The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

VALNEMULIN

SUMMARY REPORT

1. Valnemulin is an antibiotic belonging to the pleuromulin group, which acts by inhibition of bacterial protein synthesis. Its chemical structure is similar to that of tiamulin. It has been proposed for oral administration to pigs for the treatment of bacterial diseases. Various dosing regimes were proposed; the highest dose, 10 to 12 mg/kg bw per day for up to 4 weeks, was indicated for treatment of swine enzootic pneumonia.

2. The pharmacokinetics of the substance ^{3}H-labelled in the vinyl moiety was studied in Beagle dogs following oral administration at 10 and 30 mg/kg bw and intravenous administration at 3 mg/kg bw and in Sprague-Dawley rats following oral administration at 20 mg/kg bw and intravenous administration at approx. 6 mg/kg bw. In both species the substance was rapidly and completely absorbed after oral administration with bioavailability around 100%. The substance was widely distributed to the tissues. In rats killed 3 hours after oral dosing, high concentrations were found in the lung, liver and gastro-intestinal tract. Radio HPLC profiling indicated 22 different metabolites in rat plasma, liver, urine and faeces. There were considerable inter-animal variations in the amounts of the different metabolites found in the tissues. In dogs given daily oral doses of 30 mg/kg bw per day for 7 days and killed 2 hours after the last dose, high concentrations were found in liver and in bile. After both oral and intravenous dosing, the substance and its metabolites were excreted, predominantly in the faeces. Excretion of expired $^{3}H_2O$ was minimal. The metabolites in tissues and excreta were not identified. However comparison of the radio HPLC profiles for rats and dogs with those for pigs indicated that there were no major qualitative differences in biotransformation between the 3 species. Quantitative differences were mainly noted in the liver with unmetabolised parent compound accounting for approximately 17% in the dog up to 16% in pig liver (depending on time-point) and 46% in rat liver.

3. The substance was of moderate to low acute oral toxicity. In male and female Sprague-Dawley rats, the acute oral LD_{50} was greater than 1000 mg/kg bw but less than 2000 mg/kg bw. The acute oral LD_{50} values in mice were 1710 and 1482 mg/kg bw for males and females respectively. Overt signs of toxicity included reduced activity, piloerection, ataxia, hunched appearance and laboured breathing.

4. Groups of 20 to 25 Sprague-Dawley rats were fed diets calculated to provide 0, 1, 20 or 200 mg/kg bw per day of the substance for 13 weeks. At termination, groups of 5/sex rats from the 0 and 200 mg/kg bw group were retained on untreated diet for a further 4 weeks to check for reversibility of any effects. In the 200 mg/kg bw group, body weight gain and food consumption were significantly reduced in both sexes and the mean cell haemoglobin content (MCHC) was slightly reduced. There were significant increases in gamma-glutamyl transpeptidase (GGT), aspartate transaminase (AST), alanine transaminase (ALT), blood-urea nitriogen (BUN) and potassium concentrations in males given 200 mg/kg bw. At necropsy, the incidence and severity of hepatic lesions were increased in the 20 and 200 mg/kg bw groups and the incidence of thyroid follicular epithelial hyperplasia was increased at 200 mg/kg bw. Periportal vacuolation of the liver was also observed in the 200 mg/kg bw group at the end of the recovery period. The NOEL was 1 mg/kg bw per day.

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A second 13-week study with no recovery phase was carried out to determine a more definitive NOEL. The rats were fed diets calculated to provide 0, 8, 16, 32 or 64 mg/kg bw. Toxic effects on the liver were observed, these were very similar to those in the first study. However there were no effects on the thyroid. The NOEL was 8 mg/kg bw per day.

5. Groups of 5/sex CD-1 mice were fed diets calculated to provide 0, 20, 100, 300 or 1000 mg/kg bw per day for 4 weeks. Because of severe toxicity (emaciation, weight loss), the 1000 mg/kg bw dose was reduced to 700 mg/kg bw but the toxic effects continued and the group was terminated on day 21 of the study. In the 300 mg/kg bw group, body weight gain was significantly reduced, liver weights were significantly increased and there were histopathological changes in the liver. Microscopic changes attributable to treatment were also observed in the livers from mice given 20 and 100 mg/kg bw. In this study no haematology, clinical chemistry or urinalysis investigations were carried out and the pathological examinations did not cover the full range of tissues. The study was designed as a range-finding study and no NOEL was established.

6. Groups of 4/sex Beagle dogs were given daily oral doses of 0, 10, 30 or 100 mg/kg bw per day, in gelatin capsules, for 13 weeks. The doses were selected on the basis of results from a range-finding study in which doses of 120 mg/kg bw and above caused reduced food consumption and weight loss. One male dog given 100 mg/kg bw had severe convulsions after dosing on the 3rd day of the study and was euthanased. Body weight gain and food consumption were reduced in dogs given 100 mg/kg bw and plasma alkaline phosphatase values were significantly increased in this group during weeks 6 and 12. There were some significant changes in haematology values in males (but not females) but these were not dose-related and were not consistent throughout the study; they probably represented chance findings. There were no gross- or histopathological findings attributable to treatment. The NOEL was 30 mg/kg bw per day.

7. Large White pigs were fed diets containing the equivalent of 75 mg/kg bw per day for 28 consecutive days. This was stated to be approximately 5 times the proposed indicated dosage. The pigs were initially reluctant to feed due to the unpalatibility of the test substance. However the pigs remained in good health, faecal consistency was unaffected and the animals gained weight normally.

8. Groups of Sprague-Dawley rats were given daily oral doses of 0, 8, 40 or 200/160 mg/kg bw throughout the breeding of 2 generations, with 2 litters per generation. Eleven days into the study, the top dose level of 200 mg/kg bw was reduced to 160 mg/kg bw due to severe toxicity. However signs of toxicity (convulsions preceding death in 2 male parental animals) and reduced parental body weight gain were still seen after the dose was reduced. At necropsy of the F0 and F1b adults, the incidence of liver lesions (prominent lobulation and/or pale focus) was increased in the 200 mg/kg bw group compared with the controls. There were no effects on mating performance, fertility, litter size, pup weight or pup survival at any dose level. The NOEL based on parental toxicity was 40 mg/kg bw per day.

9. Groups of 30 female CD-1 mice were given daily oral doses of 0, 10, 30 or 100 mg/kg bw per day from days 6 to 15 of gestation. Two dams given 100 mg/kg bw showed piloerection, hunched posture, ataxia and dull eyes. Body weight gain and food consumption were reduced at 30 and 100 mg/kg bw. There was no evidence of teratogenicity at any dose level. Foetal examinations were complicated because some dams littered before the scheduled time of necropsy. Excluding these foetuses from the calculations showed a slight increase in the incidence of retarded ossification at 30 and 300 mg/kg bw. The NOELs for foetotoxicity and maternal toxicity were 10 mg/kg bw per day.

10. In a range-finding study, 31.25 or 150 mg/kg per day of the test substance was administered to non-pregnant rabbits. Dosing was scheduled for 5 consecutive days. However, due to severe toxicity, treatment was discontinued after 3 and 4 days for the 2 groups respectively. It was concluded that the rabbit was not a suitable subject for a teratology study with the test substance and no definitive experiment was carried out.

11. Groups of pregnant female Sprague-Dawley rats were given daily oral doses of 0, 25, 75 or 225 mg/kg bw per day from days 6 to 16 of gestation. The dose of 225 mg/kg bw caused both maternal toxicity and foetotoxicity (increased incidence of wavy ribs and delayed ossification). There was no evidence of teratogenicity at any dose level. The NOEL for both foetotoxicity and maternal toxicity was 75 mg/kg bw per day.

12. No evidence of mutagenicity was observed in an *in vitro* bacterial assay for gene mutation in *S. typhimurium*, an *in vitro* assay for gene mutation in Chinese hamster ovary (CHO) cells nor in an *in vitro* UDS assay in primary rat hepatocytes. Weak positive results were obtained at toxic concentrations in the absence of metabolic activation in an *in vitro* assay in L5178Y mouse lymphoma cells; several deficiencies were noted in the study and it was concluded that a weak clastogenic effect under these conditions was meaningless. A weak clastogenic effect was observed in the presence of metabolic activation in an *in vitro* cytogenetics assay in CHO cells at toxic concentrations, but with no dose-response. In a second *in vitro* cytogenetics assay in CHO cells, negative results were again obtained in the absence of metabolic activation but an increased frequency of cells with structural chromosome aberrations was observed in the presence of S9; however, the effect was small, not reproduced between experiments and of doubtful biological significance. Evidence of DNA-binding was observed in CHO suspensions; however the design of the assay did not have the necessary power to distinguish covalent binding of the substance from artefactual increases. A well conducted *in vivo* micronucleus test gave negative results. Clear negative results were obtained in an *in vitro/in vivo* UDS assay in liver following oral doses of 800 and 2000 mg/kg bw to Crl:CD rats. Overall, it was concluded that there was no good evidence for the induction of gene mutations by the test substance and that there was marginal evidence of clastogenicity *in vitro* but not *in vivo*.

13. No data on carcinogenicity were provided. The substance had a similar chemical structure to that of tiamulin, which had been shown to be non-carcinogenic in rats and mice. It was agreed that carcinogenicity studies were not required.

14. In the repeated-dose studies, no effects were observed which were indicative of an effect on the immune system. Studies with the related substance, tiamulin, on the formation of antibodies in mice and chickens, indicated that tiamulin did not suppress the immune system.

15. Skin sensitisation was investigated in a Magnussen & Kligman maximisation test in the guinea pig. No evidence of skin sensitisation was seen in 9 out of 10 test animals. An equivocal response was observed in the remaining animal. According to a published report, the related substance tiamulin causes skin sensitisation in around 2% of exposed humans.

16. The substance is not proposed for use in human medicine and there are no data concerning possible effects in humans.

17. A toxicological ADI of 80 µg/kg bw per day (i.e. 4.8 mg per person per day) was calculated by applying a safety factor of 100 to the NOEL of 8 mg/kg bw per day, based on hepatotoxicity, which was established in the 13-week repeated-dose study in rats.

18. *In vitro* MIC values were determined for more than 90 isolates of 10 bacterial species which were representative of those found in the human gut. For the assessment of microbiological risk, use was made of the formula that was recommended by the CVMP:

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

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

$$ADI = \frac{\frac{0.053 \times 3}{1} \times 150}{0.05 \times 60} = 7.95 \text{ µg/kg bw i.e. } = 477 \text{ µg/person}$$

where :

- 0.053 µg/ml was the geometric mean MIC_{50} value (omitting insensitive strains)
- CF1 = 1. There was no information on the likely mechanism of resistance because it was not possible to induce resistance to valnemulin in laboratory experiments; resistance to the related substance tiamulin is slow to develop and rarely encountered in the field.
- CF2 = 3 to correct for differences in growth conditions between the in vitro and in vivo situation. No correction was necessary for changes in pH. For 32 strains (representing 8 genera) there was an increase in geometric mean MBC value of 7 on increasing the inoculum density from 10^6 to 10^8 cfu/ml.
- 150 ml = the daily faecal bolus
- 60 kg = human adult bodyweight
- 0.05 = fraction of dose available to the flora at the distal part of the gastrointestinal tract, based on data in pigs which showed that approximately 2% of the orally (in-feed) administered dose of valnemulin was excreted in the faeces as microbiologically-active residues.

19. Following oral administration to pigs, valnemulin was rapidly absorbed. After a single oral dose of 10 mg/kg bw of the hydrochloride, t_{max} was 1.85 hours, c_{max} was 1.29 µg/ml and area under curve (AUC) was 5.58 µg/ml.h; these figures increased to 2.9 hours, 2.67 µg/ml and 18.23 µg/ml.h when the dose given was 25 mg/kg bw, and 4.15 hours, 6.23 µg/ml and 67.3 µg/ml.h when the dose given was 50 mg/kg bw. Following repeated doses of 5 mg/kg bw twice daily, a plateau in plasma levels had been approached by 7.5 days. No information is available on absolute bioavailability since intravenous studies have not been carried out.

20. Distribution of valnemulin to the tissues also occurred rapidly in pigs as demonstrated by radio-tracer methodology using valnemulin labelled with tritium in the vinyl moiety. In the main study, 15 animals were each given a dose of 5 mg/kg bw twice a day for 7.5 days by oral gavage. The greatest amounts of radioactivity were found in the liver (1 day after the final dose the mean concentration was 3650 µg equivalents/kg which represents around 92% of the total amount found in the edible tissues) with less in the kidney (240 µg equivalents/kg at day one, representing around 6%), very small amounts in muscle (70 µg equivalents/kg at one day one, representing around 2%) and undetectable levels in skin and fat. The last time point was 8 days and at this stage, radioactive residues were 400 µg equivalents/kg in liver, 20 µg equivalents/kg in kidney and undetectable in muscle.

21. Valnemulin was also excreted rapidly, mostly via the bile and faeces (around 87% of the total dose by 120 hours after the last dose when pigs were given 5 mg/kg bw twice daily for 7.5 days). Excretion in urine over the same period was around 3%.

22. Pigs were given oral doses of 25 mg/kg bw valnemulin, twice a day, for 7.5 days and the residues in bile and in liver were characterised using various HPLC methods, LC-MS and ^{1}HNMR. Eleven metabolites were identified in bile and 6 of these were also found in liver. These represented approximately 50% of the total residues in liver and 60% of those in bile. All these metabolites retained the intact valnemulin skeleton and were oxidised either in the side chain or the pleuromutilin ring. No epoxides were found. Only 2 of the metabolites (accounting for 4.4% of the metabolites identified) had antimicrobial activity and this was approximately 70% that of valnemulin.

23. Six pigs were given oral gavage doses of 5 mg/kg bw ^{3}H-valnemulin, twice a day for 5 days. The pigs were killed (3 per time-point) 2 or 24 hours the last dose. Total residues in tissues were determined by liquid scintillation counting. Residues of valnemulin were determined using the proposed routine analytical method based on HPLC with a limit of quantification of 25 µg/kg. Mean total residues in liver depleted from 19300 µg/kg at 2 hours to 4300 µg/kg at 24 hours. Valnemulin accounted for 8% and 2% of the total residues in liver at these time-points. Mean total residues in kidney depleted from 1000 µg/kg at 2 hours to approximately 400 µg/kg at 24 hours with valnemulin accounting for approximately 6% of the total residues at both time-points. Although residues in some samples of muscle were below the Limit of Quantification; it was estimated that valnemulin accounted for approximately 6% of the total residues in muscle over the time period 2 to 24 hours. Residues in samples of skin + fat were undetectable in all samples except for one (89 µg/kg).

24. The main residues depletion study in pigs used a nominal dose of 5 mg/kg bw/day or 15 mg/kg bw/day in the feed for 28 days. Actual doses received were approximately 3.8 and 11.6 mg/kg bw/day, respectively. Five pigs at each dose level were killed at each time point (2 hours, 8 hours, 1 day, 2 days, 3 days and 5 days). Parent compound was measured using the validated HPLC assay which has been proposed for routine monitoring. The highest levels of valnemulin were found in the liver of animals given 11.6 mg valnemulin/kg bw/day: mean of 455 µg/kg 8 hours after final treatment, 113 µg/kg 1 day after the final treatment, reducing to less than 25 µg/kg 3 days later. The equivalent levels for kidney were 94 µg/kg reducing to 63 µg/kg and to less than 25 µg/kg and for muscle 33 µg/kg reducing to 26 µg/kg and to less than 25 µg/kg. Valnemulin was undetectable in skin + fat at all time points.

25. Samples of pig liver from the previous study were re-analysed by different laboratories using both the HPLC method and a microbiological assay with *Micrococcus luteus* ATCC 9341 as the indicator organism. The limits of quantification and detection of the microbiological assay were 170 and 100 µg/kg respectively. The results indicated that residues of valnemulin in liver did not deteriorate when stored at -20°C for up to 30 months. The results from the HPLC assay were very similar to those obtained using the microbiological assay. From these results it was concluded that the ratio of residues of valnemulin to total microbiological residues in liver was constant over the period 2 hours up to 1 day after the end of treatment and was approximately 85%.

26 An analytical method for routine monitoring has been provided which measures parent compound only. It involves liquid-liquid extraction followed by derivatisation and HPLC with fluorescence detection and quantification against an internal standard. This method is clearly described and has been adequately validated. The limit of quantification was 23 µg/kg for all edible porcine tissues. Residues of tiamulin and trimethoprim did not interfere in the analysis.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 477 µg per person per day was established for valnemulin,
- valnemulin represents 85% of the total microbiological residues in porcine liver over the period 2 hours up to 1 day after the end of treatment,
- although similar data were not available for kidney and muscle, it was reasonable to assume that valnemulin would represent approximately 85% of the total microbiologically active residues in these tissues,
- residues were undetectable in skin + fat at all time-points and so it was not necessary to establish MRLs for these tissues,
- a validated analytical method based on HPLC was available for the determination of residues of valnemulin in porcine tissues ;

the Committee recommends the inclusion of valnemulin in Annex I to 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
Valnemulin	Valnemulin	Porcine	50 µg/kg 500 µg/kg 100 µg/kg	Muscle Liver Kidney	

Based on these MRLs, it was calculated that the consumer intake of microbiologically active residues would represent approximately 17% of the microbiological ADI.

Based on a ratio of 6% for the value of marker to total residues for all tissues, it was calculated that the consumer intake of total residues, based on these MRLs, would represent 24% of the toxicological ADI of 4800 µg/person/day.