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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

LUPROSTIOL

SUMMARY REPORT

1. Luprostiol is a synthetic prostaglandin F2α analogue. It is used for control of oestrus or induction of parturition in cattle, sheep, goat, horse, and pig. It is usually administered as a single intramuscular injection. The therapeutic dosages used in the target species are between 3 mg and 15 mg per animal.

2. In cattle it is used in oestrus control, suboestrus, induction of abortion, induction of parturition, chronic endometritis, pyometra and mummified or macerated foetuses. In the horse it is used in the induction of "second heat" 20 days post partum, anoestrus, treatment following early foetal death and resorption, pyometra and endometritis and induction of parturition. In the sheep and goat it is used for the luteolytic effect in planned breeding. In pigs it is used in the induction of parturition.

3. Studies in which the main pharmacological effects of luprostiol are investigated demonstrate that the pharmacodynamic parameters of luprostiol are essentially the same as those of prostaglandin F2α: (a) the spasmogenic effect of luprostiol on the rat uterus is approximately 5 times that of F2α; (b) the luteolytic effect of luprostiol in rats, hamsters and heifers is approximately 10, 15 to 30 and 2 times that of prostaglandin F2α, respectively; (c) the fully effective abortive dose of luprostiol in rats is 10 mg/kg bw after oral administration and 1 mg/kg bw after subcutaneous administration; (d) luprostiol doses of 5 to 15 mg per animal given twice intramuscularly with a 10 to 12 day interval and luprostiol given 15 mg per animal intramuscularly are able to induce luteolysis in heifers and lactating cows under farm conditions; (e) the minimum effective luteolytic dose in heifers is between 5 and 15 mg/animal intramuscularly and in cows an intramuscular dose of 15 mg per animal will render satisfactory results under practical conditions; (f) in mares and ewes the fully effective dose is 3.75 to 7.5 mg of luprostiol intramuscularly per animal; (g) in pigs the maximum effect on partus synchronisation is observed at an intramuscular dose of 7.5 mg per animal.

4. The pharmacodynamic profile of luprostiol is qualitatively the same as that of prostaglandin F2α. The dose-effect relationship shows a comparatively stronger effect of luprostiol on luteolytic and spasmogenic effect. Compared to the natural component prostaglandin F2α, the luteolytic effect is more pronounced, while the other effects - with the exception of a spasmogenic effect on the uterus *in vitro* - are similar or less than those of prostaglandin F2α.

5. Pharmacokinetic studies have been performed in rats, lactating cows, horses, pigs, sheep and goats. All the kinetic studies have been performed with ^{35}S-luprostiol and concentrations were determined by radioactivity determination and expressed as ng equivalents luprostiol per ml. Radioactivity consists of unchanged luprostiol and of metabolites, with a limit of detection in plasma and milk below 1 ng/ml and a TLC technique to investigate metabolites.

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Tel: (+44-171) 418 84 00 Fax: (+44-171) 418 84 16

6. In rats after single doses of 0.5 mg/kg bw (intravenously, intramuscularly or orally) the pharmacokinetic profile shows a rapid absorption and bioavailability after intramuscular and oral administration (t_{max} of 15-30 minutes) compared to intravenous administration of 80-100%. The terminal half life of radioactivity (luprostiol and products) is 5-7.5 hours. Within 24 hours after administration the majority of the dose is excreted ($^2/_3$ via the kidney and $^1/_3$ via the intestinal tract). Luprostiol is largely metabolised; only a small fraction is excreted as unchanged drug (2-3 % in urine and faeces). The radioactivity was eliminated from the organs at approximately the same rate as from the plasma (autoradiograms demonstrated preferred locations of radioactivity in liver, kidney and intestinal tract and rapid excretion within 24 hours).

7. Plasma and milk kinetics were studied in 5 cows after intramuscular administration of 0.047 mgkg bw ^{35}S-luprostiol (approximately 15 mg per animal) in a two step study (11 days apart). In plasma a C_{max} of 35.58 (t_{max} = 20 minutes) and 31.96 (t_{max} = 15 minutes) was obtained in the first and second study, respectively, expressed as ng luprostiol equivalent/ml, and in milk, a C_{max} of 17.25 (t_{max} = 2 hours) and 14.37 (t_{max} = 3 hours) was obtained. At 24 hours, plasma concentrations were 0.443 ± 0.278 and 0.229 ± 0.058 (1st and 2nd study) and at 48 hours the values were 0.354 ± 0.216 and 0.247 ± 0.035 (1st and 2nd study) ng luprostiol equivalent/ml. In milk the values obtained were 0.328 ± 0.145 (0.371 ± 0.225 in the 2nd study) µg luprostiol equivalent/kg at 24 hours and 0.278 ± 0.303 (0.148 ± 0.035 in the 2nd study) µg luprostiol equivalent/kg at 48 hours. Several metabolites were detected by HPLC/MS in urine, liver and kidney. The major pathway of degradation (and inactivation) was found to be the β-oxidation of the carboxylic acid side chain.

8. Plasma kinetics, urinary excretion and metabolism was studied in two Shetland ponies after a single intramuscular dose of 0.025 mg/kg bw (^{35}S-luprostiol). The higher plasma levels were 23.7 and 16.7 ng luprostiol equivalent/ml and at 8 hours after administration 68.7% of the dose was excreted in the urine (50-60% of the radioactivity was attributable to the unchanged drug), while in plasma, 70-80% was attributable to the unchanged drug 24 hours after administration. Both in urine and plasma, a less polar metabolite was observed.

9. Plasma kinetics, urinary and faecal excretion and metabolism were studied in mini-pigs after an intramuscular administration of 0.05 mg of ^{35}S-luprostiol/kg bw with a C_{max} of 27 ng/ml at 30 minutes. Excretion in urine and faeces were 66.5% and 17.5% at 24 hours, and 67.7% and 21.3% at 48 hours after treatment respectively. Metabolites in plasma were, at 4 hours, 50-60% of a less polar metabolite and 20-30% of unchanged drug. After 8 hours, 40% was excreted in urine as unchanged drug and the remainder mostly as a less polar metabolite; in faeces no unchanged drug but mainly a less polar metabolite was excreted.

10. In sheep, pharmacokinetics were studied after a single intramuscular administration of 0.125 mg ^{35}S-luprostiol/kg bw in two animals. Plasma C_{max} was 87 ng/ml (radioactive luprostiol equivalent) at 30 minutes and 3.1 ng/ml at 8 hours (at 24 hours the value was below 1.3). In milk C_{max} was 54-82 µg/kg, with a t_{max} between 2 and 5 hours, and at 48 hours the milk concentration was 1.6 µg/kg. Mean values of urinary excretion were 72.7% at 24 hours, and at 8 hours 70-80% was in the form of unchanged drug. In plasma 70-80% was as unchanged drug with a less polar metabolite increasing in time within 24 hours after administration. In milk at 24 hours, 10% was unchanged and mainly consisted of a less polar metabolite, probably the same as in plasma.

11. In goats (2 to 6 animals), after a single intramuscular administration of 0.02 mg luprostiol/kg, plasma and milk kinetics, urine and faecal excretion were determined. C_{max} was 6.2-15.6 ng/ml radioactive luprostiol equivalent (t_{max} of 15 - 30 minutes) and at 8 hours plasma concentration was less than 0.8 to 1.3 ng/ml. In milk at 4 hours, the concentration was 3-8 µg/kg. Total excretion in the first 24 hours was 76% (70% of the less polar metabolite (A) and 20% for two other more polar metabolites). Urinary excretion was 69% and faecal 18%. The ratio of unchanged luprostiol to metabolite A in plasma was 3:2 (15 minutes) and 2:3 (2 hours).

12. In all species studied, absorption after intramuscular administration occurred rapidly. Distribution studies showed that luprostiol is not specifically retained in any organ, and it was rapidly excreted within 24 hours. Residues depletion in tissues was comparable to the decline in concentration observed in plasma. Luprostiol is rapidly metabolised, and although the metabolites were not identified structurally, chromatographic positions suggests that they are closely related to luprostiol. Excretion was 80-100% via the urine and faeces within 24 hours. In lactating animals (cows, sheep and goats) a very small fraction was excreted via the milk.

13. Single acute toxicity studies were performed in mice and rats after oral, subcutaneous, intramuscular, intraperitoneal and intravenous administration. From these studies it is concluded that intravenously the LD_{50} is 116 mg/kg bw for male rats and 130 mg/kg bw for female rats. Organ microscopy did not reveal abnormalities. The parenteral no effect level is 1 mg/kg (rat) to 3 mg/kg bw (mouse) and the oral NOEL is 75 mg/kg bw (rat) to 150 mg/kg bw (mouse).

14. Subchronic toxicity was studied in rats and pigs. In rats, a 2 week oral study of 10 mg luprostiol/kg/day did not show drug related effects. At 100 mg/kg/day a decrease in bodyweight was observed. Subcutaneous administration of 100 mg/kg/day for 2 weeks in rats caused a reduction in body weight and food consumption. From a 13 week oral toxicity study in rats a NOEL of 0.1 mg/kg/day can be established (doses of 3 and 100 mg/kg/day caused effects in blood forming organs, a slight elevated plasma urea-N and renal morphologic changes related with the plasma urea-N levels). All observed effects were reversed within a 5 week period. In pigs, after oral administration for 30 days, a NOEL of 0.1 mg/kg/day was established (doses of 1.5-30 mg/kg/day retarded growth, reduced food intake and animals showed polydipsia, polyuria and a tendency to have higher urea-N levels and morphologic renal changes). All effects were reversed within a 4 week period.

15. Tolerance in target species have been performed in cattle, horses, pigs and sheep. From these studies it can be concluded that luprostiol is well tolerated in the species for which it is intended, even at high dose levels. Local reactions at the site of injection were not seen in the studies, and have not been reported in field trials. Some reactions were noted in animals in the repeated toxicity testing, however, in these cases, the injections were repeated daily at high doses.

16. In a fertility study, including teratogenicity, in rats, male and female animals were treated orally (0, 0.1, 0.3 and 1.0 mg luprostiol/kg bw/day). The males were treated for 9 weeks before mating and during the mating period. The females were treated for 2 weeks prior to mating and throughout pregnancy and lactation. No adverse effects were noted on the condition, behaviour, body weight, mating performance and fertility of the treated animals. Females receiving the higher doses (0.3 and 1.0) delivered prematurely, which was not observed in the 0.1 group. Otherwise there were no differences between the treated and control animals. No teratogenicity effects were observed.

17. In an embryotoxicity study (including teratogenicity) in rabbits (6 or 12 animals per dose) luprostiol was administered orally from day 6 to 18 or day 10 to 18 of pregnancy (doses of 0, 0.01, 0.02, 0.03 and 0.3 mg/kg bw/day). No maternal toxicity was noted at any dose, and doses up to 0.02 mg/kg/day showed no embryotoxicity. A dose of 0.03 mg/kg bw/day clearly interrupted pregnancy, apparently at a very early stage, because no foetuses were noted although the animals had been pregnant and no abortion occurred. The oral NOEL is 0.02 mg/kg bw/day.

18. In the hamster abortion test, the abortive effect of luprostiol administered orally on day 6 of pregnancy was studied in groups of 9-10 animals. A dose of 0.025 mg/kg bw did not lead to abortion, while a dose of 0.80 mg/kg bw did result in abortion. This abortifacient effect of luprostiol is part of the pharmacological activity, as a prostaglandin F2α analogue, and does not have the adverse significance which might otherwise be attributed to it.

19. Two mutagenicity tests were carried out on luprostiol: an *in vitro* test (Ames) and an *in vivo* test (Micronucleus) in rats by the oral route (doses : 0, 0.1, 3 and 100 mg/kg bw/day). The conclusion from these studies showed that luprostiol has no mutagenic potential. Luprostiol belongs to the class of prostaglandins, without known or suspected carcinogenicity activity.

20. Immunotoxicity studies were not performed.

21. No specific studies were performed with luprostiol in humans.

22. Based on the NOEL of 0.02 mg/kg bw/day from the embryotoxicity study in rabbits and a safety factor of 100, an ADI of 0.2 µg/kg bw/day (12 µg/person) is established.

23. Depletion studies were performed in cows (n=5) after intramuscular administration of ^{35}S-luprostiol/kg (15-30 mg/animal) in milk, liver, kidney, fat and muscle at 12 hours, 1 day and 3 days after administration with values of 0.92 µg/kg (liver), 0.85 µg/kg (kidney) at day 3, and 0.04 µg/kg of radioactivity luprostiol equivalent at 48 hours in milk. In horses, pigs, sheep and goats the results were similar. From these studies it can be concluded that radioactivity is rapidly eliminated from milk and organs (no residues were observed in muscle and fat). Radioactivity was mainly localised in the excretory organs (liver and kidney).

Conclusions and recommendation

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

- luprostiol is a synthetic analogue of prostaglandin F2α, a naturally occurring prostaglandin,
- it is used in animals for oestrus control and parturition and therefore not intended to be used in animals slaughtered immediately after treatment,
- it is rapidly absorbed from the injection site, rapidly metabolised and excreted and is not specifically retained in any organ or tissue,

the Committee considers that there is no need to establish an MRL for luprostiol 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
Luprostiol	All mammalian species	