



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### PIRLIMYCIN

#### SUMMARY REPORT (2)

1. Pirlimycin (CAS-no 78822-40-9) is a lincosamide antibiotic closely related to lincomycin and clindamycin. Pirlimycin is effective against common mastitis pathogens, mostly Gram-positive bacteria such as *Staphylococci* (e.g. *Staphylococcus aureus*) and *Streptococci* (*Streptococcus agalactiae*, *S. uberis*, *S. dysgalactiae*). As its hydrochloride salt it is intended for treatment of subclinical and clinical staphylococcal and streptococcal mastitis in lactating dairy cows at a dose of 50 mg/quarter administered in 2 treatments at 24-hour interval by intramammary infusion of an aqueous gel.

Pirlimycin is currently included in Annex III 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                          |
|---------------------------------------|----------------|----------------|----------------------------------------------------------------|------------------------------------------|-------------------------------------------|
| Pirlimycin                            | Pirlimycin     | Bovine         | 100 µg/kg<br>100 µg/kg<br>1000 µg/kg<br>400 µg/kg<br>100 µg/kg | Muscle<br>Fat<br>Liver<br>Kidney<br>Milk | Provisional<br>MRLs expire on<br>1.7.2000 |

Further data have now been provided to support the establishment of final MRLs for pirlimycin.

2. The mechanism of action of pirlimycin is the inhibition of protein synthesis of the bacterial cell by binding to the 50S ribosomal subunit and thereby inhibiting peptidyl transferase.
3. In rats given orally 29 mg/kg bw of <sup>14</sup>C-pirlimycin hydrochloride for 5 days, at the end of treatment highest mean concentration was found in liver (18 000 µg/kg), followed by kidney (7500 µg/kg), muscle (1500 µg/kg) and fat (1000 µg/kg). The parent compound accounted for 57 to 76% and pirlimycin sulphoxide for 21 to 42% of liver radioactivity; the levels of the metabolite were higher in males. At the end of treatment, urinary elimination accounted for about 5% of the administered dose, whereas about 60% was eliminated in the faeces.
4. Human serum protein binding determined *in vitro* was approximately 45%.

Male volunteers were treated with a single oral administration of approximately 1.8 mg pirlimycin hydrochloride/kg bw in capsules or as solution. Mean peak plasma concentrations were lower than (capsule) or approximately equal to (solution) the limit of detection of the microbiological assay used (0.156 µg/ml). Urinary excretion in 48 hours averaged 4.4% and 7.3% of the total dose for capsule and oral solution, respectively.

In a further study, male adult volunteers (5 subjects per treatment) received each oral doses of approximately 0.83, 2.1, 4.15 and 8.3 mg pirlimycin/kg bw. Plasma peak values for pirlimycin, 4 hours after administration, were lower than 0.01, 0.1, 0.2 and 0.6 µg/ml after the dose of 0.83, 2.1, 4.15 and 8.3 mg/kg bw, respectively. Urinary excretion during 24 hours ranged from 2.8 to 6.9% of the total dose. Faecal excretion (measured by bioassay over 72 hours) averaged 32% of the administered total doses.

According to the faecal recovery data, the unabsorbed fraction of pirlimycin remaining in the gastrointestinal tract of humans can be conservatively estimated at 50%.

5. The oral LD<sub>50</sub> in rats was 2524 mg/kg bw (range from 1976 to 4414 mg/kg bw). Necropsy of non-surviving rats revealed congestion of the glandular stomach and of kidney medulla.
6. In a 30-day oral toxicity study, rats were treated with doses of 50, 160 or 500 mg pirlimycin hydrochloride/kg bw/day. At 500 mg/kg bw, a few myeloid figures and a slight increase in lysosomes were noted in hepatocytes. Serum levels of glutamic oxaloacetic transaminase and of glutamic pyruvic transaminase were slightly elevated in all dose groups. A dose related increase of stomach histological lesions suggestive of focal irritation was observed at 500 and 160 mg/kg bw, whereas at 50 mg/kg bw histological examination was not performed. Stomach lesions were not observed in control animals. Due to the lack of histological examination at the lower dose level, a NOEL could not be established.

In a 13-week oral toxicity study, rats were treated with doses of 10, 30, 100 or 300 mg pirlimycin hydrochloride/kg bw/day. Haematological changes (higher mean corpuscular haemoglobin concentration) and plasma biochemical changes (such as reduced total protein, globulin, albumin, and creatinine) were present in males at dose levels of 30 mg/kg bw and higher. Lower relative liver weight was also noted in males at dose levels of 30 mg/kg bw and higher. The NOEL was 10 mg/kg bw.

7. In a preliminary, 30-day oral toxicity study, Beagle dogs were dosed orally with doses of 30, 100 and 300 mg/kg bw. Increased serum levels of aspartate aminotransferase and of alanin aminotransferase levels and hepatocytic hydropic degeneration were observed at 100 and 300 mg/kg bw; myeloid bodies were also detected by electron microscopy in the hepatocytes at the same dose levels. No effects were detected at 30 mg/kg bw/day.

In a 13-week toxicity study, Beagle dogs were treated orally with doses of 4, 16, 40 or 160 mg/kg bw/day of pirlimycin hydrochloride in gelatin capsules. Serum aspartate aminotransferase and alanin aminotransferase levels were elevated in the highest dose group. Salivation and vomiting were observed in animals treated with 160 and 40 mg/kg. Histological lesions suggestive of gastric irritation (inflammation, lymphoid hyperplasia) were noted in females at 160 and 40 mg/kg bw. The NOEL was 16 mg/kg bw/day.

8. No effects on fertility or pup survival and growth were observed in a 2-generation reproduction study on rats treated by gavage with doses of 100, 200 or 400 mg pirlimycin hydrochloride/kg bw/day. Salivation, irritability, and reduced weight gain were observed in adult animals at 200 and 400 mg/kg bw. The NOEL for general toxic effects was 100 mg/kg bw/day.
9. In an embryotoxicity/foetotoxicity study, pirlimycin hydrochloride was administered by gavage to pregnant rats at dose levels of 200, 400 or 800 mg/kg bw/day from day 6 to 15 of gestation. Maternal toxicity, as evidenced by soft stools, salivation and reduced weight gain during the dosing period, was observed at 400 and 800 mg/kg bw. The mean number of implantation per litter was slightly decreased at 400 and 800 mg/kg bw, and the resorption rate was slightly increased at the upper dose level; as a consequence, the number of live foetuses per litter was slightly, but not significantly, reduced at 800 mg/kg bw. A significant, dose-related increase in minor skeletal anomalies of the sternebrae was observed at all dose levels. A NOEL for embryotoxicity/foetotoxicity could not be identified.

In a further study, pregnant mice were treated by gavage with doses of 100, 400 or 1600 mg/kg bw from day 6 to 15 of gestation. At the upper dose level maternal toxicity was evidenced by moderate to severe diarrhoea with a few fatalities. At the same dose level, the only treatment-related effect was a moderate, but statistically significant, reduction in foetal weight. In mice, the NOEL for both maternal toxicity and foetotoxicity was 400 mg/kg bw.

It is concluded that pirlimycin is devoid of any teratogenic potential.

10. Negative results were observed in the following *in vitro* tests both with and without metabolic activation: the *Salmonella*-microsomal assay (2 tests in strains TA97, TA98, TA100, TA102, TA1535, TA1537, TA1538), in a mammalian cell forward gene mutation assay (HPRT locus) on Chinese hamster ovary (CHO) cells and AS52 cell lines, and in the unscheduled DNA synthesis assays (UDS) in primary rat hepatocytes.

Negative results were also found in the micronucleus tests by intraperitoneal route in rats and mice (at 90 to 360 mg/kg bw and 150 to 320 mg/kg bw of free base, respectively) and in a sex-linked recessive lethal assay in *Drosophila melanogaster*.

As pirlimycin hydrochloride gave negative results in a comprehensive battery of *in vitro* and *in vivo* genotoxicity assays, the range of endpoints tested *in vitro* and *in vivo* provided adequate evidence that pirlimycin is devoid of any mutagenic/genotoxic potential.

11. No carcinogenicity studies have been carried out. However, since pirlimycin does not possess any mutagenic or genotoxic potential and is not related to known carcinogenic agents, no further information on carcinogenicity is required.
12. An *in vitro* study on pirlimycin inhibitory activity against intestinal bacteria was performed. Pirlimycin was poorly active or almost inactive against Gram-negatives such as *Escherichia coli*, whereas the lowest MIC<sub>50</sub> values were obtained against Gram-positive anaerobes.

At standard inoculum density (10<sup>8</sup> cfu/ml), the following MIC values were obtained: 0.50 µg/ml for *Lactobacillus*, 0.25 µg/ml for *Bacteroides* and *Eubacterium*, 0.06 µg/ml for *Fusobacterium*, *Peptococcus/Peptostreptococcus* and 0.03 µg/ml for *Bifidobacterium*.

The same spectrum of activity was observed at 10<sup>10</sup> cfu/ml inoculum density, which is closer to human gut situation, *Bifidobacterium* was the most sensitive organism with a MIC<sub>50</sub> of 0.12 µg/ml.

Therefore, the MIC<sub>50</sub> for *Bifidobacterium*, as it was the most sensitive microorganism, at high inoculum density was selected to derive the microbiological ADI.

4. The *in vitro* activity of pirlimycin hydrochloride and its main metabolite, pirlimycin sulphoxide, was investigated. The lowest MIC<sub>50</sub> for pirlimycin hydrochloride was observed in *Bifidobacterium* spp. and *Eubacterium* spp. (0.06 µg/ml). Pirlimycin sulphoxide was much less active, with a lowest MIC<sub>50</sub> of 1.0 µg/ml in *Eubacterium* spp.

Therefore, in these conditions of assay the parent compound showed a 16-fold higher antimicrobial activity as compared to pirlimycin sulphoxide.

14. Healthy male volunteers (5 subjects per treatment) received each oral doses of approximately 0, 0.83, 2.1, 4.15 and 8.3 mg/kg bw of pirlimycin. One and/or 6 days after dosing *Clostridium difficile* was found in stools of 2, 4, 5 and 3 of the subjects treated with pirlimycin at 50, 125, 250 or 500 mg, respectively and in 0, 0, 1 and 0 in the control subjects. Although *Clostridium difficile* is a relevant parameter to assess effects on human gut flora, the data were too limited to reach any definitive conclusion about a level of pirlimycin without effect on human gut flora *in vivo*.
15. The effects of milk concentrations of pirlimycin of 40 to 2400 µg/l on the decrease of pH after incubation were examined with 5 dairy starter cultures (1 yoghurt, 3 Italian cheese and 1 buttermilk/sour cream starters). It was calculated that adverse effects on production processes based on these starters could be expected at average concentrations of 140 to 590 µg/l. A mathematical model was used to predict average level of pirlimycin for the individual starter cultures which causes the time to pH 4.6 to equal twice that of the negative controls. In addition, a lower 95% prediction limit on the average value was calculated. The lowest of the 95% prediction limit values for the different starters was 130 µg/l.

In an additional study the effect of milk concentrations of pirlimycin of 20 to 1280 µg/l on the decrease of pH after incubation were examined with a commercial Italian cheese culture, HC-13. The mean pirlimycin concentration and lower 95% prediction limit for culture HC-13 were determined to be 200 and 155 µg pirlimycin/l, respectively.

A further study evaluated the effect of lincomycin in milk on 7 dairy starter cultures and identified those most sensitive to lincomycin. Three types of yogurt starter and 2 types of Italian cheese cultures were examined. Yogurt starter cultures had the lowest mean lincomycin concentration (160 µg/l) resulting in a clotting time ratio of 2. These starter cultures were thus identified as being the most sensitive to lincomycin. A comparison of the mean lincomycin concentrations resulting in a clotting time ratio of 2 to the mean for pirlimycin for common starter culture types was made. These data demonstrated that mean levels of lincomycin were higher than those for pirlimycin when the same starter cultures and manufacturers were compared.

16. No specific studies on immunotoxicity were provided. However, in repeated dose toxicity studies pirlimycin did not cause any significant effect on white blood cells or organs relevant to immune function. Lymphoid hyperplasia concomitant with focal irritation was observed in the stomach of female dogs treated orally for 13 weeks with dose levels of at least 40 mg/kg bw. The NOEL for such effect was 16 mg/kg bw.
17. In humans, mild headache was observed in one out of 10 subjects after single oral administration of 125 mg pirlimycin hydrochloride (approximately 1.8 mg/kg bw). No gastrointestinal disturbances were observed. Healthy male volunteers (5 subjects per treatment) received each oral doses of approximately 0, 0.83, 2.1, 4.15 and 8.3 mg/kg bw of pirlimycin. The treatment did not elicit any clinical (including gastrointestinal) disturbances. Eosinophils, serum inorganic phosphorus and urine specific gravity showed, in pirlimycin-treated subjects, statistically significant increases.
18. A toxicological ADI can be determined on the basis of the NOEL of 10 mg/kg bw observed in the 13-week rat study, with lower relative liver weight and haematological and serum biochemical changes occurring at dose levels of 30 mg/kg bw and higher. Since the data are of adequate quality, and there is no evidence of effects on reproduction, teratogenicity or mutagenicity/genotoxicity, a safety factor of 100 is adequate to derive the ADI value. Based on this data, a toxicological ADI of 0.100 mg/kg bw (i.e. 6 mg/person) was established. However, a lower ADI value can be derived for the effects on human gut flora *in vitro*.
19. For the assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP:

$$\text{ADI} = \frac{\frac{\text{Geometric mean MIC}_{50} \times \text{CF}_2}{\text{CF}_1} \times (\mu\text{g/ml}) \times \text{daily faecal bolus (150 ml)}}{\frac{\text{Fraction of an oral dose available for microorganisms}}{\text{x weight of human (60 kg)}}} \quad (\mu\text{g/kg bw})$$

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

$$\text{ADI} = \frac{\frac{0.12 \times 10}{1} \times 150}{0.5 \times 60} = 6 \mu\text{g/kg bw i.e. } 360 \mu\text{g/person}$$

The following assumptions were made:

- 0.12 µg/ml is the MIC<sub>50</sub> in the most sensitive microorganism (*Bifidobacterium*);
- CF<sub>1</sub> = 1 justified by the use of the most sensitive relevant species and the evidence of a lack of emerging resistance (neither common nor rapid in over 10 millions strains surveyed) to lincosaminides;
- CF<sub>2</sub> = 10 to take into account the differences between *in vitro* and *in vivo* findings; the *in vivo* data show that a CF<sub>2</sub> = 1 would be too conservative. Pirlimycin is stable at low pH;
- 0.5 was the fraction of an oral dose available for microorganisms;
- 150 ml was the weight of the daily faecal bolus.

20. After intramammary infusion in dairy cows of 200 mg  $^{14}\text{C}$ -pirlimycin hydrochloride/quarter in 4 quarters two times at 24-hour interval plasma pharmacokinetic parameters estimated following non-compartmental analysis showed a two-phase kinetic. Mean values were:  $\text{AUC}_{0-120}$ : 2270 to 7110  $\mu\text{g}\cdot\text{hr}/\text{l}$ ;  $t_{1/2}$  of absorption phase: 2.89 hours;  $C_{\text{max}}^1$ : 83  $\mu\text{g}/\text{l}$ ;  $t_{1/2}$  of terminal phase: 37 hours;  $C_{\text{max}}^2$ : 131  $\mu\text{g}/\text{l}$ ;  $K_{\text{el}}$ : 0.0224. Approximately 50% of the intramammary administered dose reached the systemic circulation.

Pirlimycin comprised 80.6% of the total residue excreted in urine, pirlimycin sulfoxide 8.0% and 2 polar unknown compounds 3.8% and 6.7%. In faeces 44.6% of the total residue was pirlimycin, 1.5% pirlimycin sulfoxide, 32% and 18% the 2 polar unknown compounds.

20. Milk residue depletion kinetics were described in four studies. According to the study, the milk samples were analysed for residues of pirlimycin either by liquid scintillation counting, and/or by HPLC method (limit of detection: 40  $\mu\text{g}/\text{l}$ ; limit of quantification: 100  $\mu\text{g}/\text{l}$ ) and/or by microbiological method based on the activity against *M. luteus* and sensitive to 20  $\mu\text{g}/\text{l}$ .

In a first GLP study, 12 dairy cattle in mid-lactation were administered  $^{14}\text{C}$ -pirlimycin hydrochloride by intramammary infusion at a dose rate of 200 mg/quarter into 4 quarters, twice, at 24 hour interval. The mean concentrations of total pirlimycin, parent pirlimycin and microbiological active residues were in the same magnitude : 43 900  $\mu\text{g}$  equivalents/l, 40 700  $\mu\text{g}$  equivalents/l and 41 500  $\mu\text{g}/\text{l}$  respectively 12 hours after the end of the treatment. At 96 hours after treatment, the mean concentrations were : 140  $\mu\text{g}/\text{l}$  for total radioactivity, 110  $\mu\text{g}$  for the parent compound and 150  $\mu\text{g}/\text{l}$  for the microbiologically active residues.

In another GLP study, 23 dairy cattle in mid-lactation were administered  $^{14}\text{C}$ -pirlimycin hydrochloride by intramammary infusion at the recommended dose of 50 mg/quarter of pirlimycin hydrochloride twice at 24-hour interval. In milk, the mean concentrations of total radioactivity were 18 400, 2 030, 420, 170, 110 and 80  $\mu\text{g}$  equivalent/l at 12, 24, 36, 48, 60 and 72 hours after treatment, respectively. The concentrations of the microbiologically active residues ranged from 17 000  $\mu\text{g}/\text{l}$  (12 hours after dosing) to 70  $\mu\text{g}/\text{l}$  (72 hours after dosing).

In 2 non-radiometric studies, cows, mastitic in 1 quarter ( $n=26$ ) and non mastitic ( $n=20$ ), were treated in all 4 quarters at the recommended dose of 50 mg/quarter of pirlimycin hydrochloride twice at 24-hour interval. For both studies, the mean concentrations of microbiologically active residues in milk were in the magnitude 850, 230, 110 and 70  $\mu\text{g}$  microbiological active compound/l, at 24, 36, 48 and 60 hours after the end of the administration.

The ratio of pirlimycin to total residues and microbiologically active residues was estimated at 100%.

22. The depletion of pirlimycin in edible tissues was followed in 2 radiometric studies and 1 non-radiometric study in cattle.

$^{14}\text{C}$ -pirlimycin hydrochloride was administered by intramammary infusion at a dose regimen of 2 treatments at 24 hour interval and a dose of 200 mg ( $n=12$ ) or 50 mg/quarter ( $n=23$ ) into 4 quarters.

After administration of 200 mg  $^{14}\text{C}$ -pirlimycin/quarter, the concentrations of total residues were 7130, 780, 50 and 20  $\mu\text{g}$  pirlimycin equivalents/kg in liver, kidney, muscle and fat respectively at 6 days after treatment. Then they declined to reach 3570, 260, 20 and 10  $\mu\text{g}$  pirlimycin equivalents/kg in liver, kidney, muscle and fat, respectively at 14 days after treatment and 500, 10  $\mu\text{g}$  pirlimycin equivalents/kg in liver and kidney at 28 days after treatment and were below 2  $\mu\text{g}/\text{kg}$  for muscle and fat at this sampling time. The concentrations in udder were 130, 30 and lower than 2  $\mu\text{g}/\text{kg}$  on days 6, 14 and 28.

After administration of 50 mg <sup>14</sup>C-pirlimycin/quarter, the mean concentrations of total residues were 2180, 300, 20, and 10 µg pirlimycin equivalents/kg in liver, kidney, muscle and fat respectively at 6 days after the end of the treatment. Then, they declined to reach 990, 60, 10 and 10 µg pirlimycin equivalents/kg in liver, kidney, muscle and fat respectively at 14 days after the end of the treatment and 890 and 40 µg pirlimycin equivalent/kg in liver and kidney, respectively at 18 days after the end of the treatment and were below 5 µg/kg for muscle and fat for this sampling time. In udder, significant amounts of pirlimycin could be measured 330, 160 and 40 µg equivalents/kg in udder at 6, 14 and 18 days after the end of treatment, respectively.

In a non-radiometric study after intramammary infusion into all 4 quarters of 2 doses of 50 mg/quarter of pirlimycin hydrochloride at 24-hour intervals to 20 dairy cows significant amounts of pirlimycin could be measured 2 days after the end of the treatment: 1470 µg/kg in liver, 460 µg/kg in kidney, 20 µg/kg in muscle and lower than 25 µg/kg in fat. At 7 days, pirlimycin could only be measured in liver (240 µg/kg) and in kidney (60 µg/kg). At later sampling times (14, 21, 28 days), pirlimycin concentrations were below the limit of quantification. In liver, pirlimycin represented 24.9%, of the total residues radioactivity pirlimycin sulfoxide 63.9% and pirlimycin sulfone 8.1%. In kidneys, pirlimycin represented 43.1%, pirlimycin sulfoxide 46.1%, and pirlimycin sulfone 7.2%.

23. Three specific depletion studies of pirlimycin amounts in liver were conducted. The concentrations of pirlimycin in the liver determined by the *M. luteus* microbiological method (limit of detection: 20 µg/kg; limit of quantification: 30 µg/kg) or the HPLC/TSP/MS method (limit of quantification and limit of detection: 25 and 20 µg/kg)

After administration of 50 mg/quarter of pirlimycin hydrochloride into all 4 quarters twice of 31 cows at 24 hour interval, the concentrations of microbiologically active residues in the liver were: 1650 µg/kg, 600 µg/kg, 280 µg/kg, 240 µg/kg and 160 µg/kg at 2, 6, 10, 14 and 21 days after treatment, respectively.

After administration of 50 mg/quarter of pirlimycin hydrochloride administered twice at 24-hour interval in 2 quarters of 24 animals, the mean concentrations of microbiologically active residues in liver at 14, 21 and 30 days were 50, 30 and 30 µg/kg and those of the parent compound 90, 40 and 50 µg/kg respectively.

After administration of 50 mg/quarter of pirlimycin hydrochloride administered twice at 24-hour interval in 2 quarters of 33 animals, the mean concentrations of microbiologically active residues in liver were 430, 80, 60 and 40 µg/kg and those of pirlimycin 490, 70, 40 and 60 µg/kg at 7, 14, 21 and 30 days, respectively.

24. The interconversion of pirlimycin in special conditions was studied.

When administered by intramammary route into all 4 quarters as 2 doses of 50 mg/quarter of pirlimycin hydrochloride at 24 hours interval, the mean concentration of parent pirlimycin in the liver of the cows slaughtered at 2 and 7 days were 1470 and 240 µg/kg. For later sampling times (14, 21 and 28 days), the concentrations in liver were below 25 µg/kg.

When a incubation phase was introduced during the assay (incubation at 37°C for 24 hours) the mean concentrations of parent pirlimycin in the liver of the cows slaughtered at 2, 7, 14 and 21 days increased to 1690 µg/kg, 610 µg/kg, 210 and 60 µg/kg, respectively. At 28 days, the concentrations remained below 25 µg/kg. The relative increase in concentrations of parent pirlimycin was greater for samples with low concentrations (7, 14 and 21 days) whereas only minor increases were observed for samples with higher concentrations (2 days).

A further series of experiments showed that the increased concentration of parent pirlimycin occurred at the expense of the sulphoxide; the relative degree of the increase of pirlimycin was dependent upon the initial concentration of residue as a function of the slaughter interval following treatment of the dairy cow with pirlimycin; the greatest increase occurred in the first 6 to 16 hour period, but may require 22 to 24 hours to reach a plateau for maximum parent pirlimycin residue measurement. Experiments on frozen liver showed a slight increase in the concentration of parent pirlimycin; an initial freeze-thaw cycle had a slight impact upon the concentration of parent pirlimycin, but subsequent cycles produced no further apparent increase in concentration; the interconversion of pirlimycin sulphoxide to parent pirlimycin was likely to be due to residual activity of reductase enzymes within the liver.

25. The ratio of the marker residue to total microbiologically active residues was estimated to equal 1 in muscle, fat, liver and kidney. The parent compound was selected as marker residue.
26. Analytical methods for the determination of pirlimycin residue in milk and bovine tissues (liver, muscle, kidney and fat) based on mass spectrometric (MS) detection, were provided in an ISO 78/2 format with supporting validation data. The limits of quantification were 25 µg/kg for liver and 50 µg/kg for muscle, kidney, fat and milk.

### Conclusions and recommendation

Having considered that:

- a microbiological ADI of 6 µg/kg bw (i.e. 360 µg/person) has been established,
- pirlimycin is the marker residue representing 100% of the microbiologically active residues in all edible tissues including milk,
- a post-slaughter interconversion of pirlimycin sulphoxide into the parent compound may occur in liver,
- validated HPLC/MS analytical methods are available;

the Committee recommends the inclusion of pirlimycin 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 |
|---------------------------------------|----------------|----------------|----------------------------------------------------------------|------------------------------------------|------------------|
| Pirlimycin                            | Pirlimycin     | Bovine         | 100 µg/kg<br>100 µg/kg<br>1000 µg/kg<br>400 µg/kg<br>100 µg/kg | Muscle<br>Fat<br>Liver<br>Kidney<br>Milk |                  |

Based on these MRLs values, the daily intake will represent about 85% of the microbiological ADI