *Bifidobacterium* spp., *Clostridium* spp., *Enterococcus* spp., *Eubacterium* spp., *Fusobacterium* spp., *Lactobacillus* spp. and *Peptostreptococcus* were used to obtain the following values:

- MIC_{90} = 5.44
- t = 1.397
- Lower 90% CL = 3.76
- MIC_{calc} = 1.698

Mass of colon content: A value of 220 g is based on the colon content measured from humans.

Fraction of oral dose available to microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo

measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine.

The mix of metabolites reaching the colon would have 35% of the activity of tylosin A based on results in pigs as a surrogate model for the human. The faecal inactivation study indicates that at least 36% of tylosin A is bound to faeces, so 64% is available to microorganisms. Multiplying these two "availability" factors together (i.e. 0.35 × 0.64) gives 0.224. This is the fraction of an oral dose available to microorganisms.

Body weight: The body weight of an adult is assumed to be 60 kg.

Therefore, the upper bound of the ADI is calculated as follows:

$$\text{Upper bound of ADI} = \frac{1.698 \times 200 \text{ g}}{0.224 \times 60 \text{ kg}}$$

$$= 25.3 \text{ µg/kg bw}$$

Therefore, a microbiological ADI of 0–30 µg/kg bw (rounded to one significant figure) could be derived from in vitro MIC susceptibility testing and faecal binding data.

4. EVALUATION

The sponsor addressed the concerns expressed by the Committee at its thirty-eighth meeting, which enabled a reconsideration of all the toxicological and related information and a review of new studies on microbiological effects. The Committee considered that microbiological effects were more relevant than toxicological effects for the establishment of an ADI for tylosin. Therefore, the Committee established an ADI of 0–30 µg/kg bw on the basis of the MIC data. This ADI is significantly lower than it would be if it were based on a toxicological end-point and is 6400-fold lower than the lowest dose associated with increased pituitary tumours in rats.

5. REFERENCES

Anderson, R.C. (1960) *Tylosin absorption and excretion studies*. Unpublished report No. 893/FAANIM/AM from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Anderson, R.C. & Worth, H.M. (1961) *The acute toxicity of tylosin phosphate*. Unpublished study No. 893/TACUTE/AM from Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Anderson, R.C., Harris, P.N., Lee, C.C., Maze, N., Small, R.M. & Worth, H.M. (1966a) *The toxicology and pharmacology of tylosin, an antibiotic, and some salts of tylosin*. Unpublished report No. VAR.100/c/9 from Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Anderson, R.C., Worth, H.M., Small, R.M. & Harris, P.N. (1966b) Toxicological studies on tylosin: Its safety as a food additive. *Food Cosmet. Toxicol.*, **4**, 1–15.

Berkman, R.N. & Van Duyn, R.L. (undated) *Tylosin toxicity study in poultry*. Unpublished study No. VPR-53-418 from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Broddle, W.D., Gossett, F.O., Adams, E.R., Hoffman, D.G., Gries, C.L., Gibson, W.R. & Morton, D.M. (1978a) *Chronic toxicity of tylosin fed to rats for one year.* Unpublished study No. R-307 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Broddle, W.D., Gossett, F.O., Adams, E.R., Hoffman, D.G., Kitchen, D.N., Gibson, W.R. & Morton, D.M. (1978b) *A study of a parental population of rats bred to produce offspring assigned to one- and two year dietary studies of tylosin.* Unpublished study No. R-1176 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Cameron, D.M. (1990a) *Tylosin (Tylan Soluble) dose tolerance study in broiler chickens*. Unpublished study No. LLY 24/90799 from the Department of Animal Science, Huntington Research Centre, Huntington, Cambridgeshire, England. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Cameron, D.M. (1990b) *Tylosin (Tylan Soluble) dose tolerance study in turkeys*. Unpublished study No. LLY 25/90800 from the Department of Animal Science, Huntington Research Centre, Huntington, Cambridgeshire, England. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Cameron, D.M. (1990c) *Tylosin (Tylan Soluble) dose tolerance study in pigs*. Unpublished study No. LLY 22/90797 from the Department of Animal Science, Huntington Research Centre, Huntington, Cambridgeshire, England. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Cameron, D.M. (1990d) *Tylosin (Tylan Soluble) dose tolerance study in calves*. Unpublished study No. LLY 23/90798 from the Department of Animal Science, Huntington Research Centre, Huntington, Cambridgeshire, England. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Chamberland, S. (1993) *Intracellular accumulation of tylosin in primary swine and bovine alveolar macrophages*. Unpublished study from the Département de Microbiologie, Centre de Recherche du CHUL, Université Laval, Ste-Foy, Québec, Canada. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Clive, D., Johnson, K.O., Spector, J.F.S., Batson, A.G. & Brown, M.M.M. (1979) Validation and characterization of the L5178Y/TK$^{+/-}$ mouse lymphoma mutagen assay system. *Mutat. Res.*, **59**, 61–108.

CLSI (2004) *Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard*, 6th ed. Wayne, PA, USA, Clinical and Laboratory Standards Institute (CLSI Document M11-A6).

Codex Alimentarius Commission (2007) *Report of the 17th Session of the Codex Committee on Residues of Veterinary Drugs in Foods, Breckenridge, CO, USA, 3–7 September 2007*. Rome, Italy, Food and Agriculture Organization of the United Nations (ALINORM 08/31/31; http://www.codexalimentarius.net/web/archives.jsp?year=08).

Delaforge, M., Jaouen, M. & Mansuy, D. (1983) Dual effects of macrolide antibiotics on rat liver cytochrome P-450. Induction and formation of metabolite-complexes: A structure–activity relationship. *Biochem. Pharmacol.*, **32**(15), 2309–2318.

Downs, O.S. & Negilski, D.S. (1985a) *The acute oral, dermal, and ocular toxicity of granulated tylosin concentrate*. Unpublished studies Nos R-0-365-79, R-0-366-79, B-D-109-79 and B-E-94-79 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Downs, O.S. & Negilski, D.S. (1985b) *The acute oral, dermal, ocular and inhalation toxicity of Tylan 200 Injection*. Unpublished studies Nos R-0-344-79, R-0-343-79, B-D-103-79, B-E-87-79 and R-H-39-79 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Garriott, M.L., Michaelis, K.C. & Gries, C.L. (1991a) *The effect of tylosin on the induction of forward mutation at the HGPRT$^+$ locus of Chinese hamster ovary cells*. Unpublished study No. 910612CHO3279 from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Garriott, M.L., Oberly, T.J. & Gries, C.L. (1991b) *A revision to the summary of genetic toxicology studies with tylosin.* Unpublished studies Nos 891109CTX3279, 891129CAB3279, 891220CAB3279, 891212MNT3279, 891011MLT3279, 891017MLA3279, 891114MLA3279, 910501CHT3279, 910605CHT3279 and 910612CHO3279 from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Gries, C.L. (1980a) *The toxicological evaluation of tylosin (compound 27892) given to Wistar rats in the diet for two years*. Unpublished studies Nos R-287 and R-297 from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Gries, C.L. (1980b) *Compound 27892 (tylosin), studies R-287 and R-297*. Unpublished memorandum to Dr M.E. Amundson from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Gries, C.L. & Young, S.S. (1982) Positive correlation of body weight with pituitary tumor incidence in rats. *Fundam. Appl. Toxicol.*, **2**(3), 145–148.

Gries, C.L., Parton, J.W. & Garriott, M.L. (1990) *The effect of tylosin (compound 027892) on the in vivo induction of micronuclei in bone marrow of ICR mice.* Unpublished study No. 891212MNT3279 from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Harris, P.N. (1961) *Chronic toxicity study R3-59*. Unpublished study No. R3-59 from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Horiuchi, Y. (undated) *Antigenicity of tylosin lactate*. Unpublished study from Faculty of Medicine, Hokkaido University, Sapporo, Japan. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Hoyt, J.A. & Higdon, G.L. (1991) *A reproduction study of rats fed diets containing tylosin: Data tables for reproduction and progeny indices.* Unpublished report No. R-1176 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Jeong, S.-H., Kang, J.-W., Cho, J.-H., Choi, B.-K., Kim, E.-J., Jean, Y.-H., Woo, G.-H. & Chung, G.-S. (2007) *Toxicological study of tylosin tartrate in rats and mammalian cells.* Unpublished report from Toxicology and Chemistry Division, National Veterinary Research and Quarantine Service, Anyang, Republic of Korea. Submitted to WHO by Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

Kehr, C.C., Hamelink, J.L., Brannon, D.R. & Amundson, M.E. (1978a) *The toxicity of compound 27892 (tylosin) to bobwhite quail in an acute oral study.* Unpublished study No. 7017-78 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Kehr, C.C., Hamelink, J.L., Brannon, D.R. & Amundson, M.E. (1978b) *The toxicity of compound 27892 (tylosin) to bobwhite quail in an eight day dietary study.* Unpublished study No. 7025-78 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Kehr, C.C., Hamelink, J.L., Brannon, D.R. & Amundson, M.E. (1978c) *The toxicity of compound 27892 (tylosin) to mallard ducks in an eight-day dietary study.* Unpublished study No. 7022-78 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Kelich, S.L. & Byrd, R.A. (1991) *Teratology studies of tylosin in mice and rats: Summary tables and figures.* Unpublished report from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Kennington, A.S. & Donoho, A.L. (1994) *^{14}C-tylosin rat metabolism study*. Unpublished study No. T1X759102 from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Kindig, D.E.F. (1990) *The effect of tylosin on the in vitro induction of chromosome aberrations in Chinese hamster ovary cells*. Unpublished studies Nos 891109CTX3279, 891129CAB3279 and 891220CAB3279 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Kuwabara, S. (undated) *A study on the effect of a continuous and minute amount of tylosin on human intestinal flora*. Unpublished study from the Medical Department, Toho University, Tokyo, Japan. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Lacey, R.W. (1987) *Rarity of tylosin resistance in human pathogenic bacteria.* Unpublished report from the Department of Microbiology, University of Leeds, Leeds, England. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Malin, B. & Silliker, J.H. (1966) *Low level tylosin and the emergence of antibiotic-resistant bacteria in humans*. Abstract, American Society of Microbiology 6th Interscience Conference on Antimicrobial Agents and Chemotherapy, Philadelphia, PA, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Michaelis, K.C. (1990) *The effect of tylosin on the induction of forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells.* Unpublished studies Nos 891011MLT3279, 891017MLA3279 and 891114MLA3279 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Moore, M.M., Clive, D., Howard, B.E., Batson, A.G. & Turner, N.T. (1985) In situ analysis of trifluorothymidine-resistant (TFT^r) mutants of L5178Y/$TK^{+/-}$ mouse lymphoma cells. *Mutat. Res.*, **151,** 147–159.

Morton, D.M. (1988) *Tylan products. Expert report on toxicological documentation.* Unpublished report from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Negilski, D.S. & Downs, O.S. (1985) *The acute oral, dermal, ocular and inhalation toxicity of Tylan Soluble.* Unpublished studies Nos R-0-367-79, R-0-368-79, B-D-94-79, B-E-90-79 and R-H-40-79 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Pridmore, A. (2006a) *Non-clinical laboratory study: Activity of tylosin against bacterial strains representing the normal human intestinal microbiota: Determination of minimum inhibitory concentration (MIC).* Unpublished study No. 039/05 from Don Whitley Scientific Limited, Shipley, West Yorkshire, England. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Pridmore, A. (2006b) *Non-clinical laboratory study: Effect of fecal binding on the antibacterial activity of tylosin.* Unpublished study No. 040/05 from Don Whitley Scientific Limited, Shipley, West Yorkshire, England. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Quarles, J.P. (1983) *Acute comparative intravenous toxicity testing of tylosin, desmycosin, and macrocin in the ICR mouse.* Unpublished studies Nos M-V-46-83, M-V-45-83 and M-V-44-83 from the Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Richards, F.A. & Berkman, R.N. (undated) *LD_{50} determination for tylosin phosphate in chickens.* Unpublished study No. VPR-116-418 from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Ross, M.H., Bras, G. & Ragbeer, M.S. (1970) Influence of protein and caloric intake upon spontaneous tumor incidence of the anterior pituitary gland of the rat. *J. Nutr.*, **100**, 177–189.

Sieck, R.F., Graper, L.K., Giera, D.D., Herberg, R.J. & Hamill, R.L. (1978) *Metabolism of tylosin in swine and rat.* Unpublished study from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Teeter, J.S. & Meyerhoff, R.D. (2003) Aerobic degradation of tylosin in cattle, chicken and swine excreta. *Environ. Res.*, **93**, 45–51.

Terashima, H. & Maeda, J. (undated) *The effect of tylosin on a fetus and a suckling-young of Wistar strain rat.* Department of Pathology, Osaka City University Medical School, Osaka City, Japan. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Tsubura, Y., Toyoshima, K., Sano, S., Nishii, Y. & Tani, M. (undated) *Effect of tylosin on mouse breeding.* Unpublished study from Second Department of Pathology, Nara Medical College, Kashihara, Japan. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Tsuchikawa, K. & Akabori, A. (undated) *On the teratogenicity of tylosin.* Unpublished study from National Institute of Genetics, Shizuoka, Japan. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Van Duyn, R.L. & Kline, R.M. (undated) *Additional studies on the pharmacology and toxicology of Tylocine® Injection in dogs and cats.* Unpublished study from Lilly Research

Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Van Duyn, R.L., Kline, R.M. & Russell, E. (undated) *Tylocine® intramuscular canine blood levels.* Unpublished study No. VPR-139-766 from Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA. Submitted to WHO by Elanco Animal Health, Division of Eli Lilly and Company, Indianapolis, IN, USA.

Veien, N.K., Hattel, T., Justesen, O. & Norholm, A. (1980) Occupational contact dermatitis due to spiramycin and/or tylosin among farmers. *Contact Dermatitis*, **6**, 410–413.

Verbov, J. (1983) Tylosin dermatitis. *Contact Dermatitis*, **9**, 325–326.

VICH (2004) *Studies to evaluate the safety of residues of veterinary drugs in human food: General approach to establish a microbiological ADI.* Brussels, Belgium, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH Guideline 36; http://www.vichsec.org/pdf/05_2004/GI36_st7_F_rev.pdf).

Zweers-Zeilmaker, W.M., Van Miert, A.S.J.P.A.M., Horbach, G.J. & Witkamp, R.F. (1999) In vitro complex formation and inhibition of hepatic cytochrome P450 activity by different macrolides and tiamulin in goats and cattle. *Res. Vet. Sci.*, **66**(1), 51–55.