European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

CLOPROSTENOL AND R-CLOPROSTENOL
(Extension to goats)

SUMMARY REPORT (2)

1. Cloprostenol is a synthetic racemic analogue of prostaglandin F2α. Normally a racemic mixture of both the enantiomers R-cloprostenol and S-cloprostenol is obtained by the chemical synthesis. By means of a specially designed synthetic path and a chromatographic method, the enantiomer can be separated and pure R-cloprostenol can be isolated. Both cloprostenol and the pure R-cloprostenol are used in veterinary medicinal products. Cloprostenol acts as a luteolytic agent causing functional and morphological regression of the *corpus luteum* followed by return to oestrus and normal ovulation in cattle. It may also be used for the induction of parturition in pregnant cows, sows and mares. The usual therapeutic doses for cloprostenol are 500 µg in cattle, 175 µg in pigs and 125 to 500 µg in *Equidae*. However, for R-cloprostenol, the following dosages are recommended: 150 µg in cattle and 75 µg in pigs. In all cases, the product is administered by the intramuscular route.

   Cloprostenol and R-cloprostenol are currently entered into Annex II of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Cloprostenol	Bovine, porcine, equidae	
R-Cloprostenol	Bovine, porcine, equidae	

2. A request was submitted to the EMEA for the extension of the existing entry in Annex II of Council Regulation (EEC) No. 2377/90 to caprine species. The scientific justification for this extension was assessed in accordance with the Position Paper Regarding Availability of Veterinary Medicines - Extrapolation of MRLs (EMEA/CVMP/457/03-FINAL) and taking into account the Notes for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL) and on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL)

3. In setting the ADI in the original assessment of cloprostenol, the following data were considered:

   In rats, after subcutaneous administration of doses ranging from 20 to 200 µg/kg bw of cloprostenol, 60 % of the administered radioactivity was recovered in urine and 14 % in faeces within 48 hours. Excretion was complete in 7 days.

   Urinary major metabolites were identified by GC-MS: tetranor acid of 9-keto-cloprostenol, δ-lactone of the tetranor acid of cloprostenol. Unchanged cloprostenol (less than 10 %) and an acid labile conjugate were minor components. No precise information was given about the relative percentage of metabolites.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 47
E-mail: mail@emea.eu.int http://www.emea.eu.int

In marmosets, 55 % of the administered radioactivity was recovered in urine and 16 % in faeces within 72 hours after subcutaneous administration of 100 µg of ^{14}C-cloprostenol per kg bw. Two major urinary metabolites were identified as unchanged cloprostenol (approximately 40 %) and its dinor acid (approximately 57 %) formed by one stage of β-oxidation of the parent compound.

4. The acute toxicity of cloprostenol and R-cloprostenol is low. In rats no LD_{50} values could be established after the intravenous, subcutaneous or intramuscular administration of racemic cloprostenol. Parenteral LD_{50} values were greater than 5 mg/kg bw. The oral LD_{50} values were higher than 25 mg/kg bw. The LD_{50} of R-cloprostenol was higher than 50 mg/kg bw by subcutaneous or intramuscular routes.

   In mice, the LD_{50} value of R-cloprostenol after intramuscular administration was close to 350 mg/kg bw. In this test, the acute toxicity of R-cloprostenol was similar to that of racemic cloprostenol.

5. After subcutaneous administration of cloprostenol at doses of 0, 12.5, 25, 50 µg/kg bw/day for one month in rats, vacuolisation of the luteal cells of the corpora lutea was the only significant consistent drug-related change observed for all the doses tested. This effect was reversible one month after the end of the treatment.

6. In the 3-month oral toxicity study carried out in rats (0, 10, 50, 150 µg/kg bw/day of cloprostenol), 50 µg/kg bw was the NOEL, ovarian vacuolisation being observed at the highest dose.

   In the 3-month oral repeated study in marmosets (0, 10, 50, 150 µg/kg bw and 0, 10, 20, 50 µg/kg bw/day of cloprostenol), an induction of myocardial changes and a statistical increase in testicular weights were reported at 150 µg/kg bw/day. A NOEL of 50 µg/kg bw/day could be retained.

7. In cattle, 200 times the dose of cloprostenol sodium caused only mild and transient scouring.

   In heifers, no adverse effects were noted after two intramuscular administrations, 11 days apart, of R-cloprostenol (as the sodium salt) at the recommended dose (150 µg) or at a ten-fold dose (1500 µg).

   In sows, no adverse effects of R-cloprostenol (as the sodium salt) were reported after single intramuscular administration at the recommended dose (75 µg), at five-fold dose (225 µg) or at ten-fold dose (750 µg).

8. In a 3 generation study carried out in rats, the oral administration of doses of 0, 10, 15, 20 and 40 µg of cloprostenol/kg bw did not induce effects on reproductive performance of the animals. The only effects seen were the slight reduction in neonatal viability attributable to the prematurity of the offspring. A NOEL of 15 µg/kg bw/day for cloprostenol was retained.

   In a series of reproductive studies, it was shown that the sensitivity of the rat to termination of pregnancy resulting from luteolysis varies according to the point in pregnancy when the compound is administered. The dose 25 µg/kg bw of cloprostenol did not terminate pregnancy.

9. No teratogenic properties of cloprostenol were reported in the two teratogenicity studies performed either in rats after oral administration of 0, 10, 25, 50 and 100 µg/kg bw/day or in rabbits after subcutaneous administration of 0, 0.025, 0.075 and 0.250 µg/kg bw/day.

10. R-Cloprostenol was devoid of mutagenic activity in two in vitro tests (Ames test and mouse lymphoma L5178Y/TK+/-). In another in vitro test (chromosome aberration assay in human lymphocytes), chromosomal aberrations were observed only at very high concentrations (2320 µg/ml). However, as this compound gave negative results in the in vivo bone marrow micronucleus test by the intraperitoneal route in mice, it can be concluded that the data provided give adequate assurance that R-cloprostenol is not genotoxic.

11. Because of its sensitivity and physiological relevance, the NOEL of 15 µg/kg bw/day obtained in the 3-generation study in rat is used for the calculation of the ADI. Using a safety factor of 100 to allow for species and individual variations in sensitivity and an additional safety factor of 2 to take into account that this study was carried out with the racemic compound, an ADI of 0.075 µg/kg bw/day (4.5 µg/person/day) was derived.

12. For the extension to include caprine species in Annex II the following information was taken into account:

13. In pigs, after a single intramuscular administration of ^{14}C-cloprostenol (acid in the form of sodium salt) at a dose of 200 µg, the highest plasma level of radioactivity (0.70 ± 0.14 µg/l) was measured at 1 hour after dosing. At 24 hours post dosing, the levels were close to 0.04 µg/l.

    Fifty percent of the dose administered was recovered either via urine or faeces. The major urinary metabolites were: the parent compound (approximately 10-14 %), the tetranor acid metabolite (approximately 37 %) and polar compounds (26-32 %).

    After intramuscular administration of 75 µg of R-cloprostenol to sows, the maximum concentration of R-cloprostenol in plasma was close to 2 µg/l and occurred between 30 and 80 minutes after injection. The half-life of elimination $T_{1/2\beta}$ was estimated to be 3 h 10 min.

14. In dairy cows, after a single intramuscular injection of 500 µg of free acid ^{14}C-cloprostenol (specific activity 122 µCi/mg free acid), the highest plasma level (0.43 ± 0.043 µg free acid equivalent/l) was reached within 30 minutes after dosing. The concentrations were lower than 0.01 µg free acid equivalents/l at 24 hours post dosing. The $T_{1/2}\beta$ was 3 hours.

    The recovery of ^{14}C in urine (52.5 ± 4.8 %) was achieved by 16 hours.

    Cloprostenol was extensively metabolised in the cow by β-oxidation to give the tetranor acid of cloprostenol, isolated as δ-lactone and as glucuronide conjugates (44 %). The parent compound represents 18 % of the radioactivity excreted.

    After intramuscular administration of 150 µg of R-cloprostenol/cow, the highest plasma concentration of R-cloprostenol was found at 90 minutes after injection (approximately 1.4 µg/l). The half-life of elimination $T_{1/2}\beta$ was estimated to be 1 h 37 min.

15. Residue depletion studies were available in pigs and cattle. In pigs, 30 minutes after intramuscular administration of 200 µg ^{14}C-cloprostenol (acid in the form of sodium salt), the highest concentrations of radioactivity were detected at the injection site and in kidney (43.98 ± 6.90 and 19.00 ± 4.13 µg cloprostenol equivalent/kg respectively).

    At 24 hours post injection, the amounts were lower than the limit of quantification (0.04 µg cloprostenol equivalent/kg) in muscle and in fat. The residues in liver and kidney were of the same magnitude (close to 0.10 µg cloprostenol equivalent/kg). At the injection site the levels were still relatively high (0.83 ± 0.53 µg cloprostenol equivalent/kg).

16. A first tissue depletion study was carried out in cows dosed with 500 µg of free acid ^{14}C-cloprostenol (122 µCi/mg free acid) by single intramuscular injection.

    At 30 minutes post injection, the residues of cloprostenol could only be detected in kidney (19.10 ± 2.730 µg cloprostenol equivalent/kg), in liver (7.25 ± 0.641 µg cloprostenol equivalent/kg)) and at the injection site (162 ± 19.10 µg cloprostenol equivalent/kg).

    At 24 hours post dose, the amounts were much lower: 0.123 ± 0.019 µg cloprostenol equivalent/kg in kidney, 0.036 ± 0.010 µg cloprostenol equivalent/kg in liver and 0.493 ± 0.198 µg cloprostenol equivalent/kg at the injection site.

    No residues were detectable after 48 hours postadministration.

    A second depletion study, carried out with unlabelled compound at the same dose, showed that all the residues were below the limit of quantification of the radioimmunoassay method used at 24 hours post administration (0.3 or 0.5 µg cloprostenol/kg according to the tissue).

    In a third non-radiometric study, at 16 hours after a single administration of 150 µg of R-cloprostenol to cows, 0.092 ± 0.063 µg R-cloprostenol/kg were measured at the injection site, 0.051 ± 0.003 µg R-cloprostenol/kg in liver and 0.120 ± 0.021 µg R-cloprostenol/kg in kidney. Only traces of R-cloprostenol were noted in muscle.

17. Less than 1 % of radioactivity administered was eliminated via cow's milk.

After an intramuscular dose of 500 µg ^{14}C-cloprostenol (specific activity 86.35 µCi/mg acid in the form of sodium salt - 22 µCi per animal) to cows, the highest levels, corresponding to 4 µg free acid equivalents/l, were found in samples collected over the 0-4 hour period. By 24 hours after dosing, levels had fallen below 0.012 µg/l.

In a second depletion study carried out with unlabelled compound no detectable cloprostenol residues (less than 0.1 µg cloprostenol/l) were found at nine hours after dosing or in subsequent samples after single intramuscular dose of 500 µg cloprostenol.

18. In cows, the ratios of parent compound to total residues were estimated from tissue samples collected at 30 minutes after intramuscular administration of 500 µg ^{14}C-cloprostenol. 90 % of the radioactivity could be extracted. The parent compound represented 85 % of total residues in muscle, 35.5 % in kidney and liver. In liver and kidney, the percentage of cloprostenol metabolites, lactone and its tetranor acid, was close to 20 %. In milk, the ratio of cloprostenol to total radioactivity ranged from 65 % (at 0 to 4 hours) to 16 % (24 hours post dosing).

19. Twenty-four hours after treatment tissue residues in cattle and pigs are generally only present at the injection site and to a lesser extent in the liver and kidney. Total radioactivity in cow's milk was less than 1% of the administered dose. The maximum intake of total residues that might be ingested from animals slaughtered 24 hours after treatment would be 6.7 % of the ADI for pig meat (including injection site) plus cow's milk and 4.8 % for cattle meat and milk. These values decrease to below 1 % without the injection site. The available pharmacokinetic and residues depletion data do not indicate any significant variability between the mammalian species that have been investigated, therefore, any possible difference in pharmacokinetics in goats, including goat's milk, would not be expected to have a significant impact on this percentage.

Conclusions and recommendation

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

- an ADI of 0.075 µg/kg bw/day (i.e 4.5 µg/person/day) was previously established for cloprostenol
- animals are unlikely to be sent for slaughter immediately after treatment,
- both R-cloprostenol and cloprostenol are eliminated rapidly in bovine and porcine species,
- the extensive metabolism results in products without pharmacological activity,
- by 24 hours after treatment, the maximum amount of total residues which might be ingested from pig or cattle meat and cows milk amounts to less than 7 % (including 300 g of the injection site) and less than 1 % (without the injection site) of the ADI for cloprostenol. The amount of total residues which might be ingested from caprine tissues and milk is considered to be similar,
- cloprostenol and R-cloprostenol are already included in Annex II of Council Regulation (EEC) No. 2377/90, as amended, for bovine and porcine species and *Equidae*,
- cloprostenol and R-cloprostenol fulfil the criteria laid down for the extension of the Annex II entry to caprines as minor species;

the Committee for Veterinary Medicinal Products recommends the inclusion of cloprostenol and R-cloprostenol in Annex II of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Cloprostenol	Caprine	
R-Cloprostenol	Caprine	