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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ISOXSUPRINE

SUMMARY REPORT

1. Isoxsuprine, a β-adrenoceptor agonist with antagonist activity at α-adrenoceptors, is used in veterinary and human medicine as a vasodilator and a relaxant of smooth muscle. In veterinary medicine, isoxsuprine is used as a peripheral vasodilator in horses (for oral administration at dosages of 0.45-2.4 mg isoxsuprine hydrochloride/kg bw/day for several weeks) and as an uterine muscle relaxant in horses, cows, pigs, sheep and goats (for intramuscular administration at dosages of 46.3-231.6 mg isoxsuprine lactate/animal).

2. Isoxsuprine is rapidly absorbed and distributed in humans, dogs and horses. After oral administration of isoxsuprine hydrochloride, serum peak levels were reached after 20-60 minutes in dogs, after 20-180 minutes in humans and after 8 hours in horses. After intramuscular administration of isoxsuprine hydrochloride serum peak levels were reached after 12-30 minutes in humans. No accumulation in plasma of horses occurred after long-term treatment. In humans, elimination half-lives were 1.7-2.9 hours after oral treatment and 2.1-2.4 hours after intramuscular treatment. Isoxsuprine is almost completely conjugated into its glucuronidate and sulphate conjugates. Excretion of isoxsuprine in humans, horses and dogs is rapid (with the major part excreted within the first 24 hours after treatment) and mainly via urine (± 70-80% in humans and dogs).

3. The pharmacodynamic activity of isoxsuprine was tested in humans and animals. The most relevant pharmacological (side) effects are on the cardiovascular system, followed by (therapeutic) effects as uterus relaxation and vasodilation in extremities.

   In dogs, a NOEL of 0.2 mg/kg bw was found, based on effects on heart rate. However, oral treatment of human patients with 40 mg isoxsuprine hydrochloride (0.67 mg/kg bw/day) during 16 weeks revealed no effects on heart rate and blood pressure. Based on these results it can be concluded that humans are less susceptible to the cardiovascular effects than dogs. In humans, vasodilation in the extremities is found at lower doses than cardiovascular effects: in patients with peripheral circulatory problems the LOEL for peripheral vasodilatory effects is 0.5 mg/kg bw/day.

4. Administered orally, isoxsuprine is moderately to slightly toxic in acute toxicity tests. For both rats and mice, the oral LD_{50}-values varied between 0.9 and greater than 6 g/kg bw, depending on age, strain and formulation used.

5. In a number of oral repeated-dose toxicity studies in rats (4 and 13 weeks, 14 months) and dogs (3 and 13 weeks, 12 months), only effects on blood parameters (decrease in haemoglobin concentration, packed cell volume and erythrocyte count) and slight vasodilation were found.

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The overall LOEL in the oral repeated-dose toxicity studies was 50 mg isoxsuprine/kg bw/day. No overall NOEL could be established.

6. In a 2-generation reproduction study in rats, in which only one isoxsuprine concentration (150 mg/kg bw/day) was tested, a decrease in bodyweight gain of the dams, a decreased incidence in pregnancy rate, and a decrease in mean number of pups per litter were found.

   In rat teratology studies doses of 100 mg/kg bw/day or more produced an increase in body weights of dams and in one study of pups. This effect was not found in teratology studies with mice and rabbits. Isoxsuprine was not teratogenic. The overall NOEL for maternal- and foetotoxicity was 20 mg/kg bw/day.

7. A number of mutagenicity tests (Ames test, micronucleus test, test for gene mutation *in vitro* and tests for chromosomal aberrations *in vitro* and *in vivo*) have been performed. There was no evidence for any gene mutations in the Ames test and mouse lymphoma assay. Isoxsuprine induced a dose dependent increase in chromosomal aberrations in CHO cells *in vitro*. However, the *in vivo* chromosomal aberration test in the bone marrow of rat (intraperitoneal) and the *in vivo* micronucleus test in mice (oral) indicated that the clastogenic potential was not expressed *in vivo*. Based on these results, isoxsuprine is not considered to be mutagenic. Therefore a carcinogenicity experiment was not deemed necessary.

8. Based on the overall toxicological NOEL of 20 mg/kg bw/day and a safety factor of 100, a toxicological ADI of 0.2 mg/kg bw can be established for isoxsuprine. However, since isoxsuprine is a substance of low oral toxicity, the pharmacological effects are most relevant for the safety evaluation of isoxsuprine. Based on the NOEL of 0.2 mg/kg bw for effects on heart rate in dogs, and applying a safety factor of 100, a pharmacological ADI of 0.002 mg/kg bw/day (equivalent to 0.12 mg/day for a 60 kg person) can be established for isoxsuprine. This ADI provides a 250-fold safety margin to the LOEL of 0.5 mg/kg bw/day for peripheral vasodilation in human patients with peripheral circulatory problems.

9. Residue data with horses have been provided after oral administration of the commercial product at dosages of 2.4 mg isoxsuprine hydrochloride/kg bw/day for 14, 28 or 65 days. Total isoxsuprine residues (= isoxsuprine + conjugates) were determined in liver, kidney, muscle and fat by means of HPLC (limit of quantification 0.01 mg/kg) after withdrawal times of 8 hours, 1, 3 and 7 days. In kidney, total isoxsuprine residues depleted from 0.21 mg/kg at 8 hours, via 0.075-0.165 mg/kg at 1 day and 0.02-0.05 mg/kg at 3 days, to below the limit of quantification at 7 days. In liver, only at 8 hours were residues found (0.22 mg/kg), thereafter total isoxsuprine residues were below the limit of quantification. In muscle and fat no residues could be detected at any time point.

10. Residue data with cattle have been provided after intramuscular administration of the commercial product at a dosage of 231.6 mg isoxsuprine lactate/animal, three times with intervals of 2 hours. Isoxsuprine residues were determined in liver, kidney, muscle, fat and injection site by means of HPLC (limit of quantification 100 µg/kg; limit of detection 50 µg/kg) after withdrawal times of 12 hours, 2, 5 and 10 days. At 12 hours, residues were found in kidneys of 2 out of 4 treated animals at concentrations of 117-126 µg/kg and at the injection site in one animal (3369 µg/kg), but were below the the quantification and detection limits in all other tissues sampled. At all other time points, no residues were detected in any tissue.

11. Milk residue data have been provided after intramuscular treatment of cattle with the commercial product at a dosage of 231.6 mg isoxsuprine lactate/animal, three times with intervals of 2 hours. Milk samples were taken twice daily up to 4 days after treatment and total

isoxsuprine residues were determined by means of HPLC (limit of quantification 100 µg/l). Only in 1 out of 8 samples from the first milking after treatment residues were found (129 µg/l). In all other milk samples residues were below the detection limit (50 µg/kg).

12. No data on pharmacokinetics and residue depletion have been provided for pigs, sheep and goats.

13. HPLC-methods are available for the determination of total isoxsuprine (= isoxsuprine + conjugates) in tissues of the horse (HPLC/MS) and tissues and milk of cattle (HPLC/UV). The limits of quantitation are 100 µg/kg in tissues and milk of cattle and 10 µg/kg in tissues of the horse.

Conclusions and recommendation

Having considered the criteria laid down by the Committee for inclusion of substances in Annex II of Council Regulation (EEC) No 2377/90, and in particular that:

- isoxsuprine is used for infrequent or non-regular treatment,
- the animals are unlikely to be sent for slaughter during or immediately after treatment,
- isoxsuprine is rapidly absorbed, distributed and excreted,
- total isoxsuprine residues in tissues and milk deplete to below the pharmacological ADI within 1-2 days;

the Committee considers that there is no need to establish an MRL for isoxsuprine and recommends for cattle and horses its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table. The data available for pigs, sheep and goats do not allow any conclusion for these species.

Pharmacologically active substance(s)	Animal species	Other provisions
Isoxsuprine	Bovine, equidae	