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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DENAVERINE HYDROCHLORIDE

SUMMARY REPORT

1. Denaverine hydrochloride is a benzilacid derivative (2,2-diphenyl-2-(2-ethylbutoxy)-acetic acid-2-(dimethylamino)-ethylester-hydrochloride). In veterinary medicine denaverine hydrochloride is used to regulate myometrial contractions during parturition. The substance is intended for single parenteral (intramuscular) use in heifers and cows at the dose level of 200 - 400 mg per animal (approximately 0.8 mg/kg bw), in sheep at the dose level of 80 - 120 mg per animal (approximately 0.8 mg/kg bw) and in pigs at the dose level of 200 mg per animal (approximately 1.2 mg/kg bw). The application can be repeated after approximately 60 minutes. Denaverine hydrochloride has been used in humans (including infants) at oral doses of 60 to 270 mg per person, at doses of 100 to 400 mg per rectum per person and parenterally at 40 to 160 mg per person.

2. Denaverine hydrochloride has a relaxant effect on the smooth muscle. It has a relaxing effect on the prepartum uterus and provides an increased flexibility of the soft birth canal. In addition, denaverine hydrochloride has a surface anaesthetic effect and an anticonvulsive effect as well as a slightly tranquilizing and antipyretic effect. After subcutaneous, intramuscular and intraperitoneal application of denaverine hydrochloride to pigs, cows and sheep onset of the effect was seen after 15 to 30 minutes. The muscle relaxant effect lasted up to several hours but the analgesic effect persisted no longer than 90 minutes. It also supports the action of oxytocin and shows a purely additive reaction with morphine. Denaverine has been shown to induce liver enzyme activity in rats.

3. In rats, after oral treatment, 33% of unchanged denaverine was detected in the urine within 24 hours. Only low levels of unchanged substance were traceable in the urine after 48 hours. There was no indication of accumulation of denaverine in the body. After oral treatment with denaverine twelve metabolites have been detected in urine from rats. The identified metabolites show that denaverine is metabolized by ester cleavage, oxidative O-dealkylation and N-dealkylation. Eight metabolites were identified by mass spectrometry: benzilic acid, 2,2-diphenyl-(2-dimethylaminoethyl)acetate, N-demethyl-1 and 3,3- diphenyl-morpholin-2-one~~,~~ diphenylacetic acid~~,~~ methyl- and ethyl-(2-(2-ethylbutoxy)-2,2-diphenyl)acetate and methylbenzilate. In rats benzilic acid and 3,3- diphenyl-morpholin-2-one represents the main metabolic products.

4. The acute toxicity of denaverine is low. The LD_{50} has been determined for different application routes in mice and rats. The LD_{50} in mice after administration by the oral route was 4425 mg/kg bw, by the intraperitoneal route 500 mg/kg bw, by the subcutaneous route 370 mg/kg bw and by the intravenous route 138 mg/kg bw. The LD_{50} in Sprague Dawley rats was, after oral treatment more than 4650 mg/kg bw, after intramuscular treatment more than 46.4 mg/kg bw and after intravenous treatment 32.3 mg/kg bw. Acute toxic effects showed an excitatory action in the form of clonic convulsions and terminally, tonic-tetanic convulsions.

5. Toxicity studies with repeated oral doses (100 to 1000 mg/kg bw) of denaverine for two weeks or subcutaneous injections (10 to 300 mg/kg bw) of denaverine for one week were conducted in rats. In addition to clinical signs of general toxicity the bodyweight gain and food consumption were dose dependently reduced in the treated groups. The major findings were dose dependent histopathological

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liver changes such as vacuolation of the hepatocytes, eosinophilic and enlarged centrolobular hepatocytes, dilated sinusoids, focal or diffuse dissociation of hepatocytes, small focal subcapsular necroses and small parenchymal necroses, in all the treated groups.

A 90-day repeated dose oral toxicity study in Wistar rats has been performed in accordance with current requirements. Groups of rats were treated daily by gavage with 0 (n=20 of each sex) 3 (n=15 of each sex), 20 (n=15 of each sex) and 100 (n= 20 of each sex) mg denaverine hydrochloride /kg bw for 90 days. In addition rats from the control group and the highest dose group were selected for a 28-day recovery period. Haematological parameters were not affected by treatment. At the two highest doses of denaverine hydrochloride a dose dependent hepatocytic degeneration in rats were recorded. This finding was supported by an increase in the liver enzyme activity and increase in the liver weights as well as the histological findings. The alterations were not reversible and were more severe and more frequent in male rats than in female rats. A NOEL of 3 mg/kg bw could be established.

6. Denaverine was studied for reproduction effects in a two-generation study in rats with dose-levels of 10, 33 or 100 mg/kg bw orally. The reproduction parameters were not affected either in the F0 or F1 generation. Furthermore, the embryo development (embryotoxicity) and postnatal development (foetal toxicity) was not affected by the treatment in the F1 generation. The highest dose-level had a clear toxic effect on the treated parental generation, similar to that seen in the subacute toxicity studies. The dose-level of 33 mg/kg bw resulted in a decrease in body weight gain and food consumption in both females and males but the liver changes occurred only in the male rats. Similar, but less pronounced toxic effects were also seen at the dose level 10 mg/kg bw. In this study the liver changes were both time- and dose-dependent since the males were dosed for a longer period (10 weeks before mating and during mating) than the females (2 weeks before mating, during mating, pregnancy and lactation).

7. The substance was tested in the Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1537 and TA1535 with and without metabolic activation. Denaverine hydrochloride was not mutagenic in this test system. Furthermore, an *in vitro* mammalian cytogenetic test was performed with human lymphocytes. No increase in the frequency of chromosomal aberrations was found in any of the test groups.

   Denaverine hydrochloride was also tested in the *in vitro* Mammalian Cell Mutation Test (Mouse Lymphoma L5178Y cells). Cultures with concentrations of 50 µg/ml, without metabolic activation, showed a statistically significant increase in mutation frequency in two of three test series. At this dose level there was a pronounced cell toxicity (stagnation or regression in cell density). No indication of mutagenicity was found at the dose 40 µg/ml or lower with or without metabolic activation.

   Denaverine hydrochloride was negative in the Mouse Micronucleus Test at intravenous doses of 50, 25 and 12.5 mg/kg bw. The two highest doses were clearly toxic to the animals. A statistically significant decrease in the percentage of polychromatic erythrocytes in bone marrow was obtained in all test-groups at both 24 and 48 hours after the last administration, indicating that the erythropoiesis was depressed.

   It could be concluded that denaverine hydrochloride is not mutagenic.

8. No carcinogenicity studies were provided.

9. A toxicological ADI of 0.03 mg/kg bw or 1.8 mg per person can be established from the presented studies. The NOEL of 3 mg/kg bw was based on the hepatotoxic effects and derived from the 90-day study in rats. Furthermore, a safety factor of 100 was used.

10. The anticonvulsant effect of denaverine was tested in humans for 4 months with 90 - 180 mg/day orally, and reduced the frequencies of convulsions. In other studies patients were treated with denaverine hydrochloride for pain of spastic and inflammatory origin in the urogenital region and the gastrointestinal tract, post-operative pain as well as birth pain, milder forms of spastic bronchitis and bronchial asthma in children, migraine and migrainoid headaches and as an antiepilepticum The effect was stronger in nonpregnant than in pregnant women. The incidence of side effects was low~~,~~ (3.3% of treated patients). After oral treatment, the only side effects shown in a few cases were drowsiness, giddiness, fits of perspiration and nausea. A NOEL for pharmacological effects in humans could not be established from these studies. However, pharmacologically effective doses in humans are higher than the calculated toxicological ADI.

11. The absorption of denaverine after oral treatment in the target species (cow, sheep and pig) was poor. A residue study was performed with cows in different lactation periods. Eight cows were treated with 400 mg denaverine hydrochloride intramuscularly twice with one hour interval. Four cows were slaughtered at 1 and 3 days and samples of muscle, lung, liver, kidney and fat were analysed. Denaverine hydrochloride and two metabolites (2,2-diphenyl-(2-dimethylaminoethyl)-acetate and benzilic acid) were analysed with an HPTLC method. The detection limits of denaverine hydrochloride, (2,2-diphenyl-(2-dimethylaminoethyl)acetate and benzilic acid in muscle were less than 0.13, 0.012 and 0.011 mg/kg, respectively, in liver less than 0.013, 0.060 and 0.011 mg/kg, respectively, in kidney less than 0.015, 0.033 and 0.015 mg/kg, respectively and in fat less than 0.031, 0.031 and 0.027 mg/kg , respectively. Residue levels were below the limit of detection in all tissues after 1 or 3 days after the last treatment. It is concluded that denaverine hydrochloride is excreted rapidly from the treated animals. No data on residues in milk were presented. However, it is unlikely that milk from treated cows will be used for human consumption directly after parturition.

    Since no data on pharmacokinetics and residue depletion have been provided for pigs and sheep no conclusions for these species can be given.

Conclusions and recommendation

Having considered that:

- denaverine hydrochloride is used in a small number of individual animals and for infrequent use, on parturient females only,
- treated animals are unlikely to be sent for slaughter immediately after treatment,
- denaverine hydrochloride is rapidly excreted in cows since no residues could be detected at 24 hours after the end of treatment,
- residues in animal products are not likely to be of a toxicological concern to humans;

the Committee considers that there is no need to establish an MRL for denaverine hydrochloride and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Denaverine hydrochloride	Bovine	