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August 1999

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TIAMULIN

SUMMARY REPORT (1)

1. Tiamulin is a diterpene antimicrobial with a pleuromutilin chemical structure similar to that of valnemulin. The activity of tiamulin is largely confined to gram-positive micro-organisms and *mycoplasma*. Tiamulin acts by inhibiting protein synthesis at the ribosomal level. In veterinary medicine, tiamulin is used for treatment and prophylaxis of dysentery, pneumonia and mycoplasmal infections in pigs and poultry. Tiamulin is available as a 2, 10 or 20% premix for pigs and poultry, a 12.5% solution or 45% water soluble powder for addition to drinking water for pigs and poultry, or a 10% injectable formulation for pigs. Dietary doses are 100 to 200 mg/kg feed for 5 to 10 days, and up to 6 weeks at 50 mg/kg feed for pigs, and 160 to 320 mg/kg feed for poultry. In drinking water, doses of 4 to 25 mg/kg bw for up to 1 week are used for pigs, and doses of 30 to 60 mg/kg bw for 3 to 5 days in poultry. Intramuscular doses of 10 to 20 mg/kg bw may be given daily for up to 5 days.

   Tiamulin is not used in human medicine.

2. A series of tests was carried out to investigate secondary pharmacodynamic effects. Tiamulin had no effect on motility, cardiazol-induced convulsions, body temperature or pupil diameter in the mouse following oral administration of doses of up to 200 mg/kg bw. In the rat, there were no progestational, uterotropic, diuretic or anabolic effects at oral doses of up to 100 mg/kg bw. However there was a decrease in testosterone-stimulated growth of seminal vesicles at 50 mg/kg bw; the NOEL was 15 mg/kg bw. This effect was considered to be of doubtful significance because of the lack of effects on reproductive performance, fertility, gonadal weight or pathology in the repeated-dose toxicity and reproductive toxicity studies in rats which used dose levels of up to 20 times the NOEL for this study.

   Electrocardiogram effects were observed in dogs given a single oral dose of 5 mg/kg bw but not at the lower dose of 1 mg/kg bw. A NOEL of 3 mg/kg bw/day based on this effect was established in a repeated-dose toxicity study in dogs. No electrocardiogram effects were observed in rhesus monkeys given an oral dose of up to 45 mg/kg bw tiamulin nor in humans given an oral dose of up to 10.7 mg/kg bw tiamulin.

[1] *The summary report was updated in April 2017 to correct information in paragraph 22 relating to the time points at which residue levels were measured*

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3. A plasma C_{max} value of 2.6 µg/ml was observed in dogs 40 to 60 minutes after an oral dose of 10 mg ^{3}H-labelled tiamulin /kg bw. Elimination was bi-phasic with an initial elimination half-life of 5.5 hours followed by a slower elimination half-life of 7.5 days. Around 100% of the dose was recovered in excreta within 10 days of dosing with approximately 33% of this in the urine. Tiamulin underwent extensive metabolism in the liver. No antimicrobial activity was observed for 67% of the metabolites. In rats given an oral dose of 50 mg/kg bw ^{3}H-labelled tiamulin, 92% of the dose was recovered from excreta within 2 days of dosing. In urine 15 to 30% of the dose was eliminated and 45 to 63% was eliminated via bile. Comparison with intravenous dosing indicated that the oral bioavailability in rats was 95 to 100%. Rat urine contained several metabolites together with a small amount of unmetabolised tiamulin.

4. In male and female Sprague-Dawley rats, the acute oral LD_{50} values for tiamulin were 2740 and 1830 mg/kg bw, respectively. In male and female NMRI mice, the acute oral LD_{50} values were 770 and 650 mg/kg bw. The substance was of high toxicity when administered intravenously (LD_{50} values were 19 to 20 mg/kg bw in rats and approximately 50 mg/kg bw in mice).

5. Groups of CFE Carworth rats were fed diets containing 0, 5 or 30 mg tiamulin/kg bw/day for 26 weeks. Further groups of rats received 180 mg/kg bw/day for 10 weeks, followed by 270 mg/kg bw/day for 16 weeks; one group was necropsied at the end of treatment, the remaining rats were maintained on untreated control diets for a further 4 or 8 weeks. There were increases in serum cholesterol and in water intake in the 180 mg/kg bw group. When the dose was increased to 270 mg/kg bw/day, the effects included increased serum alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. Abdominal distension, dense faeces and increased urine specific gravity were also observed. Absolute and relative liver weights were increased in both sexes and fatty infiltration of the liver was observed on histopathological examination. The effects on the liver were ameliorated in the recovery groups. The NOEL was 5 mg/kg bw/day.

6. Groups of 4 male and 4 female Beagle dogs were given daily oral doses of 0, 3 or 10 mg/kg bw/day of tiamulin in gelatin capsules for 26 weeks. Further groups were given 30 mg/kg bw/day for 13 weeks and the dose was then increased to 45 mg/kg bw/day. Two male dogs died within 2 hours of administration of the third dose of 45 mg/kg bw/day and the dose was therefore reduced to 30 mg/kg bw/day for the surviving animals. Further groups of 2 males and 2 females were subsequently added to the top dose group and were maintained on untreated diets for 4 or 8 weeks at the end of the study. Emesis was observed in all groups including the controls. Serum alanine aminotransferase activity was significantly increased at 10 mg/kg bw/day. Liver weights were increased in surviving dogs given the top dose and fatty deposits were found in the liver of one of these dogs. Changes in electrocardiograms including prolongation of the QT interval and an increased incidence of double peaked T-waves were observed at 10 mg/kg bw/day and above. The NOEL was 3 mg/kg bw/day.

7. Groups of 4 male and 4 female Beagle dogs were given daily oral doses of 0, 3, 10 or 30 mg/kg bw/day of tiamulin in gelatin capsules for 54 weeks. In the groups given 10 and 30 mg/kg bw/day, occasional emesis was observed, serum potassium concentrations were decreased and electocardiograms showed prolongation of the QT interval. Serum lactate dehydrogenase (LDH) was significantly increased in some groups at some time-points but a consistent dose-relationship was not observed and there was no increase in the cardiac-related isoenzyme LDH1. It was concluded that the NOEL was 3 mg/kg bw/day.

8. A study into the effects of tiamulin on male fertility was carried out prior to the introduction of GLP but was well documented. Groups of 15 male OFA rats were given daily oral doses of 0, 30, 55 or

100 mg/kg bw/day of tiamulin for 71 days prior to mating with untreated females. Each male was mated with 2 females. Thirteen days after mating 50% of the females were killed, the remainder were allowed to litter down and rear their offspring to weaning. There were no substance-related effects on fertility or on survival or growth of the offspring. A similar study was carried out to investigate effects on female fertility and employed the same dose levels. In this study, the females were treated for 14 days prior to mating with untreated males. Treatment of the females continued throughout pregnancy and gestation. There were no substance-related effects on fertility and growth and survival of the offspring were unaffected by treatment.

9. A GLP-compliant 3-generation study was carried out in Charles River CD rats with 2 litters bred per generation. Daily doses of tiamulin equivalent to 0, 2, 8 or 32 mg/kg bw/day were administered in the diet. There were no overt signs of toxicity and no effects on behaviour or reproductive performance. There were no substance-related gross or histopathological changes in either the parents or the offspring.

10. Several studies were carried out to investigate the potential effects of tiamulin on reproductive performance in pigs. In these studies, breeding sows were fed a diet containing 200 mg/kg feed from days 84 to 92 of gestation, another group was maintained on a diet containing the equivalent of 16 mg/kg bw/day from 2 days after mating for 6 weeks, and further groups were given tiamuilin in the drinking water at a dose of 8.8 mg/kg bw/day for various periods during gestation and in some cases up to weaning of the offspring. There were no adverse effects on health of the sows, pregnancy, parturition, litter size, growth and survival of the piglets, oestrus cycle or subsequent breeding performance. In another study, breeding boars were maintained on diets containing 16 mg/kg bw/day of tiamulin for 14 days. There were no effects on health status, libido or semen quality.

11. Groups of pregnant female Yellow-silver rabbits were given daily oral doses of 0, 30, 55 or 100 mg/kg bw/day from days 6 to 18 of gestation. Although the study pre-dated the introduction of GLP, it was well documented. Doses of 55 mg/kg bw/day and above caused the deaths of some dams and maternal body weight gain was reduced. Litter size and foetal weights were reduced at 55 mg/kg bw/day and above. There was no evidence of teratogenicity at any dose level. The NOEL was 30 mg/kg bw/day for both foetotoxicity and maternal toxicity.

12. Groups of pregnant female OFA rats were given daily oral doses of 0, 30, 100 or 300 mg/kg bw/day from days 6 to 15 of gestation. Although the study pre-dated the introduction of GLP, it was well documented. At 300 mg/kg bw/day there were minor signs of maternal toxicity such as poor coat. At this dose level, the mean foetal weight was reduced and there was an increased incidence of retarded skeletal development. There was no evidence of teratogenicity. The NOEL was 100 mg/kg bw/day for both foetotoxicity and maternal toxicity.

13. Tiamulin did not induce gene mutations in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 or TA 1538. An *in vitro* assay for gene mutation at the HPRT locus of V79 Chinese hamster cells also gave negative results. In an *in vivo* micronucleus test, Swiss mice were given a single oral dose of 420 mg tiamulin/kg bw and bone marrow samples were harvested 24, 48 and 72 hours after dosing. Tiamulin had no effect on the frequency of micronucleated polychromatic erythrocytes. It was concluded that tiamulin was not genotoxic.

14. Groups of 65 Charles River CD rats per sex and dose were fed diets containing tiamulin at concentrations designed to provide intakes of 0, 2, 8 or 32 mg/kg bw/day of tiamulin for 30 months. The study was completed in 1981 and a complete histopathological examination on the 2 and 8 mg/kg bw/day groups was not carried out at that time. In 1993 the original histology

slides were reviewed together with new sections taken from archived wet tissues or wax blocks. There was no significant dose-related trend in the incidence of any tumour type. A NOEL for non-neoplastic effects of 8 mg/kg bw was identified, based on statistically significant increases in cholangiofibrosis, bile duct proliferation and hepatocellular vacuolation in both sexes at 32 mg/kg bw, accompanied by a significant decrease in bodyweight in females at this dose.

15. In another study, groups of 70 CD1 COBS Charles River mice per sex and dose were fed diets containing the equivalent of 0, 1, 6 or 48 mg/kg bw/day of tiamulin for up to 123 weeks. Interim sacrifices of 5 mice per sex and dose were carried out after 26, 53 and 78 weeks. The histology slides were reviewed in the light of advances in tumour classification, 13 years after the original study was completed. There was no significant dose-related trend in the incidence of any tumour type.

    In vitro MIC data were provided for more than 100 strains representing 10 different genera of bacteria of human gut origin. The geometric mean MIC_{50} for sensitive strains was calculated to be 0.32 µg/ml.

    For the establishment of the microbiological ADI the following standard formula was used:

$$\text{ADI (µg/kg bw)} = \frac{\dfrac{\text{geometric mean } MIC_{50} \times CF2}{CF1} \text{ (µg/ml)} \times \text{daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}}$$

    And therefore the microbiological ADI was calculated as indicated below:

$$\text{ADI} = \frac{\dfrac{0.32 \times 2}{1} \times 150}{0.05 \times 60} = 32 \text{ µg/kg bw i.e.} = 1920 \text{ µg/person}$$

The following assumptions were made:

- CF1 = 1 because resistance to tiamulin was very difficult to induce *in vitro*; tiamulin showed no tendency to induce resistance to *Enterobacteriaceae in vivo*, the prevalence of resistance to tiamulin was very low after 20 years of use, the pleuromutilins were not used in human medicine and their spectrum of activity was relatively narrow, the data indicated that a plasmid-mediated mechanism of resistance was unlikely,
- CF2 = 2 because MIC values for tiamulin against strains of organisms representing 8 different genera of human gut origin showed a mean 2-fold increase on increasing the inoculum density from 10^6 to 10^8 cfu/ml,
- 150 g was the weight of the daily faecal bolus,
- from pharmacokinetic studies in pigs, rats and dogs, a conservative figure of 5% was adopted as the fraction of the oral dose available to micro-organisms at the distal part of the gastrointestinal tract.

16. Tiamulin is not used in human medicine. In a patch study in humans, topical administration of a 0.05% formulation did not cause skin irritation or sensitisation. Another study was carried out in 6 healthy male human volunteers. Three volunteers were given 5 oral doses progressing from 0.125 to 7.2 mg/kg bw with 72 hours between each dose. The remaining volunteers were given a single oral dose in the range 8.2 to 10.7 mg/kg bw tiamulin. There were no substance-related changes in blood pressure, serum chemistry or electrocardiograms.

17. A toxicological ADI of 0.03 mg/kg bw, i.e. 1.8 mg per person, was established by applying a safety factor of 100 to the NOEL of 3 mg/kg bw/day, established in the 26-week and 54-week

studies in dogs, based on electrocardiogram changes and raised serum alanine aminotransferase and lactate dehydrogenase concentrations at higher doses. The toxicological ADI was lower than the microbiological ADI and was considered the most relevant for the safety evaluation.

18. In pigs orally dosed with 10 mg ^{3}H-tiamulin hydrogen fumarate/kg bw/day for 5 consecutive days, over 15 metabolites were detected in liver but no individual metabolite accounted for more than 5% of the total residue. The total residue concentrations, as determined by liquid scintillation counting (LCS), in liver, kidney, muscle and skin + fat were 41 000, 4100, 500 and 800 µg equivalents/kg, respectively, 2 hours after dosing and 20 000, 900, 200, and 400 µg equivalents/kg, respectively, after 24 hours.

    In rats and pigs orally dosed with ^{3}H-tiamulin, the metabolites present in urine, faeces and bile, as determined by nuclear magnetic resonance and mass spectroscopy, were quantitatively and qualitatively similar. No single metabolite accounted for more than 7% of the total residue.

    In pigs (2 animals per sex and group), following oral administration of 5 mg ^{14}C,^{3}H-tiamulin base/kg bw/day for 10 consecutive days, approximately 35% of the dose was eliminated in urine and 65% in faeces. The total residue concentrations in liver, kidney, muscle and fat were 21 880, 600, 720 and 720 µg equivalents/kg, respectively, 10 days after dosing and 480, 220, 430, 910 µg equivalents/kg after 25 days.

19. In laying hens, broilers and turkeys (6 animals per group) orally dosed with 10 mg ^{3}H-tiamulin hydrogen fumarate/kg bw/day for 5 consecutive days, over 15 metabolites were detected in tissue extracts but most of the residue was accounted for by 4 metabolites. No individual metabolite represented more than 30% of the total residue in poultry tissues.

20. In pigs (2 animals per sex and group), following oral administration of 10 mg ^{3}H-tiamulin hydrogen fumarate/kg bw/day for 5 consecutive days, the average total residue concentrations in liver, kidney, muscle and fat were 3223, 150, 55, and 150 µg equivalents/kg, respectively, 3 days after dosing. When 15 mg ^{3}H-tiamulin hydrogen fumarate/kg bw/day was administered intramuscularly for 3 consecutive days, the average total residue concentrations in liver, kidney, muscle and fat were 6853, 618, 135, and 378 µg equivalents/kg, respectively, 3 days after dosing. At the injection sites, the average total residue concentrations were 20 725 and 38 955 µg equivalents/kg in the penultimate and last administration sites, respectively. The average concentrations of residue with antimicrobiological activity present in the penultimate and last injection sites were equivalent to approximately 6 and 45% of the total residue.

    In pigs orally dosed with ^{3}H-tiamulin, 6-desmethyltiamulin accounted less than 1% of the total residue in bile and urine samples and had 67% of the antimicrobiological activity of tiamulin when tested by agar plate diffusion. Four other metabolites were found to have antimicrobiological activities relative to tiamulin of between 0.7 and 3.3% and all other metabolites had relative activities of less than 0.3%.

    In pigs (4 animals per sex and group) given *ad libitum* access to feed containing tiamulin at a concentration of 39 mg /kg for 10 consecutive days, the average concentrations of metabolites in liver that could be hydrolysed to form 8-α-hydroxymutilin, as detected by gas chromatography with electrochemical detection, were 447 and 247 µg equivalents/kg at 2 and 12 hours after dosing, respectively. In animals dosed for 18 consecutive days, the average concentrations of 8-α-hydroxymutilin in liver were 184, 256, 214 and 175 µg equivalents/kg at 12, 16, 20 and 24 hours after dosing, respectively.

In the liver of pigs orally treated with tiamulin, the percentage of the metabolites that can be hydrolysed to 8-α-hydroxymutilin (i.e. marker residue) to total residues was 3.5, 3.6 and 5.7% at 4, 24 and 96 hours after treatment, respectively.

21. In laying hens (6 animals per group) given 50 mg ^{3}H-tiamulin/kg bw/day for 5 consecutive days, the mean total residues, in liver, muscle and skin + fat, as determined by liquid scintillation counting, were 93 800, 3800 and 5200 µg equivalents/kg, respectively, 2 hours after dosing. Eight hours after dosing, mean total residues were 106 700 and 1350 µg equivalents/kg in liver and muscle but were not determined in skin + fat. At 8 hours after the end of dosing, the mean residues of the metabolite 8-α-hydroxymutilin accounted for 8.5%, 2.5% and 0.5% of the total residues in liver, muscle and skin + fat, respectively. Though tiamulin accounted for a much larger proportion of the total residues than the metabolite 8-α-hydroxymutilin it was agreed that 8-α-hydroxymutilin was an appropriate marker residue for chicken tissues as this was the analyte for which there was a validated routine analytical method. Mean 8-α-hydroxymutilin concentrations were 17 500, 50 and less than 50 µg equivalents/kg in liver, muscle and skin + fat, respectively, at 8 hours after dosing.

22. Laying hens were given 50 mg ^{3}H-tiamulin/kg bw/day for 5 consecutive days and the residues in eggs were determined. The mean total residues determined by scintillation counting were 8100, 15 400 and 19 100 µg equivalents/kg in yolk, on days 3 and 5 of dosing, and 24 hours after the end of treatment, respectively. Over the same time period, the mean total residues in egg white were 8000, 9400 and 7900 µg equivalents/kg, respectively. Residues of the metabolite 8-α-hydroxymutilin in egg yolk were below the limit of quantification on days 3 and 5 of treatment and were 70 µg/kg at 24 hours after treatment. Residues of 8-α-hydroxymutilin in egg white were 60, 110 and 50 µg/kg at 3 and 5 days of dosing and 24 hours after the end of treatment, respectively. Residues of tiamulin were much higher. Mean tiamulin residues in egg yolk were 4300, 8400 and 7600 µg/kg on days 3 and 5 of dosing and 24 hours after the end of treatment, respectively and in egg white were 4000, 4100 and 4000 µg/kg at the same time points. It was concluded that the percentage of tiamulin marker residue to total residue in eggs from laying hens orally dosed with tiamulin was approximately 50%.

23. In broilers, given 50 mg ^{3}H-tiamulin/kg bw/day for 5 consecutive days, the mean total residue concentrations in liver, muscle and skin + fat, as determined by liquid scintillation counting, were 108 000, 550 and 6500 µg equivalents/kg, respectively, 2 hours after dosing. At this time point, the mean residues of tiamulin in liver, fat and muscle were 15 500, 1400 and 2200 µg/kg, respectively. Residues of the metabolite 8-α-hydroxymutilin in broiler tissues accounted for approximately 7%, 3% and 2% of the total residues in liver, muscle and skin + fat, respectively.

24. In turkeys (6 per sex per time point), given *ad libitum* access to drinking water containing 0.025% w/v for 5 consecutive days, the concentrations of metabolites that could be hydrolysed to form 8-α-hydroxymutilin, as detected by gas chromotography with electrochemical detection, were less than 50 µg/kg in muscle. In 1 out of 6 skin + fat samples 8-α-hydroxymutilin concentrations of 72, 90 and 71 µg/kg were detected at 0 hours, 8 hours, and 1 day after treatment, respectively. Two days after treatment all skin + fat samples contained a concentration of residues that can be hydrolysed to 8-α-hydroxymutilin of less than 50 µg/kg. The average 8-α-hydroxymutilin concentrations in liver, 6 combined samples from 6 pairs of birds per time point, were 905, 518, 527, 253 and 228 µg/kg at 0 hours, 8 hours, 1 day, 2 days and 3 days after treatment, respectively. Thus 8-α-hydroxymutilin represented not more than 3% of the total residues in turkey tissues.

25. In a study in which turkeys were given 50 mg ^{3}H-tiamulin/kg bw/day for 5 consecutive days, the total residue concentrations, as determined by liquid scintillation counting, in liver, muscle and

skin + fat were 87 000, 3150, and 4600 µg equivalents/kg, respectively, 2 hours after dosing. At the same time point, mean residues of 8-α-hydroxymutilin were 8300, 110 and 120 µg/kg in liver, muscle and skin + fat, respectively. It was concluded that 8-α-hydroxymutilin was an appropriate marker residue for turkeys and represented approximately 3%, 1% and 1% of the total residues in liver, muscle and skin + fat, respectively.

26. Routine analytical methods for monitoring the use of tiamulin, based on gas chromatography with electrochemical detection, validated for porcine and chicken tissues, were available.

    Metabolites in pig tissues were extracted in organic solvent, hydrolysed to a common α-hydroxymutilin derivative and measured as 8-α-hydroxymutilin equivalents. The limits of quantification of the routine analytical method were 50 µg/kg for porcine muscle and liver and chicken muscle, skin + fat and liver.

    Tiamulin in chicken eggs was extracted in organic solvent and measured. The limit of quantification of the routine analytical method was 500 µg/kg for chicken eggs.

    Though the use of the routine analytical method for determining 8-α-hydroxymutilin in turkey tissues had been demonstrated, no validation data were available for turkey tissues.

Conclusions and recommendation

Having considered that:

- an ADI of 0.03 mg/kg bw, i.e. 1.8 mg per person, was established,
- the sum of the residues that can be hydrolysed to form 8-α-hydroxymutilin were identified as the marker residue in tissues of pigs, chicken and turkeys,
- tiamulin was identified as the marker residue in chicken eggs,
- total residue concentrations in the liver of pigs, chicken and turkeys were more than ten times higher than those found in kidney, muscle or skin + fat,
- the marker residue represents 4% of the total residues in pig liver between 4 and 96 hours after dosing with tiamulin,
- the marker residues represent approximately 9, 3 and 2% of total residues in chicken liver, muscle, and skin + fat 2 and 8 hours after treatment; 3, 1, and 1% of the total residues in turkey liver, muscle and skin + fat 2 hours after treatment, and 50% in chicken eggs between 3 and 6 days after treatment,
- MRLs were not elaborated for pig kidney and skin + fat or chicken and turkey kidney as residue concentrations in these tissues were considered too low and of minor significance to the daily residue intake,
- validated analytical methods for monitoring 8-α-hydroxymutilin in pig liver and muscle and chicken liver, muscle, skin + fat and for monitoring tiamulin in chicken eggs are available,
- the use of a routine analytical method for determining 8-α-hydroxymutilin in turkey tissues had been demonstrated but the validation data did not meet the requirements of Volume VI,
- MRLs for pig muscle, chicken muscle, skin + fat and eggs were established at twice the limit of quantification of the routine analytical methods for determining the presence of the marker residue;

the Committee for Veterinary Medicinal Products recommends the inclusion of tiamulin in porcine species and chicken 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
Tiamulin	Sum of metabolites that may be hydrolysed to 8-α-hydroxymutilin	Porcine	100 µg/kg 500 µg/kg	Muscle Liver	
		Chicken	100 µg/kg 100 µg/kg 1000 µg/kg	Muscle Skin + fat Liver	
	Tiamulin	Chicken	1000 µg/kg	Eggs	

Based on these MRLs values, the daily intake will represent about 90% of the ADI.

And recommends the establishment of provisional maximum residue limits for the above mentioned substance in turkey in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Tiamulin	Sum of metabolites that may be hydrolysed to 8-α-hydroxymutilin	Turkey	100 µg/kg 100 µg/kg 300 µg/kg	Muscle Skin + fat Liver	Provisional MRLs expire on 1.7.2001

Based on these MRLs values, the daily intake will represent about 75% of the ADI.

Before the Committee for Veterinary Medicinal Products can consider the inclusion of tiamulin for turkey in Annex I of Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

List of questions

1. The routine analytical method for determining the presence of 8-α-hydroxymutilin should be validated for the relevant tissues of turkeys in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community and the CVMP Note for Guidance on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL).