COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BROTIZOLAM

SUMMARY REPORT

1. Brotizolam is a triazolo-1,4-thienodiazepine and exerts antiemotional, anticonvulsant, muscle relaxant and sedative-hypnotic effects. brotizolam is used in human medicine as a hypnotic agent. In veterinary medicine Brotizolam is recommended for the treatment of anorexia and after surgery in cattle using a single intravenous dose of 2 µg/kg bw.

2. The central nervous system was identified as the main site of action of brotizolam but the cardiovascular and the respiratory system can be also affected. Electroencephalogram changes following i.v. treatment of rabbits were the most sensitive parameter observed for brotizolam. On this basis a pharmacological NOEL of 1 µg/kg bw (intravenous) was proposed by the applicant.

   In cats (intraperitoneal) and cattle, horse, and sheep (intravenous), an orexogenic activity was demonstrated following doses of 2 - 8 µg brotizolam/kg bw. Higher doses produce sedation, drowsiness and loss of motocoordination. The observed signs of restlessness in dogs, cats and monkeys can be explained by the cardiovascular effect of brotizolam.

   The metabolites of brotizolam were found to be less potent than parent compound.

3. The pharmacokinetic properties of Brotizolam were examined in the rat, mouse, dog, monkey, target species cattle and man.

   After oral dosing, brotizolam was rapidly absorbed in all examined species. Tmax in blood ranged from 0.25 h - 2h. Following oral or intravenous administration of [^{14}C]-Brotizolam to rats, dogs and monkeys, total radioactivity in blood declined in a biphasic manner with the half-lives of 0.4 h-1.3 h for the first phase and of 14.8 h-20.8 h for the terminal phase.

   Brotizolam was extensively metabolized in all examined species including humans. Metabolism occurred mainly via ring oxidation and oxidation of the methyl group. The main urinary metabolites in humans and monkeys following oral dosing are We 964 (hydroxylated methyl group) and We 1061 (hydroxylated diazepine ring), in dogs We 964 and We 1064 (rearranged We 1061). The main metabolites in rat bile were We 1061 and "Structure I" (hydroxylated phenyl ring), in mouse bile We 964 and We 1061. Almost all products are conjugated, however the site and type of conjugation were not clearly identified. After intravenous administration of brotizolam, the main metabolites found in excreta from rat and dog were We 964 and We 1061.

   Excretion is practically complete within a few days after treatment. In rat, faecal excretion was predominant while elimination in other species occurred via faeces and urine in approximately equal proportions.

   In rats, brotizolam and its metabolites crossed the placental barrier and were detectable in the foetus. Metabolites were also found in the milk.

4. Brotizolam showed low single dose toxicity in rats, mice, dogs, and primates following oral, intravenous or intraperitoneal administration. In mice and rats LD50 values were reported to be >10 000 mg/kg bw following oral, >1000 mg/kg bw following intraperitoneal and >20 mg/kg bw after intravenous treatment. Following oral application, LD50 values of >2000 mg/kg bw were found in dogs and of >4000 mg/kg bw in monkeys. The main clinical signs were sedation and sleep (sometimes preceded by restlessness, ataxia, tremor) caused by the central depressing activity of the agent.

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5. Repeated dose studies for periods of up to 18 months were carried out in rats (oral, up to 1000 mg/kg bw/day), dogs (intravenous, 0-0.3 mg/kg bw/day) and monkeys (oral, 0-100 mg/kg bw/day). The predominant findings - hyperactivity, aggressiveness (except for monkeys), voracity, sedation, ataxia, increased motility, and behavioural changes - were related to the central nervous effects of brotizolam. Only in an oral rat study a NOEL of 0.3 mg/kg bw could be established.

6. Fertility studies in rats (0.05-100 mg/kg bw/day, per gavage) showed no effect of brotizolam on reproduction.

7. Teratogenicity studies in rats (0.05-500 mg/kg bw/day, per gavage) revealed foetal variations and malformations in pups at maternotoxic doses. A NOEL of 2.5 mg Brotizolam/kg bw/day for maternal and foetal toxicity was derived.

   In rabbit teratogenicity studies (0.05-9 mg/kg bw/day, per gavage), Brotizolam showed no adverse effects upon foetuses even at doses leading to significant weight loss in females. A NOEL of 0.05 mg brotizolam/kg bw/day was established on the basis of maternal toxicity.

8. Peri- and postnatal studies in rats (0.05-400 mg/kg bw/day, per gavage) indicated an increased number of stillbirths. Increased pup deaths during the first days after parturition were mainly caused by poor mothering. Maternal effects also influenced the physical, functional and behavioural development of the pups. A NOEL of 0.05 mg brotizolam/kg bw/day was derived.

9. Brotizolam gave negative results in all submitted mutagenicity tests (Ames test, mitotic gene conversion assay, microbial gene mutation assay, HGPRT test, cell transformation assay, unscheduled DNA synthesis test, *in vivo* mammalian bone marrow cryogenetic test and dominant lethal test).

10. Carcinogenicity studies were conducted in mice (18 months duration) and rats (24 months duration) at doses of 0, 0.3, 10, 200 mg/kg bw. Fifty % of the male mice of the 10 and 200 mg/kg bw/day dose groups showed a dilatation of the urinary bladder probably caused by the muscle relaxing and sedative effect of the substance. No oncogenic effects were observed. Male rats showed nephritis, kidney necrosis, cystitis, prostate abscesses and seminal vesiculitis. In females increases in hyperplasia of the mammary gland and in ovarian cystic follicles, paw acanthosis and hyperkeratosis were observed. In the high dose group, a significant increase in thymic lymphomas and neurilemmomas of the uterus was found in females and an increase in adenomas of the thyroid in males. The occurrence of thyroid adenomas explained as due to the species specific physiology and histology of the rodent thyroid and is valued to have no relevance for human safety. The neurilemmomas in the uterus remained unexplained and the lymphoma were interpreted to a consequence of treatment induced stress and malnutrition.

11. Special studies were conducted in rats and humans to investigate the effect of brotizolam on thyroid hormone metabolism. In one rat study, blood levels of T_4 were found decreased and that of TSH were found increased beginning at oral doses of 200 mg/kg bw/day. After 13 weeks of treatment, an equilibrium of thyroid regulation was observed.

    Brotizolam had no effect on thyroid function in healthy volunteers given brotizolam at the therapeutic level (0.25 mg/day) for 14 days.

    In humans, the lowest psychoactive oral dose appeared to be approximately 0.1 mg/person/day corresponding to 1.7 µg/kg/bw/day.

12. The applicant applies for brotizolam to be included into Annex II of Council Regulation Nº. 2377/90/EEC. He proposes an ADI of 0-0.01 µg/kg bw/day based on a NOEL of 1 µg brotizolam/kg bw derived from electroencephalogram studies in rabbits and on a safety factor of 100.

13. Following both intravenous and intramuscular administration of [^{14}C]-brotizolam (2 or 10 µg/kg bw) to lactating cows plasma elimination occured rapidly. A biphasic plasma kinetic was observed for the recommended intravenous administration. Radioactivity was initially eliminated with a half-life of approximately 0.5-1.2 h, followed by a second phase of slower depletion after about 3 h post dose (half life ca. 5 h). After intramuscular administration peak plasma concentrations occured at 30 min post dose and the plasma half life was found to be 3 h.

14. Excretion of the dose in cows was rapid and occured predominantly via the faeces. Within the first day after intravenous administration of [^{14}C]-brotizolam more than 70 % of the dose was recoverd in the faeces and over 20% in the urine. Excretion was practically complete after 6 days.

15. Investigations on the metabolites of [^{14}C]-brotizolam in excreta of cows indicated that brotizolam was metabolised mainly by hydroxylation, and also by conjugation. It appeared from the metabolic profiles in urine, faeces and bile samples of intravenous dosed cows that the parent compound and WE 964 and WE 1061, which were two of the major metabolites in laboratory animals, were also present in the exctreta from the target animal. The information provided on tissues and milk metabolites was incomplete and did not allow definite conclusions on the identity and quantitative composition of the drug residues.

16. Distribution and depletion of total [^{14}C]-brotizolam tissue and milk residues was similar after intravenous and intramuscular treatment of cows. The highest residue concentrations and most prolonged were found in liver, followed by kidney. In the main total residue study cows were treated with the recommended single intravenous dose at 2 µg/kg b.w. At 6.5 h post dose the mean residue concentration was 3.54 µg/kg in liver, and 1.12 µg/kg in kidney; in muscle and fat the residue concentrations were below the limit of detection. At 24 h post dose only in liver quantifiable total residue concentrations (0.56 µg/kg) were detected. The highest total milk residues were observed at 7 h post dose (0.08 µg/l), and depleted to 0.02 µg/l at 23 h and were below the limit of quantitation at 31 h.

17. The mean total residue intake from edible tissues and milk from animals treated with the recommended intravenous dose was calculated to be 0.576 µg, 0.186 µg and 0.065 µg using the standard food consumption figures. This is below the proposed ADI of 0.6 µg/person/day. From this result brotizolam is considered as a substance for which MRLs are not required, and inclusion into Annex II is recommended.

18. Since inclusion of brotizolam into Annex II was applied for by the applicant a routine analytical method was not provided.

    As Brotizolam is intended for use in small numbers of animals, in view of its low toxicity in animals and the fact that the levels present as residues will not result in the ADI being exceeded, and because the substance is rapidly metabolised and excreted, it is recommended that it be placed into Annex II of Council Regulation (EEC) N° 2377/90 for cattle as the target species.