



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### PREDNISOLONE

(as free alcohol)

### SUMMARY REPORT

1. Prednisolone is a synthetic glucocorticosteroid. In veterinary medicine, prednisolone (as the free alcohol) is included as an ingredient in a number of antibiotic preparations, which are indicated for intramammary administration for the treatment of bovine mastitis. The usual dose corresponds to 10 mg prednisolone per infected quarter. Treatment may be repeated at 12-hour intervals for a maximum of 3 treatments.

Prednisolone is also used in human medicine, as the free alcohol and as various 21-ester derivatives, including the acetate, hexanoate, pivalate, sulfobenzoate, succinate and phosphate.

2. The pharmacological activity of prednisolone lasts longer than that of hydrocortisone but less than that of the longer-acting glucocorticoids such as dexamethasone. The gluconeogenic potency of prednisolone is equivalent to 400% of that of hydrocortisone but only around 13% of that of dexamethasone. It has very limited mineralocorticoid activity. Inhibitory concentration (IC)<sub>50</sub> values were determined for a number of pharmacodynamic parameters in humans. The lowest IC<sub>50</sub> value was obtained for cortisol suppression: 10.26 ± 3.83 ng/ml, corresponding to a dose of around 2160 µg/day. A special study was carried out in which groups of Wistar rats were given oral doses of prednisolone in the range 10 to 100 µg/kg bw. The rats were killed 2, 3 or 4 hours after dosing and liver samples were taken for determination of tyrosine aminotransferase activity. A dose-related increase in tyrosine aminotransferase activity was observed at 40 µg/kg bw/day and above. The NOEL was 20 µg/kg bw/day.
3. Pharmacokinetic studies in different species showed that prednisolone sodium phosphate, prednisolone succinate and prednisolone acetate were rapidly converted *in vivo* to prednisolone and that prednisolone was responsible for the pharmacological activity. Similar data for prednisolone-21-(3-sulfobenzoate) sodium were not provided.

Pharmacokinetic data in cattle were available for intramammary administration of the free alcohol only. Lactating cows were given intramammary infusions of a commercial product containing prednisolone into 2 quarters, equivalent to 11 mg prednisolone per quarter. The treatment was repeated 24 hours later. Plasma samples were taken from the cows and analysed using HPLC. Peak plasma concentrations of prednisolone were found 1 to 2 hours after treatment and were in the range 23.2 to 40.2 µg/l. Only traces of the inert metabolite prednisone were found 1 to 4 hours after treatment (up to 3.26 µg/l). Within 12 hours of the first infusion, 2.35 to 4.56% of the administered dose was excreted in the urine as prednisolone and 0.26 to 0.46% as prednisone.

For horses, pharmacokinetic data were provided following intramuscular and intravenous administration of prednisolone-21-succinate sodium and prednisolone-acetate. The dose was 0.6 mg/kg bw, expressed as the alcohol. Both substances were well, though slowly, absorbed with bioavailability in the region of 100%. In another study, horses received oral doses in the range of 0.5 to 2.1 mg prednisolone/kg bw in the feed and were also given intramuscular doses in the range of 0.2 to 0.4 mg/kg bw. Excretion of prednisolone was complete within 3 days, regardless of the route of administration. Urine samples contained approximately equal amounts of prednisolone, prednisone, 20 $\beta$ -dihydroprednisolone and 20 $\beta$ -dihydroprednisone.

In humans, the pharmacokinetics of prednisolone showed diurnal variation, which was related to the concentrations of endogenous hydrocortisone. Mean peak plasma concentrations of 0.466  $\mu$ g/ml prednisolone were obtained around 1 to 2 hours after oral administration of 40 mg prednisolone in the morning. Oral bioavailability was dose-dependent and showed considerable individual variations; bioavailability ranged from 60% to 92% for a 10 mg tablet. Prednisolone was bound to plasma proteins, mostly to the corticosteroid binding globulin, transcortin, and a smaller percentage to albumin; the extent of the binding was concentration-dependent. Prednisolone competed with endogenous hydrocortisone for the binding site.

Prednisolone was widely distributed to the tissues and crossed the placenta. Of an oral dose of 5 mg <sup>3</sup>H-prednisolone, 0.14% was recovered per litre of breast milk during the following 48 to 61 hours. Following oral or parenteral dosing with <sup>14</sup>C-prednisolone, more than 90% of the administered dose was excreted in urine within 48 hours of treatment; only 1 to 2% of the dose was recovered from faeces.

In mammals, there is interconversion of prednisone and prednisolone. Residues of both steroids were found after oral or parenteral administration of either. In humans, the interconversion favoured prednisolone with prednisolone concentrations higher by 4 to 10-fold over those of prednisone. After intravenous administration of 0.8 mg prednisolone/kg bw to humans, 30% of the material in urine was prednisolone and 2.5% was prednisone. In males and females, 8% and 5% of the material in urine was 6 $\beta$ -prednisolone, regardless of dose or route of administration.

Published metabolism data for humans, including some studies with <sup>3</sup>H-prednisolone and some limited metabolism data for horses, cattle, rats and rabbits indicated partial metabolism of prednisolone to the biologically inert substance prednisone. In humans, a number of other metabolites were also identified; on structural grounds these were not expected to possess corticosteroid activity.

4. The acute oral LD<sub>50</sub> of prednisolone was 1680 mg/kg bw in male and female Swiss mice.  
After subcutaneous injection of prednisolone to male Sherman rats, deaths were delayed for at least 10 days after dosing; the LD<sub>50</sub> value following a 21-day observation period was 147 mg/kg bw. The acute intraperitoneal LD<sub>50</sub> of prednisolone in mice was reported to be 767 mg/kg bw but the acute intraperitoneal LD<sub>50</sub> of prednisolone acetate was greater than 1000 mg/kg bw in the same species.
5. Two repeated dose toxicity studies were carried out in Sprague-Dawley rats using oral (gavage) doses of 0, 0.6, 2 and 6 mg/kg bw/day for 63 days and the same dose levels for 151 days. The main findings were reduced body weight gain and food consumption, reduced leukocyte counts, and decreased thymic, spleen and adrenal weights. Slight to moderate myeloid depletion of the bone marrow in rats given 6 mg/kg bw was the only significant pathological finding. The studies were carried out during the late 1950s according to the standards of the day. No NOELs were established due to the adverse effect on bodyweight gain and reductions in some organ weights at the lowest dose.
6. Groups of Mongrel dogs (2 animals per sex and dose) were given daily oral doses of 0, 2.5 or 5 mg/kg bw per day for 6 weeks. Bodyweight gain was reduced at 5 mg/kg bw. There were no significant effects on haematology or clinical chemistry values. There were dose-related increases in urine volume and mean urine sodium and potassium concentrations and a corresponding reduction in urinary specific gravity. At necropsy, glycogen deposition in the liver and adrenal cortical atrophy were found in treated dogs. A NOEL was not established due to the effects on urinalysis at the lowest dose.

7. Repeated dose toxicity studies were also carried out in rabbits (intramuscular doses of 0.5 to 2.5 mg prednisolone acetate/kg bw, for up to 22 doses) and guinea pigs (intramuscular doses of 2.2 mg prednisolone/kg bw for up to 8 doses and oral doses of 0, 1, or 10 mg prednisolone/kg bw administered in the drinking water or 0, 10, or 100 mg/kg bw administered in the feed for 24 weeks). No NOELs were determined in any of these studies. Hepatotoxicity was observed at the lowest dose tested in rabbits and was dose-related in severity; rabbits appeared to be the species most susceptible to this effect. In guinea pigs the main effects were reduced body weight gain (apparent at the lowest dose of 1 mg/kg bw), increases in haematocrit and haemoglobin values and decreased bone mineral concentrations.
8. Formulations based on prednisolone were well tolerated in the target species, when administered according to the recommended dose regimes.
9. Groups of 24 male Sprague-Dawley rats were given daily subcutaneous doses of 0, 0.04, 0.2 or 1 mg prednisolone farnesylate/kg bw/day for 63 days prior to mating. Groups of 24 females received the same doses for 14 days prior to mating. Treatment continued up to day 7 of gestation. The females were killed on day 20 of gestation and the uterine contents examined. Signs of toxicity such as fur loss or thin fur were observed in males given 0.2 mg/kg bw and above and females given 1 mg/kg bw. Atrophy of the thymus was observed at 0.2 mg/kg bw and above. Body weight gain and food consumption were reduced in both sexes given 1 mg/kg bw. There were no effects on oestrus cycle, male or female fertility, numbers of corpora lutea, implants, implantation loss, foetal weight or foetal sex ratio. There were no substance-related effects on the incidence of foetal malformations or variations. The NOEL was 0.04 mg/kg bw/day.
10. A study was carried out to investigate the induction of cleft palate in rabbits. Few other parameters were reported and the study did not comply with current guidelines for group sizes and period of dosing. Intramuscular injections of 1.5 to 8 mg prednisolone/day from days 13 to 16 of gestation induced cleft palate. No cases of cleft palate were observed in the foetuses from dams receiving 1 mg/day, equivalent to approximately 360 µg/kg bw/day. In a poorly conducted study in hamsters, a single intramuscular administration of 7 to 20 mg prednisolone/kg bw on day 11 of gestation induced a dose-related incidence of cleft palate together with reductions in the numbers of live foetuses and reduced foetal weights. No effects were observed at the lower dose of 5 mg/kg bw.

In a study in Sprague-Dawley rats, animals were given subcutaneous injections of various corticosteroids, including prednisolone which was administered at doses of 0, 12.5, 25, 50 or 100 mg/kg bw/day on days 14 and 15 of gestation. The numbers of foetuses with cleft palate and palatal slit were significantly increased in the groups given 100 and 50 mg prednisolone/kg bw respectively. Effective doses (ED)<sub>50</sub> values for producing palatal slits and cleft palate were calculated for a range of corticosteroids and it was shown that the ranking followed a similar pattern to the ranking of therapeutic activity. It was shown that prednisolone possessed only 5% of the potential of dexamethasone to induce palatal slit.

In further study, groups of 27 to 29 pregnant female Sprague-Dawley rats were given daily oral doses of 0, 3, 30 or 100 mg prednisolone/kg bw/day from days 6 to 15 of gestation and 2 further groups were administered 200 mg prednisolone/kg bw/day. 200 mg/kg bw caused severe maternal toxicity. Doses of 30 mg/kg bw/day and above caused increased embryoletality and reduced foetal weight. Two (out of 344) foetuses in the 30 mg/kg bw group were malformed; one had cleft palate, the other omphalocele. The NOEL was 3 mg/kg bw/day.

Teratogenicity was also investigated in a study over 2 generations in Sprague-Dawley rats. Groups of mated females were given daily subcutaneous doses of 0, 1, 5 or 25 mg/kg bw/day of prednisolone farnesylate from days 7 to 17 of gestation. From each group 26 or 27 dams were killed on day 20 of gestation and the uterine contents examined. There was a dose-related reduction in maternal body weight gain and food consumption. There was no evidence of teratogenicity or foetotoxicity. The remaining 14 or 15 dams from each group were allowed to litter naturally and rear the offspring. The weanlings were monitored for developmental landmarks and behavioural tests such as righting, bar-holding, open field behaviour and water maze learning were carried out. There were no substance-related effects on the survival or development of the offspring and no effects on mating and pregnancy outcome following mating of the F<sub>1</sub> generation.

As a group the corticosteroids have not been associated with congenital malformations in humans. Administration of prednisolone during 200 human pregnancies resulted in normal offspring. Normal physical and mental development was reported for 83 children aged 6 years whose mothers had been treated during pregnancy with prednisolone. The mothers had received 30 mg prednisolone/day for 3 days.

Studies in mice, rabbits, hamster and rats showed that prednisolone caused malformations including cleft palate when administered parenterally. The teratogenic potential was much less when administered orally and a NOEL of 3 mg/kg bw/day was established in rats following oral dosing.

11. No mutagenic effect of prednisolone was observed in an *in vitro* mouse lymphoma assay for gene mutation in both presence and absence of metabolic activation. Prednisolone gave positive results in the presence of metabolic activation in a rapid screening assay based on the measurement of the proportion of single to double-stranded DNA by alkaline unwinding and hydroxyapatite elution in mouse lymphoma cells treated *in vitro*. No increase in the frequency of sister chromatid exchanges (SCE) was observed when peripheral lymphocytes from 4 healthy humans and 4 patients with cancer were incubated with prednisolone for 74 hours. Negative results were also obtained in a cytogenetic assay in peripheral lymphocytes taken from 9 human patients treated with 3 mg prednisolone/kg bw for 3 months followed by 0.5 to 1 mg prednisolone/kg bw for up to 120 months. Prednisone, the precursor of prednisolone, gave negative results in an *in vitro* assay for gene mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, in both presence and absence of metabolic activation. Negative results were also obtained in an *in vivo* assay for chromosomal aberrations in rat bone marrow cells, following administration of doses up to 800 mg prednisone/kg bw (route not specified). It was concluded that prednisolone was not genotoxic.
12. A carcinogenicity study was carried out to compare the potential carcinogenicity of prednimustine (the prednisolone ester of chlorambucil) with equivalent doses of its single moieties (chlorambucil and prednisolone) and the unlinked mixture of these 2 substances. The study used 30 female Sprague-Dawley rats per dose and treatment group, the animals were treated for a period of 18 months. The doses of unlinked prednisolone used were 0 (untreated controls), 9 times 3 mg/kg bw, 4.5 times 3 mg/kg bw, twice 3 mg/kg bw and once 3 mg/kg bw per month. As expected, administration of prednimustine was associated with an increase in tumours of the auditory canal and administration of chlorambucil was associated with an increase in 4 different tumour types. Administration of prednisolone alone did not increase the incidence of any tumour type.

13. In a special study to investigate the immunotoxicity of prednisolone. groups of 4 Beagle dogs were given oral doses of 0, 1 or 10 mg prednisolone/kg bw for a 3-week period (twice per day for the 1st week, once per day for the 2nd week and on alternate days during the 3rd week). The dogs were vaccinated on day 20 and challenged with virulent canine distemper virus on day 24. Another group of 4 dogs remained untreated, was not vaccinated, and was challenged with virulent canine distemper virus on day 24; these dogs showed clinical signs typical of the infection. There was no effect on the magnitude and time of appearance of antibodies in the dogs given 1 mg prednisolone/kg bw but the group given 10 mg/kg bw showed a relative delay in antibody production. Histopathological examination revealed mild peripheral lymphoid depletion in spleen and lymph nodes from the dogs given 10 mg/kg bw but not in the dogs given 1 mg prednisolone/kg bw. Although a NOEL was not established due to changes in leukocyte morphology and inhibition of peripheral blood lymphocyte-phyto mitogen responses in the low dose group *in vitro*, it was clear that dogs given 1 mg/kg bw prednisolone showed a normal *in vivo* immunogenic response to challenge with virulent canine distemper virus.
14. A pharmacokinetic-pharmacodynamic model was applied to investigate the relation between the time course of prednisolone concentration and the lymphocytopenic effects in humans. Healthy human volunteers were given single or multiple oral doses of 30 mg prednisolone in the early morning or the evening, when the endogenous hydrocortisone levels are high and low, respectively. There were no significant differences in the pharmacokinetic parameters. The results suggested that hydrocortisone and prednisolone were equipotent with respect to the lymphocytopenic effect.
15. *In vitro* MIC values were determined for prednisolone against 7 species of Gram positive and 8 species of Gram negative microorganisms (a total of 51 isolates). The MIC values were all greater than 64 µg/ml, confirming that prednisolone had no significant antimicrobial activity.
16. Preparations containing prednisolone are available for oral, intra-articular, intramuscular and topical administration to humans. A number of different dosage regimes are employed depending on the indication, daily doses ranging from 5 mg/person up to 150 mg/person. Daily oral doses of up to 10 mg/person are generally well tolerated but the risk of adverse effects markedly increases above that dose level. The adverse effects are similar to those of the other corticosteroids and include acute adrenal insufficiency, which may be precipitated by an infection or trauma, and indications of glucocorticoid overactivity such as increased appetite, obesity and facial rounding. Growth retardation may occur in children receiving long-term oral treatment.
17. A pharmacological ADI of 0.0002 mg/kg bw (i.e. 0.012 mg/person) was calculated by applying a safety factor of 100 to the NOEL of 20 µg/kg bw/day which was established for induction of tyrosine aminotransferase activity in the rat.

Although clear NOELs had not been established in the repeated-dose toxicity studies, it was agreed that the effects in these were mainly attributable to the pharmacological activity of prednisolone and no useful information would be obtained by repeating these studies. It was noted that there was a difference in magnitude of 15 000 between the pharmacological ADI and the NOEL of 3 mg/kg bw/day established for teratogenicity in rats following oral dosing.
18. No radiometric residue depletion studies with prednisolone in the target species were provided. Evidence from published data suggested that, like dexamethasone, prednisolone was metabolised in both humans and the target species, to substances with no pharmacological activity. It was therefore agreed that radiometric studies were not required and parent compound is the marker residue.
19. Lactating cows were given intramammary infusions of a commercial product containing prednisolone into 2 quarters, equivalent to 11 mg prednisolone per quarter. The treatment was repeated 24 hours later. In the milk 0.045 to 1.42% of the administered prednisolone was recovered as prednisolone. The cows were killed 4 or 7 days after the last dose (4 cows per time point) and tissue samples were taken for residue analysis using HPLC. Residues of prednisolone in all tissues were below the respective limits of quantification. The limits of quantification in this study were 1.28 µg/kg for kidney and liver, 1.22 µg/kg for muscle and 1.23 µg/kg for fat.

20. In another study using a different commercial intramammary preparation, 8 lactating dairy cows, 4 medium yielding and 4 low yielding, were given an intramammary infusion into all 4 quarters. The dose corresponded to 9.85 mg prednisolone per quarter. Treatments were given at 3 consecutive milkings, approximately 12 hours apart. The cows were slaughtered 4 or 7 days after the last dose (4 per time point). Residues of prednisolone in all tissues, determined by HPLC, were below the respective limits of quantification. The limits of quantification in this study were 2.41 µg/kg for kidney, 1.20 for liver, 1.28 µg/kg for muscle and 1.25 µg/kg for fat.
21. Residues of prednisolone were determined using HPLC in samples of milk taken from 12 lactating dairy cows. The cows, 6 high milk yielders and 6 low milk yielders, were given intramammary infusions of a commercial product containing prednisolone, at a rate of 11 mg prednisolone/quarter, into 2 quarters. The treatment was repeated 24 hours later. At the first milking after the first treatment, residues in milk from the 2 treated quarters were in the range of below 0.81 to 235 µg/l. They depleted to below 0.81 to 4.30 µg/l by the second milking. At the first milking after the second treatment, residues of prednisolone in milk from treated quarters were in the range of below 1.28 to 502 µg/l. Residues in most milk samples were undetectable (below 0.81 µg/l) at the second milking after the second treatment. Low residues (up to 10.7 µg/l) were found in milk samples from untreated quarters. Residues of the inactive metabolite prednisone were undetectable (below 0.85 µg/l) in most milk samples but a few samples taken at the first treatment after milking contained measurable residues (up to 80 µg/l). Hydrocortisone was not detected in any of the milk samples (below 1.04 µg/l).
22. Similar results were obtained in a study using a different commercial formulation and 6 lactating cows. Intramammary infusions of 9.85 mg prednisolone per quarter were made, into 2 quarters, and residues in milk were determined using HPLC. Residues of prednisolone in the range 3.3 to 292 µg/l were found in samples taken at the first milking after the last infusion. In this study, no residues were detected in milk from untreated quarters.
23. The residue data for horses, pigs, sheep or goats and for cattle following administration other than by the intramammary route of administration were inadequate. These studies used only one or 2 animals per time point and the analytical method used to determine residues was not sufficiently sensitive. Consequently, only consumer intake arising from the intramammary use of prednisolone in cattle could be estimated. Following intramammary use in cattle, residues of prednisolone in tissues were very low and MRLs could be set at values corresponding to twice the limit of quantification.
24. The proposed routine analytical method for the determination of residues of prednisolone in bovine edible tissues and in milk was based on HPLC with UV detection. The specificity of the method was satisfactory and residues of penicillin G, dihydrostreptomycin, neomycin, bacitracin, tetracycline, novobiocin, prednisone and hydrocortisone did not interfere with the prednisolone quantification. The limits of quantification were 3.1 µg/kg for bovine milk, 5 µg/kg for kidney and liver and 1.3 µg/kg for muscle and fat. The method was described in the ISO 78/2 format.
25. There was no validated analytical method for the determination of residues of prednisolone in the edible tissues of horses, pigs, sheep or goats.

## Conclusions and recommendation

Having considered that:

- a pharmacological ADI of 0.0002 mg/kg bw (i.e. 0.012 mg/person) was established for prednisolone,
- prednisolone was considered to be the marker residue and accounted for all the residues with corticosteroid activity,
- a validated analytical method for the determination of residues of prednisolone in the milk and edible tissues of cattle is available,
- there was no information to demonstrate the likely extent of consumer exposure to residues other than those arising from the intramammary use of prednisolone in bovines; following intramammary use in cattle, residues of prednisolone in tissues were very low and MRLs could be set at values corresponding to twice the limit of quantification,

the Committee recommends the inclusion of prednisolone in Annex I of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Prednisolone	Prednisolone	Bovine	4 µg/kg 4 µg/kg 10 µg/kg 10 µg/kg 6 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRL values, the theoretical maximum daily intake will represent 99% of the ADI.