



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

MECILLINAM

SUMMARY REPORT

1. Mecillinam is a derivative of 6 β -amidinopenicillanic acid. It is intended to be used, in association with the first-generation cephalosporin cephapirin, as uterine bolus for prophylactic and therapeutic treatment of endometritis in cows. The bolus will contain 125 mg of mecillinam and 125 mg of cephapirin. The intended treatment is a single intra-uterine administration of two boluses, i.e. 250 mg of each active ingredient.
2. The antimicrobial activity of mecillinam differs significantly from that of most other β -lactam antibiotics, including the aminopenicillins, because of its high activity against Gram-negative bacteria, especially *Enterobacteriaceae*. It is less active against Gram-positive bacteria than benzylpenicillin and without significant activity against anaerobic species. Mecillinam is relatively resistant to acid and to β -lactamases produced by Gram-negative bacteria. Synergism has been demonstrated for mecillinam and other β -lactamase stable penicillins, with respect to several species of *Enterobacteriaceae*. No synergism has been observed for mecillinam and aminoglycosides, chloramphenicol, tetracyclines or polymyxins.

In laboratory animal studies employing parenteral dosing in the range 20 to 100 mg/kg bw mecillinam was practically devoid of general pharmacologic effects.

3. Mecillinam is poorly absorbed (5 to 10%) following oral administration in mice, rats, dogs and cattle. Bioavailability following subcutaneous and intramuscular administration was almost 100%. Following parenteral administration the elimination half-life in plasma was 0.8 to 1.5 hours and the volume of distribution was 0.2 to 0.5 l/kg. Binding to plasma proteins in the rat was 18%. Highest tissue concentrations were found in liver and kidneys. Passage across the blood-brain barrier and the placental barrier of mice was negligible.
4. In rats and dogs, after parenteral administration, mecillinam was excreted mainly in the urine, the major part (60 to 75%) within the first few hours after administration. One-half to two-thirds of the amount excreted during the first 24 hours period was microbiologically active. Over the same interval, 5 to 10% of the dose was recovered in the bile and an additional 3 to 9% were recovered in the faeces.
5. In the dog the proportion of microbiologically active substance in plasma (expressed as mecillinam equivalents) decreased from around 80% at 15 minutes to 25% at 4 hours. Seven metabolites were found in dog urine. Only one of these, the formyl-6-aminopenicillanic acid, was identified. The metabolite was microbiologically active, but less potent than the parent substance.

6. In non-ruminating calves 1 to 3% of an oral dose of 15 mg/kg were excreted in the first 24 hours urine in microbiologically active form, compared to 40 to 60% when the same dose was administered intravenously. Excretion into the milk of cows was low: following intravenous administration of 8 mg mecillinam/kg bw to two cows the concentration of the microbiologically active substance averaged 0.12 µg/ml at 2 hours, and 0.72 µg/ml at 6 hours.

Following single intra-uterine administration within 26 hours after calving of two boluses, corresponding to 250 mg mecillinam and 250 mg cephapirin, mecillinam was detectable in urine for up to 16 hours at concentrations varying from 15.5 µg/ml (detected in one sample collected at 4 to 6 hours) to 0.10 µg/ml (in a sample collected at 10 hours). The limit of quantification of the method was 0.05 µg/ml.

7. Several of the toxicological studies submitted with the application were carried out before the current GLP regulations were adopted.
8. The acute toxicity of mecillinam was low. In mice LD₅₀ values greater than 8 000 mg/kg bw by oral route, equal to or greater than 8 000 mg/kg bw by intraperitoneal route, equal to or greater than 5 600 mg/kg bw by intramuscular route, and of 950 to 1 400 mg/kg by intravenous route were observed. LD₅₀ values in the same order of magnitude were obtained for rats. By intravenous route LD₅₀ was approximately 1 100 mg/kg and greater than 1 000 mg/kg bw in rabbits and dogs, respectively.
9. Repeated-dose toxicity studies were carried out in rats and dogs at doses in the range of 50 to 450 mg/kg bw/day. A subcutaneous 13-week study was performed in rats, while dogs were treated intravenously for 22 days or subcutaneously for 4 or 13 weeks. One 13-week dog study (450 mg/kg bw/day) included a 25-weeks reversibility study. No repeated-dose toxicity studies by oral administration were carried out. The principal target organ of toxicity was the liver. The effects observed included a dose-related increases in serum alkaline phosphatase and in the organ weight. Histological findings included hepatocyte enlargement, deposition of eosinophilic and basophilic granules in the cytoplasm of the hepatocytes, and deposition of basophilic granules in the Kupffer cells. Except for the basophilic deposits all effects were reversible within a few weeks after cessation of treatment. The NOEL for hepatic changes in the rat was 50 mg/kg bw day, while no NOEL was established in the dog studies. However, treatment-related effects were marginal at the lowest dose employed (50 mg/kg bw day).
10. No data were provided concerning the tolerance of mecillinam in the bovine. In view of the intended use such documentation is not deemed necessary for the establishment of an ADI.
11. Reproductive studies (fertility, teratogenicity and peri-post natal development studies) with subcutaneous doses up to 550 mg/kg bw/day gave no indication of significant adverse effects on fertility, foetotoxicity/teratogenicity and on offspring of treated parents.
12. No evidence of mutagenic potential was observed in a non-GLP Ames test. No signs of mutagenic potential were found in a micronucleus test in mice treated with a single intravenous dose of 400 mg/kg. Since the *in vivo* test was negative and because no evidence of mutagenicity has been observed for other β-lactams antibiotics, no further mutagenicity studies are deemed necessary.
13. No carcinogenicity studies have been carried out. However, since the molecule does not contain any structural alert and no evidence of carcinogenic potential has been observed for other β-lactam antibiotics, no carcinogenicity studies are deemed necessary.
14. Data provided indicated that mecillinam is significantly less potent than benzylpenicillin for primary sensitizing potential and also with respect to the potential in eliciting allergic reaction in humans already sensitized to penicillin.

15. The geometric mean of MIC₅₀ for a representative selection of bacterial species of the normal human gut flora was 68.16 µg/ml at an inoculation density of 10⁹ CFU/ml. In order to take into account the range of MICs a one-tailed lower 10% confidence limit of the geometric mean was established as 24.30 µg/ml.
16. No significant inhibition was observed at concentrations up to 1.0 µg/ml on the activity of bacterial cultures commonly used in the dairy industry.
17. Mecillinam has been used therapeutically in humans since the late 1970s. The oral bioavailability is lower than or equal to 5%. Piv-mecillinam (the pivaloyloxy methyl ester of mecillinam), a prodrug of mecillinam with high oral bioavailability is available for oral administration. Piv-mecillinam, which is without antimicrobial activity, is quickly hydrolyzed following absorption. The recommended dosage of mecillinam to adults, for both oral and parenteral administration, is up to 800 mg 3 to 4 times daily, a maximum dose of 60 mg/kg per day is indicated in case of severe infections. A combination product containing piv-ampicillin and piv-mecillinam is also available. Commonly reported adverse reactions include gastrointestinal disturbance, vomiting, soft stools and diarrhoea. A less common adverse reaction is skin exanthema. Rarely occurring adverse reactions are muscle fatigue, esophagitis and urticaria. Adverse reactions in connection with intravenous administration of mecillinam appear to be rare.
18. Although no oral toxicity studies have been provided, the toxicity of mecillinam is expected to be lower after oral than after parenteral administration as the oral bioavailability is only 5% compared to the subcutaneous bioavailability of 100%. A LOEL can be set based on the results of the subcutaneous repeated-dose toxicity studies, where marginal increases in liver weights were observed in the low-dose group (50 mg/kg bw/day) in one 13-week dog study, thus leading to a high toxicological ADI in comparison with the derived microbiological ADI.
19. A microbiological ADI should be calculated based on the effect of the substance on the human gut flora. Since no suitable *in vivo* data are available, the ADI must be based on *in vitro* data.

For the assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP:

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

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

$$\text{ADI} = \frac{24.30 \times 1}{3} \times 150 = 23.8 \mu\text{g/kg bw i.e.} = 1428 \mu\text{g/person}$$

$$\frac{\quad}{0.85 \times 60}$$

The following assumptions have been made:

- the calculated geometric mean MIC of 24.30 µg/ml,
- CF1 = 3 to adjust for development of plasmid mediated resistance,

- CF2 = 1; since the MIC determinations were carried out employing a relatively dense *inoculum* no adjustment for differences between *in vitro* and *in vivo* growth conditions was considered necessary,
 - the fraction of an oral dose available for microorganisms has been set at 85%, based on the results of a study of the breakdown of mecillinam under simulated human gastrointestinal conditions.
20. Residue depletion of mecillinam in uterine contents, plasma and milk were studied after intrauterine administration of boluses containing mecillinam and cephapirin (1:1 w/w) after single treatment or after administration of two doses with an interval of 48 hours, to *postpartum* cows. Three dose levels of mecillinam were employed: 250, 500 and 1000 mg. Elimination half-life of mecillinam in uterine fluid was in the order of 1 to 2 hours following the first treatment and of 2 to 3 hours following the second treatment, which may be taken as an indication that the absorption capacity of the uterine wall decreases during the *postpartum* period. Mecillinam concentrations were below the limit of detection 24 to 48 hours after treatment. Plasma concentrations of mecillinam were generally low compared to the administered dosage, indicating limited absorption of the substance from the uterine cavity. Maximum plasma concentrations for mecillinam were obtained after 1 to 4 hours and were below the detection limit 6 to 12 hours after treatment. Mecillinam residues were detectable only occasionally in milk from the first milking following treatment (the 0 to 12 hour milk), in concentrations not exceeding 40 µg/l.
 21. Residue depletion of mecillinam in edible tissue was studied after a single intrauterine administration to 20 healthy cows within three days after calving of two boluses corresponding to a dose of 250 mg mecillinam and 250 mg cephapirin. Five cows were sacrificed at 3, 6, 24 and 48 hours after treatment. Residues of mecillinam were assayed in liver, kidney, muscle and fat by a method combining HPLC identification/separation with quantification by microbiological technique; the limit of quantification was 50 µg/kg. At 3 hours average concentration in kidney samples was 194 µg/kg (range 141 to 256 µg/kg). At 6 hours residues were detected only in one kidney sample (73 µg/kg). Residue contents in kidney samples collected at later time were below the limit of quantification. Two samples from liver collected at 3 hours contained detectable residues (62 and 108 µg/kg respectively); no residues were detected in samples collected at later time points. Residue contents were below the limit of quantification in all samples of muscle and fat.
 22. An analytical method is available for the determination of mecillinam in tissues and biological fluids. The method involves solid-phase extraction, identification of mecillinam-containing extract by HPLC with UV photo diode array detection, followed by quantification of the residues by a microbiologically based method. The method was validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community. The specificity of the method is considered acceptable. The method has been adequately described.
 23. Based on the results of the residue studies, in the worst-case situation of an animal slaughtered within 6 hours after treatment, the theoretical maximum daily intake of total residues was calculated to be 120 µg equivalent to 8% of the ADI.

Conclusions and recommendation

Having considered that:

- the systemic absorption of mecillinam following intra-uterine administration is limited and that absorbed mecillinam is rapidly excreted via the kidney,
- mecillinam is used infrequently in a small number of animals,
- animals are unlikely to be sent for slaughter immediately following treatment,
- potential residues of mecillinam in edible tissues following intra-uterine administration of mecillinam are unlikely to be of toxicological concern to the consumer,
- potential residue concentrations of mecillinam in milk from treated animals will be well below concentrations exhibiting significant effect on bacterial species used in the dairy industry;

the Committee concluded that there is no need to establish MRLs for mecillinam and recommends its inclusion into Annex II of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Mecillinam	Bovine	For intrauterine use only