COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ENROFLOXACIN

SUMMARY REPORT (1)

1. Enrofloxacin is a fluoroquinolone antibiotic which acts by inhibition of bacterial DNA-gyrase. It is administered to cattle, pigs, chickens and turkeys to control bacterial infections caused by sensitive organisms.

2. In rats, enrafloxacin was well absorbed after oral administration. It was rapidly excreted in the bile and urine, mostly as enrofloxacin and the metabolite ciprofloxacin, together with relatively small amounts of other metabolites. Ciprofloxacin was the only metabolite of significance which was found in the tissues and excreta of the species studied. Ciprofloxacin is widely used in human medicine and was reported to be well absorbed after oral administration to humans; peak plasma concentrations were achieved within 1-2 hours and the half-life was 3.5-4.5 hours. Humans excreted 30-50% of an oral dose of ciprofloxacin in the urine within 24 hours of administration and significant amounts were also excreted in faeces.

3. Enrofloxacin was of low acute toxicity. In a 13-week study in the rat in which up to 7500 mg/kg enrofloxacin was administered in the feed, the findings attributable to treatment included :

   - reduced bodyweight gain (in both sexes receiving the top dose),
   - caecal distension with no corresponding microscopic alterations (a common finding in rats given large doses of antibiotics),
   - histopathological changes in the knee joint (in 3 out of 30 animals receiving the top dose), and
   - in male rats receiving the top dose, dark, round or oval cells in the epididymides (12 out of 15 rats) and in the seminiferous tubules (5 out of 15 rats). These cells probably represented a degenerating or necrotic "cap phase" spermatid.

   The no-effect level for the study was 300 mg/kg (equivalent to 40 mg/kg bw).

4. A further study was carried out which incorporated various "recovery periods", in order to explain the testicular effects. In rats not included in a recovery phase, no abnormal spermatozoa were found at the lowest dose administered (125 mg/kg equivalent to 10 mg/kg bw per day). No abnormal spermatozoa were found in the seminiferous tubules of any animal at the end of the 90-day recovery phase and recovery of the epididymides was also apparent. This study indicated that the effects on the testes were reversible.

5. Similar effects on the male reproductive tract were observed in several other studies in rats including a multigeneration study in which reproductive performance was impaired at the top dose level of 7500 mg/kg but not at 500 mg/kg.

   However similar effects on spermatogenesis mere not observed in repeat dose studies in other species.

6. Degenerative changes, typical of those induced by fluoroquinolone antibiotics, were found in the articular cartilage after administration of enrofloxacin to immature animals such as calves, piglets and puppies. Dogs were the species most sensitive to this effect and a NOEL of 3 mg/kg bw per day was established for arthropathy in a 13-week repeat dose study in which the dogs were 3-months old at the start.

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Switchboard: (+44-171) 418 8400 Fax: (+44-171) 418 8447
E_Mail: mail@emea.eudra.org http://www.eudra.org/emea.html

7. Enrofloxacin was not teratogenic in either the rat or the chinchilla and NOELs for foetotoxicity of 50 and 25 mg/kg bw per day, respectively, were established.

8. Enrofloxacin produced no increase in revertants in an bacterial assay for gene mutation though only very low drug concentrations could be tested because of the high toxicity shown towards the tester strains of bacteria. Sporadic increases in mutant frequency were observed in a CHO HGPRT forward mutation assay but without any evidence of a dose-response and an in vitro UDS assay gave negative results. There was a small but dose-related increase in SCEs per metaphase in an in vivo SCE assay in hamster bone marrow but the increase was not statistically-significant and no evidence of mutagenicity was found in either an in vivo mouse micronucleus test nor in an in vivo cytogenetics assay in rat bone marrow.

9. In a rat carcinogenicity study, the incidence of endocardial tumours was elevated in females receiving the top dose. When the incidence of endocardial tumours was combined with the incidence of proliferative lesions, the difference from the controls was significant in both sexes receiving the top dose. Also in this study, the incidence of bile duct hyperplasia was increased in a dose-related manner. However an independent review of the histology slides indicated that both the endocardial neoplasms and the Schwann cell-like hyperplasia were not associated with administration of enrofloxacin. In a second carcinogenicity study in the same strain of rat, no neoplastic changes attributable to treatment with enrofloxacin were observed. However because evidence of bile duct hyperlasia was apparent at the lowest dose level tested, a third study was carried out; histopathological examinations after 12 months showed no evidence of a dose-related increase in bile duct hyperplasia at dose levels of up to 50 mg/kg in the diet. Although the incidence of malignant lymphoma was increased in treated groups in a carcinogenicity study in the mouse, the incidence was within the historical control range. It was concluded from these studies that enrofloxacin was not carcinogenic.

10. No data were provided on the effects of enrofloxacin in humans. The main metabolite, ciprofloxacin, has been widely used in human medicine for several years; the side effects were reported to include gastrointestinal disturbances, some hypersensitivity reactions and crystalluria. Ciprofloxacin is contra-indicated in young children and in lactating mothers because of the risk of arthropathy.

11. Data on the in vitro sensitivity of several species of human gastrointestinal flora indicated that E. coli was the most sensitive to enrofloxacin with a MIC range of 0.015-0.03 µg/ml. MIC values in the same numerical range were also obtained for the metabolite ciprofloxacin. An ADI of 0-0.3125 µg/kg bw per day for a 60 kg person (a maximum ADI of 18.75 µg/person) was derived, using the lowest MIC of 0.015 µg/ml corrected by a factor of 10 to obtain the concentration without effect on the human gut flora, and assuming :

    - a daily faecal bolus of 150 g,
    - a "safety factor" of 10, and
    - 12% for the quantity of the ingested amount which would be available to the bacteria at the distal part of the intestine.

12. Residue depletion in all tissues of all food producing species was very rapid. In pigs, residues in tissues consisted chiefly of enrofloxacin with smaller amounts of ciprofloxacin. 12 hours after withdrawal of the drug, 80-90% of the total residue present consisted of enrofloxacin + ciprofloxacin. In calves, residues consisted of almost equal amounts of enrofloxacin and ciprofloxacin. Residues in poultry also consisted of a mixture of the two compounds.

    The following MRLs were recommended:

Marker residue	Animal species	MRLs	Target tissues
sum of enrofloxacin + ciprofloxacin	Bovine, porcine, poultry	30 µg/kg	muscle, liver, kidney

    The MRLs recommended above would result in a daily maximum intake of 15 µg active ingredients based on a daily food intake of 500 g of combined tissue. This is within the ADI estimated in paragraph 11 (above).

13. An analytical method for the determination of both enrofloxacin and ciprofloxacin was developed, based on HPLC with fluorescence detection. A limit of determination of l0 µg/kg was achievable for both substances, for most edible tissues.