



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### TYLOSIN

#### SUMMARY REPORT (3)

1. Tylosin is a macrolide antibiotic that is active mostly against Gram-positive bacteria and mycoplasmas. It is ineffective against *Enterobacteriaceae*. It consists predominantly of tylosin A (factor A), but varying amounts of tylosin factor B (desmycosin), tylosin factor C (macrosin) and tylosin factor D (relomycin) may also be present, depending on the manufacturing source. Most of the microbiological activity resides with tylosin A. Tylosin factors B, C and D and dihydroxydesmycosin (metabolite) have around 50%, 70%, 30% and 15% of the activity of tylosin A respectively. The safety studies were carried out using tylosin from a commercial source with a specification of tylosin A being at least 80% and the sum of tylosin A, B, C and D : being at least 95%. Tylosin and its phosphate and tartrate salts are used in pigs, cattle and poultry for the treatment of conditions caused by sensitive organisms. It may be administered to calves, orally in the milk replacer, at a dose of 40 mg/kg bw and to cattle by intramuscular injection at a dose of 4-10 mg/kg bw. In pigs, tylosin 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 for the prevention and control of diseases such as swine dysentery and enzootic pneumonia. Tylosin is administered to poultry in the drinking water at a dose equivalent to 35 mg/kg bw. Tylosin is also authorised as a feed additive in accordance with Council Directive 70/524/EEC; the substance may be incorporated into pig feed at concentrations in the range of 5-20 mg/kg feed for animals up to 6 months of age and 10-40 mg/kg feed for animals up to 4 months of age.

Tylosin is currently entered in Annex III of Council Regulation (EEC) No 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Tylosin	Tylosin	Bovine, porcine, poultry	100 µg/kg	Muscle, liver, kidney	Provisional MRLs expire on 1 July 1997
		Bovine	50 µg/kg	Milk	

2. The oral bioavailability of tylosin in rats, dogs and cattle was low. In pigs, oral bioavailability was greater - around 22.5% based on area under curve (AUC) values determined in a cross-over design study. In most species peak plasma concentrations were attained 1-2 hours after administration (less than or equal to 1 mg/l in the rat after 50 mg/kg bw, less than or equal to 0.9 mg/l in the rabbit after 10 mg/kg bw and less than or equal to 3 mg/kg bw after 25 mg/kg in the dog). In the rat and dog significant biliary secretion was reported after parenteral administration with bile/serum concentration ratios of around 143-266 and 1230-3780, after doses of 100 mg/kg bw intraperitoneally and 10 mg/kg bw intravenously respectively. After daily oral administration of 10 mg/kg bw per day of unlabelled tylosin for 3 days followed by <sup>14</sup>C-tylosin to rats for 5 days, 99% of the radioactivity was excreted in faeces and 1% in urine; the excreted residues consisted predominantly of tylosin A, tylosin factor D and dihydrodesmycosin. A pig was given 110 mg/kg bw unlabelled tylosin orally for 2 weeks followed by <sup>14</sup>C-tylosin for 3 days;

99% of the radioactivity was excreted in faeces and 1% in urine; the majority of the excreted residues was tylosin factor D (33%), tylosin factor A (6%) and dihydrodesmycosin. Similar results were obtained in a recent GLP-compliant study using 3 pigs; in this study around 94% of the excreted radioactivity was in faeces and 6% in urine. In 2 animals, around 43% of the radioactivity in pig faeces was tylosin factor D and 44% was dihydrodesmycosin. The faeces from the third pig contained the seco acid of tylosin factor D (resulting from hydrolysis of the lactone in the macrolide ring) (56%) and tylosin D (6%).

Residues of tylosin factor D and dihydrodesmycosin were also found in pig liver and kidney. After intramuscular injection of 17.6 mg/kg bw <sup>14</sup>C-tylosin to calves, less than 50% of the administered dose was recovered from excreta. Tylosin A (29.8%), tylosin factor D (11.4%), tylosin factor C (25.2%) and demethyl-tylosin factor D (10.8%) were found in faecal extracts and cysteinyl-tylosin A was the main component (70%) in urine. Tylosin was metabolised by similar metabolic pathways in rats, pigs and cattle though there were quantitative differences in the amounts of metabolites produced.

3. Tylosin base and its salts were shown to be of low acute oral toxicity with LD<sub>50</sub> values in excess 5000 mg/kg bw in the rat and mouse and greater than 800 mg/kg bw in the dog. In dogs, overt signs of toxicity included salivation, emesis and defaecation. Comparative intravenous studies of tylosin, desmycosin and macrocin in females rats gave LD<sub>50</sub> values of 321, 193 and 189 mg/kg bw, respectively. Tremors, clonic convulsions and leg weakness were seen in all three cases, with poor grooming in animals dosed with tylosin and hypoactivity and ataxia in those dosed with desmycosin.
4. Several repeat-dose studies were conducted in rats and dogs; most of these studies were carried out some years ago and are not to modern standards. In rats exposed to daily subcutaneous doses of 10-100 mg tylosin/kg bw in an acacia gum suspension for one month, no treatment related effects were reported. In another one month study rats were exposed to doses of 100-1000 mg/kg bw as the tartrate salt using the same route and vehicle. Diarrhoea was seen at doses greater than or equal to 250 mg/kg during the first week, regressing to soft stools (also occasionally seen at 100 mg/kg). Scarring and scabbing of the injection site was occasionally seen at 100 mg/kg bw and at all other doses. In a 2-year study in the dog, oral doses of 200 mg/kg bw and above caused vomiting and diarrhoea. Mild renal effects (nephrosis and pyelonephritis) were seen in 1 out of 4 dogs at 200 mg/kg bw and 1 out of 4 dogs at 400 mg/kg bw. The NOEL was 100 mg/kg bw per day.
5. A one-year study was conducted in weanling rats of parents fed diets containing tylosin. Offspring were exposed to diets containing 0, 0.1, 0.5 or 1.0% tylosin base (equivalent to approximately 0, 50, 500 and 1000 mg/kg bw per day). No treatment-related effects on bodyweight gain, food consumption, organ weights or pathology were reported. There was a dose-related increase in lymphocyte count and corresponding decrease in neutrophils in both sexes, significantly only in the 0.5% and 1.0% females. Serum glucose was increased in the high dose (significant only in the females), and urine was more alkaline in the mid and high dose females. The NOEL was 0.1%, equivalent to approximately 50 mg/kg bw.
6. Tylosin was tested for genotoxicity in 3 *in vitro* assays and in one *in vivo* assay. Weak positive results were obtained only in the absence of metabolic activation and at cytotoxic dose levels when tylosin was tested at concentrations in the range 10-1000 µg/ml in dimethyl-sulphoxide (DMSO), in an *in vitro* mouse L5178Y TK<sup>+/−</sup> lymphoma assay for gene mutation. However no increase in mutant frequency was observed in an *in vitro* gene mutation assay at the HGPRT<sup>+</sup> locus in Chinese hamster ovary cells using concentrations ranging from 100-1500 µg/ml. Tylosin was not mutagenic in an *in vitro* chromosomal aberration assay using Chinese hamster ovary cells using doses in the range 500-1000 µg/ml in dimethyl-sulphoxide, with and without metabolic activation. An *in vivo* mouse bone marrow micronucleus test (2 daily doses of 1250-5000 mg tylosin base/kg bw in acacia gum) was also negative. Overall, it was concluded that tylosin was unlikely to present a mutagenic risk.

7. Two replicate carcinogenicity studies were carried out in Wistar rats. F<sub>1</sub> offspring of animals fed tylosin for 10 weeks prior to mating were fed diets containing 0, 0.1, 0.5 or 1.0% tylosin for 2 years (equivalent to approximately 0, 50, 500 or 1000 mg/kg bw per day). No overall treatment-related effects were seen on haematology, clinical chemistry or urinalysis. There were trends towards increasing survival, bodyweight gain and food consumption in the treated males compared to controls. The increased survival in treated groups was associated with a significant treatment-related reduction in necrotising pneumonia. No treatment-related effects on organ weights were reported. In males but not females, there was a dose-related increase in pituitary adenomas; 10% controls, 11% low dose, 22.5% mid dose and 25% high dose. The incidence of malignant tumours was unaffected by treatment. Historical control data were provided on the incidence of pituitary adenomas in thirteen 2-year studies in the same strain of rat. These data suggested that the incidence of pituitary adenomas in the controls in the tylosin studies was unusually low. A significant association was demonstrated between the incidence of pituitary adenomas and bodyweight. It was concluded that the apparent increased incidence of pituitary adenomas in the tylosin treated groups was attributable to enhanced survival and increased bodyweight in these groups rather than a carcinogenic effect of the substance.
8. A recent published paper described the effects of low doses of tylosin (0.1 and 5 mg/kg feed in the diet for up to 65 days) on the pituitary-gonadal axis of male Wistar rats. Statistically significant changes were observed in some hormone levels and in pituitary weights. However, the reported hormonal and organ weight changes lacked consistency, and there was no evidence of significant time- or dose-relationships. In addition, serious inadequacies were noted in the methodology and interpretation of the results and it was concluded that there was no clear evidence of a direct effect of tylosin on the pituitary-gonadal axis.
9. Four older long-term studies in the rat were reported with group sizes of 3 to 30 animals/sex/group, exposed to diets containing up to 20% tylosin base for 17 months to 2 years. No significant treatment-related effects were reported, but the studies and reports were inadequate for the assessment of carcinogenic risks.
10. Studies on reproductive toxicity were not carried out to modern standards and were poorly reported but gave no indication of any adverse effects on reproductive parameters such as fertility. In a 2-generation study mice were exposed to diets containing 0, 0.1 or 1.0% tylosin; there were no treatment-related effects on reproductive parameters. In a 3-generation study rats were given 0 or 1.0% tylosin base; no treatment-related effects were observed. In a special study, weanling rats received diets containing 0, 0.1, 0.5 or 1.0% tylosin base (equivalent to approximately 0, 50, 500 or 1000 mg/kg bw) for 10 weeks prior to mating and for 6 months thereafter. High dose males showed decreased white cell counts at termination. Offspring were unaffected by treatment and were assigned to the 1 year study reported in paragraph 5.
11. In a teratogenicity study pregnant mice were given doses of 0, 100, 500 or 1000 mg/kg tylosin in water by oral gavage on days 7-12 of gestation. At sacrifice on day 18, no effects on reproductive or foetal parameters were reported. No effects were observed in the offspring of pregnant mice exposed to doses of 500 or 1000 mg tylosin/kg bw on days 7-12 of gestation. Offspring were observed for 9 weeks after birth and examined for growth, survival, behavioural and morphological effects. In two studies, pregnant rats were exposed to dietary doses of 0, 0.1, 1.0 or 10% tylosin on days 1-20 of gestation (equivalent to 0, 60.5, 725 or 4800 mg/kg bw per day). In one study dams were killed on day 20 of gestation and the only adverse effects reported were slightly reduced pup weights and delayed ossification in the top dose (4800 mg/kg bw). No effects were reported at the middle dose of 725 mg/kg bw. In the second study, dams were allowed to give birth and offspring were observed during weaning. A slight decrease in growth was seen in the top dose only.

12. Sensitisation studies in the guinea pig using 10 mg tylosin base subcutaneously, or 2-10 mg tylosin HCl/kg bw intraperitoneally followed by an intravenous challenge dose of 5 mg/kg bw were considered inadequate for evaluating sensitising potential, with deaths at all doses except 2 mg/kg bw. Tylosin tartrate was reported to cause a mild skin sensitization reaction in guinea pigs challenged 14 days after exposure to 10 intracutaneous injections of 50 mg/ml. Tylosin formulations were reported to cause slight skin irritation and mild to marked ocular irritation in the rabbit. In a Swedish study 6/9 veterinary surgeons with contact dermatitis were sensitised to tylosin and there are reports of contact dermatitis in animal feed workers.
13. Tylosin has never been used in human medicine. In a human volunteer study, a daily oral dose of 20 mg of tylosin administered over a period of 6 months had only a marginal effect on the total numbers of resistant *streptococci*. No tendency towards increased resistance was reported in another study in which 2 female volunteers received oral doses of 2 or 5 mg tylosin per day for 3 months. No adverse effects were reported in either study. It was considered that a microbiological ADI could not be based on these limited data.
14. The effects of tylosin on yoghurt starter cultures were investigated by the French National Reference Laboratory. The maximum concentrations of tylosin without effect on lactic ferment were 0.075 µg/ml for *Streptococcus thermophilus* and 0.004 µg/ml for *Lactobacillus bulgaricus*. It was noted that *Streptococcus thermophilus* was always cultured first and so it was concluded that a concentration of 0.075 µg/ml was the maximum concentration of tylosin without effect on the industrial manufacture of yoghurt. However a German publication reported that tylosin at a concentration of 50 µg/kg inhibited some parameters, including the formation of D(-)-lactate, in 2 commercially-available yoghurt starter cultures.
15. A toxicological ADI of 500 µg/kg bw was calculated by applying a safety factor of 100 to the NOEL of 50 mg/kg bw which was established in the one-year repeated-dose (dietary) study in rats. The lower ADI based on a microbiological end-point was considered to be the most relevant ADI for assessing the risk to consumers.
16. For the assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP :

$$\text{ADI} = \frac{\frac{\text{geometric mean MIC}_{50} \times \text{CF2}}{\text{CF1}} \quad (\mu\text{g/ml}) \times \text{daily faecal bolus (0.15 l)}}{\frac{\text{fraction of an oral dose available for microorganisms}}{\text{x weight of human (60 kg)}}} \quad (\mu\text{g/kg bw})$$

Based on the above formula the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{\frac{0.606 \times 2}{1} \times 150}{(1 - 0.5) \times 60 \text{ kg}} = 6.06 \mu\text{g/kg bw (363.3 } \mu\text{g/person )}$$

Where :

- *In vitro* MIC values were provided for a total of 83 different strains of 9 genera of microorganisms representative of those found in the human gut. A geometric mean MIC<sub>50</sub> of 0.606 µg/ml was calculated for all the sensitive genera (*Lactobacillus*, *Bifidobacterium*, *Clostridium*, *Bacteroides*, *Peptostreptococcus*, *Eubacterium* and *Enterococcus*) which were tested at pH 6.6 and 10<sup>9</sup> cfu/ml.

- CF1 = 1 because the geometric mean MIC<sub>50</sub> value for all the sensitive species was used; nature of resistance chromosomal;
  - CF2 = 2 to correct for increasing inoculum density in the *in vivo* situation; it was not considered necessary to correct for pH;
  - 1 is the fraction of the oral dose available to the microorganisms at the distal part of the gastrointestinal tract because in some species 99% of the oral dose was excreted in faeces and there were no data for humans; this value was corrected by a factor of 0.5 to account for the nature of the residues in faeces;
  - 150g is the weight of the daily faecal bolus;
  - 60 kg is the human bodyweight.
17. Following administration of <sup>14</sup>C-tylosin to 3 male pigs in the feed at a dose level of 220 mg/kg for 5 days (equivalent to approximately 3.2 mg/kg bw per day), the animals were slaughtered 4 hours after the last dose. Mean residues in liver, kidney, fat and muscle were 450, 460, 50 and 70 µg/kg tylosin-equivalents respectively. The samples of liver and kidney were then analysed for residues of tylosin A using HPLC; residues in all samples were below the limit of quantification (50 µg/kg). The liver was then assayed using a microbiological assay which showed that more than 75% of the residues had microbiological activity. The radioactivity present in liver and kidney samples were further extracted and the extracts pooled for analysis using HPLC/ISP/MS/LSC. Around 70% of the total radioactivity was extractable. 12.3% and 7.6% of the residues present in liver and kidney consisted of tylosin A. Smaller amounts of tylosin factor D (10.3% and 6.1% in liver and kidney respectively), dihydrodesmycosin (5.4% and 4.1% in liver and kidney respectively) and cysteinyl-tylosin A (which readily converts to tylosin A) were also present.
18. Residue depletion studies were carried out in pigs following administration in the feed (200 mg/kg for 28 days) or the drinking water (250 mg/l for 10 days). In both studies, 3 males and 3 females were killed at each time point. Residues (determined by HPLC (for tylosin A) or a microbiological assay) indicated occasional low residues in liver and kidney samples taken from animals killed 6 hours of the end of treatment (around 30 µg/kg for both tissues). The limit of detection for both assay methods was 20 µg/kg. Residues were undetectable in all samples taken 3 days after treatment. Following 5 daily intramuscular injections of 10 mg/kg bw to pigs, the animals were slaughtered in groups of 4 at each time point and residues of tylosin A were determined using HPLC. Residues at the injection site depleted from a mean of 6380 µg/kg 6 hours after treatment, to 148 µg/kg 3 days after treatment and were below the limit of detection (20 µg/kg) 7 days after treatment. Residues in other tissues were detectable only at the first time point (6 hours); the mean residues were 92, 67, 355 and 669 µg/kg in muscle, fat, liver and kidney respectively.
19. Following 3 daily intramuscular injections of 17.6 mg/kg bw <sup>14</sup>C-tylosin, 2 calves were slaughtered 4 hours after the last dose. Mean residues in liver, kidney, muscle and fat were 25210, 47810, 2870 and 1520 µg/kg tylosin-equivalents respectively. Tissue samples were analysed for tylosin A using the proposed routine analytical method based on HPLC; the mean residues were 2635, 6945, 705 and 940 µg/kg in liver, kidney, muscle and fat respectively, corresponding to 10.5%, 14.5%, 24.6% and 61.8% of the total residues in these tissues. When assayed using a microbiological assay, 39.3%, 33.3% and 34.5% of the total residues present in kidney, liver and muscle respectively, were shown to possess microbiological activity. From these results, it was calculated that tylosin A represented 36.7%, 31% and 70% of the microbiological residues present in kidney, liver and muscle respectively. The radioactivity present in tissues was further extracted and the extracts pooled for analysis by HPLC/ISP/MS/LSC. More than 84% of the radioactivity was extractable. 34%, 20%, 34% and 22% of the residues in liver, kidney, muscle and fat, respectively, was estimated to consist of tylosin A.

20. Following 5 daily intramuscular injections of 10 mg/kg bw to calves, 3 animals of each sex were slaughtered at each time point and the residues of tylosin A in tissues were determined using HPLC (limit of quantification 50 µg/kg). Mean residues in liver and muscle depleted from 1960 µg/kg and 470 µg/kg 6 hours after treatment, to 170 µg/kg and 280 µg/kg 3 days after treatment and were below 50 µg/kg at later time points. Residues in fat were detectable only in samples taken 6 hours after treatment (mean 230 µg/kg). Mean residues in kidney depleted from 7790 µg/kg 6 hours after treatment, to 460 µg/kg 3 days after treatment, to 70 µg/kg 7 days after treatment. Residues at the injection site depleted from 32300 µg/kg 3 days after treatment, to 1430 µg/kg 14 days after treatment, to 290 µg/kg 21 days after treatment.
21. Six dairy cows were given daily intramuscular injections of 10 mg/kg bw tylosin for 3 consecutive days, immediately after the morning milking. Residues of tylosin A in milk were determined using HPLC. On the day after treatment, residues in milk were in the range 200-630 µg/kg at the morning milking and 90-330 µg/kg at the afternoon milking. 3 days after the end of treatment, residues were found in only one milk sample at the morning milking (60 µg/kg) and were below the limit of quantification (50 µg/kg) in all samples taken at the afternoon milking. In a second study in which daily intramuscular injections of 10 mg/kg bw per day were given to 10 dairy cows for 5 consecutive days, residues in milk were in the range 300-930 µg/kg on the morning of the day after the last dose and declined to 38-150 µg/kg on the morning of the second day after the last dose. This study also used HPLC but it appeared that tylosin A and a metabolite were being measured. There were no data to indicate the composition of the residues in milk. However the depletion of residues in milk in a study employing a microbiological assay method paralleled those found in the study using the same dosage regime and an HPLC assay, suggesting that most of the microbiologically-active residues in milk were tylosin A.
22. Chickens were given drinking water containing 500 mg/l of tylosin for 8 days; groups of birds were killed 6 hours, 1, 5 and 10 days after the end of treatment and the tissues were analysed by HPLC. Residues of tylosin A were below the limit of quantification (50 µg/kg) in all samples except for one liver sample taken 6 hours after treatment (83 µg/kg). In 3 other studies in which chickens were administered tylosin in the drinking water and in the feed, microbiological assays were used to determine the residues in tissues. Residues were found only in occasional liver and kidney samples taken within a few hours of treatment. No radiometric studies were carried out in chickens.
23. Forty eight laying hens were given tylosin in the feed (400 mg/kg feed) or in the drinking water (500 mg/l or 1000 mg/l) for 5-7 days. Residues in eggs were determined using a microbiological assay. The mean residues in whole eggs declined from 370 µg/kg tylosin-equivalent on the day after the end of treatment, to 130 µg/kg 3 days after treatment, to 80 µg/kg 4 days after treatment.
24. Turkeys were given tylosin in the drinking water (5g/gallon) for 7 days. Groups of 3 birds were killed at zero (exact number of hours unspecified), 24, 48, 72 and 96 hours after the end of treatment. Residues in edible tissues were determined using a microbiological assay. Mean residues in the livers from 2 birds were 385 µg/kg tylosin-equivalent at the first time point and were below the limit of detection (350 µg/kg) in all birds 24 hours after the end of treatment. Low levels of residues (around 260 µg/kg) were found in occasional skin and fat samples. Residues in all tissues were below the limit of detection at the 48 hour time point (limit of detection values were 160 µg/kg for fat, 154 µg/kg for skin, 360 µg/kg for muscle and 178 µg/kg for kidney).

25. Routine analytical methods for the determination of residues of tylosin A in eggs, edible tissues of pigs, cattle and poultry and cows' milk were based on HPLC with UV detection. The limit of quantification of the methods was 50 µg/kg and the limit of detection was 20 µg/kg. The methods were described in the ISO 78/2 format. Carbon tetrachloride and chloroform were used in the assays. The HPLC routine analytical method for determination of residues in milk had recently been improved and the solvents carbon tetrachloride and chloroform had been replaced. This refined method had been validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community. The limit of quantification was 25 µg/kg and the Limit of Detection was 10 µg/kg. Non-specific microbiological assay methods were also available for the determination of residues in tissues, eggs and milk. These methods measured both residues of tylosin A and residues of metabolites such as tylosin D which also had some antimicrobial activity.
26. Kidney was the appropriate target tissue for routine monitoring purposes for both pigs and cattle. For monitoring purposes of the edible tissues of the carcass, MRLs need to be set for muscle and fat. It was agreed to set MRLs at values of twice the limit of quantification for all bovine and porcine tissues and for bovine milk. Information concerning the ratio of marker to total residues or the ratio of marker to total microbiologically-active residues was available only for one time point. For cattle, this was 4 hours after dosing; at this time point 70%, 36.7% and 31% of the microbiologically-active residues present in muscle, kidney and liver were present as tylosin A. The relationship was not known for the time point when the residues would have depleted to the proposed MRLs (between 3 and 7 days for cattle treated intramuscularly).
27. Taking into account the very low residues in edible tissues of poultry at all time points, it was agreed to set MRLs for this species at a value of twice the limit of quantification of the analytical method. No MRLs could be proposed for eggs because there was no reliable information concerning residues depletion and no information concerning the composition of the residues in eggs.

## Conclusions and recommendation

Having considered that:

- an ADI of 6 µg/kg bw has been established;
- tylosin A is the marker residue;
- for pigs and cattle, kidney is the target tissue for monitoring purposes;
- in cattle, mean residues of tylosin A in kidney were 70 µg/kg 7 days after intramuscular administration and were below 50 µg/kg in other edible tissues; tylosin A accounted for approximately 37%, 31% and 70% of the residues with microbiological activity present in bovine kidney, liver and muscle, 4 hours after intramuscular dosing; the relationship was not known for later time-points;
- in pigs, residues of tylosin A in liver and kidney samples 6 hours after oral dosing were around 30 µg/kg and were undetectable in other tissues; tylosin A accounted for approximately 33%, and 43% of the residues with microbiological activity present in porcine liver and kidney 4 hours after oral administration;
- residues in milk were in the range of 38-150 µg/kg at the morning milking of the second day after intramuscular treatment of dairy cows; most of the residues in cow's milk consisted of tylosin A though the exact ratio was not known;
- no reliable data were available concerning the composition of residues nor the depletion of residues in eggs,
- a validated physico-chemical analytical method for residues monitoring purposes is available;

the Committee recommends the inclusion of tylosin in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Tylosin	Tylosin A	Bovine	100 µg/kg	Muscle, fat, liver, kidney	
			50 µg/kg	Milk	
		Porcine,	100 µg/kg	Muscle, liver, kidney, skin+fat	
		Poultry	100 µg/kg	Muscle, liver, kidney, skin+fat	

Based on these MRLs, and assuming that the percentage of residues in bovine tissues which were present as tylosin A did not change significantly with time, it was estimated that the daily consumer intake would represent approximately 50% of the microbiological ADI.