



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BETAMETHASONE

SUMMARY REPORT

1. Betamethasone is a synthetic corticosteroid which has marked glucocorticoid effects and is practically devoid of mineralocorticoid effects. Its chemical structure is the same as that of dexamethasone, except for the conformation of the 16-methyl group which projects above the plane of the steroid moiety in betamethasone, the 16 β -epimer, and below the plane in dexamethasone, the 16 α -epimer. In veterinary medicine, betamethasone is administered to a range of species to treat inflammatory conditions, shock and circulatory collapse, acetonæmia, and to induce parturition in cattle. However the establishment of MRLs was only requested for cattle and pigs. Formulated products are based on betamethasone or betamethasone sodium phosphate and there is a combination product which also contains antibiotics. The products are administered by intravenous or intramuscular injection. Usually, up to 3 doses of 0.038 mg/kg bw/day are administered at 24-hour intervals. For induction of parturition, a single dose of 0.08 mg/kg bw is normally employed.
2. The pharmacological activity of betamethasone closely paralleled that of dexamethasone. The affinities of the glucocorticoid receptors in rat hepatoma tissue culture cells for dexamethasone and betamethasone ($\log K_D$) were 8.47 and 8.55, respectively. In several studies, the antirheumatic potency of the 2 substances was identical. Betamethasone produced no significant increase in tyrosine aminotransferase activity in rat liver at oral doses of up to 0.004 mg/kg bw. Higher doses produced a dose-related statistically-significant increase in tyrosine aminotransferase activity; the pharmacological NOEL was therefore 0.004 mg/kg bw.
3. The pharmacokinetics of betamethasone was studied in humans, in pregnant and lactating rats, and in *in vitro* studies. Betamethasone sodium phosphate was rapidly de-esterified *in vivo* to betamethasone. Following oral administration of 3 different tablet formulations to human volunteers at a dose of 2 mg/person, plasma C_{max} values of 24 to 25 ng/ml were obtained around 2 hours after dosing. In another study, 58 to 80% of an oral dose was recovered from the urine, as a mixture of unmetabolised betamethasone and 6 metabolites, within 48 hours of dosing. The oral bioavailability in humans was estimated to be at least 70%. *In vitro* studies in human, dog, rat and cow plasma showed that betamethasone was extensively bound to plasma proteins. Betamethasone was widely distributed to the tissues. In pregnant rats given a subcutaneous dose of 1 mg betamethasone/kg bw, concentrations of radioactivity in maternal liver, kidneys and adrenals and in the foetal membrane were higher than in maternal plasma. When the same dose was administered to lactating rats, concentrations of radioactivity in the milk peaked at 122.3 ng/ml, 6 hours after dosing. The metabolic pathways were similar to those of other corticosteroids and involved oxidation of the 11 β -hydroxyl group to ketone, reduction of the C-20 ketone to give the alcohol, hydroxylation at the C-6 position and loss of the C-17 side chain to give 17-oxosteroids.
4. The acute oral LD₅₀ values for both betamethasone valerate and betamethasone disodium phosphate in rats, mice and dogs were reported to be greater than 1000 mg/kg bw.

5. Pre-GLP repeated dose oral toxicity studies were carried out in rats using daily oral doses of 0.024 to 3 mg betamethasone (free alcohol)/kg bw and 0.24 to 30 mg betamethasone-17-valerate/kg bw. Control groups were dosed with the solvent vehicle, distilled water or aqueous carboxymethylcellulose. The duration of these studies ranged from 28 days to 9 months. The effects included reduced body weight gain, leukopenia, lymphopenia and eosinopenia and thymic and adrenal atrophy. No NOELs were established.
6. Groups of 2 animals per sex and dose (Beagle dogs) were given daily oral doses of 0, 0.5, 1 or 2 mg betamethasone-17-valerate/kg bw in gelatin capsules, 6 days per week for 6 weeks. Another group was given an oral dose of 1 mg/kg bw betamethasone free alcohol according to the same dosing schedule. All treated groups exhibited muscular wasting, pot-belliedness and polydipsia, lymphopenia and eosinopenia and adrenal and thymic atrophy. Absolute liver weights were significantly increased. No NOEL was established. To investigate the liver changes, a special study was carried out in which groups of 2 dogs were given daily intramuscular injections of solvent vehicle or 0.45 mg betamethasone/kg bw for 28 days. At the end of the treatment period, liver from treated dogs were 3 times as heavy as the livers from the control animals and contained 3 times as much glycogen.
7. A 12-month repeated dose toxicity study was carried out in Rhesus monkeys using oral doses of 0, 0.2, 0.4, 0.8 or 2 mg betamethasone alcohol/kg bw/day. The effects were a consequence of the pharmacological activity of the substance and included reduced body weight gain, lymphopenia, eosinopenia, hepatotoxicity and atrophy of the adrenals and lymphoid tissues. As only a brief summary of this study was available no conclusions could be drawn and therefore no NOEL was established.
8. In a recent fertility study, groups of Wistar-derived rats were given daily subcutaneous doses of 0, 0.01, 0.1 or 1 mg betamethasone butyrate propionate/kg bw. The males were dosed for 9 weeks prior to mating and the females for 2 weeks prior to mating and then up to day 7 of gestation. In males, body weight gain and food intake were reduced at 1 mg/kg bw and a number of organ weights were significantly reduced at termination. Thymus weights were significantly reduced in both sexes given 0.1 mg/kg bw and above. At 1 mg/kg bw, the implantation rate was significantly reduced, the incidence of resorptions was significantly increased and the mean female foetal weight was significantly reduced. There were no effects on male or female fertility or on the numbers of corpora lutea at any dose level. The overall NOEL was 0.01 mg/kg bw/day, based on reduced thymus weights at the next dose level of 0.1 mg/kg bw/day.
9. Four teratogenicity studies were carried out in rabbits using parenteral dosing. Two studies used inadequate group sizes and were only briefly reported; fetuses with cleft palate were observed in both studies and no NOELs were established. Group sizes were not mentioned in a third study in which details of gross and soft tissue abnormalities were not provided; again, only a brief summary of the study was available. A fourth study was adequately conducted and employed groups of 17 pregnant JW-KBL rabbits which were given daily subcutaneous injections of 0, 0.0001, 0.001, 0.003 or 0.01 mg betamethasone/kg bw/day butyrate propionate from days 6 to 18 of gestation. Maternal body weight gain was significantly reduced at 0.01 mg/kg bw. At 0.01 mg/kg bw, foetal weights were significantly reduced and the incidences of fetuses with malformations and skeletal variations were significantly increased. Four fetuses from the 0.01 mg/kg bw had cleft palate and 8 had flexion contracture of the carpal joint. The NOELs for both teratogenicity and foetotoxicity were 0.003 mg/kg bw/day. Studies with the corticosteroid beclomethasone (which has a chlorine atom instead of fluorine at the 9 α -position) indicated that in rabbits, the subcutaneous route was more sensitive than the oral route in detecting reproductive effects. Consequently a higher NOEL would be expected for betamethasone following oral administration.

10. According to a brief summary, subcutaneous injections of 0.05, 0.2 or 0.3 mg betamethasone/day to mated female rats from days 12 to 15 of gestation resulted in cleft palate in 17%, 46% and 85% of the foetuses, respectively. In another study, groups of pregnant female Wistar rats were given daily subcutaneous injections of 0, 0.05, 0.4 or 3.2 mg betamethasone butyrate propionate/kg bw/day from days 7 to 17 of gestation. Twenty-three dams per dose were subjected to Caesarean section on day 21 of gestation. Maternal body weight gain was significantly reduced in the groups given 0.4 and 3.2 mg/kg bw and there were significant dose-related reductions in absolute and relative adrenal, spleen and thymus weight in all treated groups. Resorptions were significantly increased in the 0.4 and 3.2 mg/kg bw groups and foetal weights were significantly reduced in all treated groups. In the 3.2 mg/kg bw group, 8 foetuses had malformations of the sternebrae. Further groups of 10 dams per dose were allowed to litter down and rear the offspring to weaning. Pup weights and survival were adversely affected at 0.4 and 3.2 mg/kg bw. No substance-related effects were observed in developmental and behavioural tests and the fertility of the pups was unaffected by treatment. The NOEL for teratogenicity was 0.4 mg/kg bw/day.
11. No conclusions regarding NOELs could be drawn from 2 poorly conducted and reported teratogenicity studies in which mice were given subcutaneous doses in the range 0.1 to 10 mg betamethasone alcohol/kg bw/day at various periods during gestation. In the first study using doses of 0.1 or 0.2 mg betamethasone/kg bw/day, 85% and 71% respectively of the foetuses in these groups had cleft palate. Studies with the corticosteroid beclomethasone (which has a chlorine atom instead of fluorine at the 9 α -position) indicated that the subcutaneous route was more sensitive than the oral route for detecting reproductive effects in the mouse. For beclomethasone, NOELs of 0.08 and 0.04 mg/kg bw/day were established for teratogenicity in ICR mice using oral and subcutaneous dosing, respectively.
12. In a peri-/post-natal study, groups of 23 pregnant Wistar rats were given daily subcutaneous doses of 0, 0.004, 0.04 or 0.4 mg betamethasone butyrate propionate/kg bw from day 17 of gestation up to day 21 of lactation. At 0.4 mg/kg bw, maternal body weight gain was reduced and there was a significant reduction in the numbers of pups born alive. There were no substance-related effects on pup weight gain, development or fertility of the pups. The NOEL was 0.04 mg/kg bw/day.
13. Negative results were obtained in an *in vitro* assay for gene mutation in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA102 and *Escherichia coli* WP2 uvrA and in an *in vitro* forward point mutation assay (HPRT locus) in of Chinese hamster ovary (CHO) cells. In an *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes, cultures treated in the presence of metabolic activation and sampled at 20 hours had significant increases in cells with structural and numerical aberrations; cultures sampled at 44 hours after treatment showed no increase in cells with structural or numerical aberrations and all tests carried out in the absence of metabolic activation gave negative results. An *in vivo* micronucleus test was carried out in which groups of 5 male and 5 female mice were given two intraperitoneal injections, 24 hours apart, of 0, 250, 500 or 1000 mg/kg bw/day and killed 24 or 48 hours after dosing. At the 48 hour sampling time, statistically significant increases in micronucleated polychromatic erythrocytes were observed in males (but not females) given 250 and 500 (but not 1000) mg/kg bw/day. Because the increases the numbers of micronucleated polychromatic erythrocytes were observed only in males and were still within the historical control range, it was considered this to be a negative result.
14. No carcinogenicity data were provided for betamethasone. However the chemical structure of betamethasone did not contain any structurally-alerting features. Published data for prednisolone and its precursor, prednisone and a very brief published report on carcinogenicity studies with triamcinolone in rats (given daily oral gavage doses of 0.00005, 0.0002 or 0.001 mg/kg bw/day) and mice (given 0.0001, 0.0006 or 0.003 mg/kg bw/day) reported no evidence of carcinogenicity. A human epidemiology study reported no increased incidence of cancer following the administration of several glucocorticoids. Although the quality of the human data were poor, it was considered that betamethasone was unlikely to be carcinogenic.
15. No data were provided on the potential effects of betamethasone on human gut flora or the organisms used in industrial food processing, however due to the nature of the compound such data were considered not necessary.

16. Like the other glucocorticoids, betamethasone has suppressant effects on the immune system. In a special study, dexamethasone and betamethasone were compared in a natural killer cell cytotoxicity assay. The MIC₅₀ values for the 2 substances were 5 x 10⁻⁶ and 4 x 10⁻⁶ mg/ml, respectively.
17. Betamethasone has been widely used in human medicine for many years as betamethasone alcohol and as various esters. Products are available for oral or intravenous or intramuscular injection to treat conditions such as rheumatoid arthritis, severe hypersensitivity reactions, Crohn's disease, haemolytic anaemia, leukaemias and malignant lymphoma. There are also topical products for the treatment of allergic and inflammatory conditions and a product administered by inhalation for the treatment of asthma. The normal oral dose is 1.5 to 5 mg/day for the first few days reducing by 0.25 to 0.5 mg/day every 2 to 5 days to a maintenance dose of around 0.5 mg/day. Dosage reduction should be completed in stages to avoid adrenal insufficiency. Betamethasone preparations are generally well tolerated but suppression of the immune system increases the susceptibility of patients to infections. Betamethasone is contraindicated during pregnancy due to the risk to the foetus of cleft palate and intrauterine growth retardation. However antenatal administration of betamethasone is used in cases of premature labour to hasten maturation of foetal organs and tissues and reduce perinatal mortality; epidemiology studies in which the children were examined up to age 12 showed no adverse outcome.
18. A NOEL of 0.004 mg/kg bw had been established for betamethasone for induction of hepatic tyrosine aminotransferase activity in the rat, which could lead to an ADI of 0.00004 mg/kg bw/day. However the chemical structure of betamethasone and dexamethasone is the same except for the conformation of the 16-methyl group which projects above the plane of the steroid moiety in betamethasone (the 16β-epimer) and below the plane in dexamethasone (the 16α-epimer) and the two substances had very similar toxicological properties and equivalent glucocorticoid activity. Therefore, it was considered that the ADI for betamethasone should be the same as that previously established for dexamethasone, 0.000015 mg/kg bw.
19. The serum kinetics of 2 medicinal products containing betamethasone were investigated in cattle. One product was administered by intramuscular injection to 4 male and 6 female cattle at the recommended dose of 0.08 mg/kg bw and residues in serum were determined by radioimmunoassay. Mean peak serum concentrations of 7.3 ng/ml were attained around 8.9 hours after dosing. The AUC_{0-∞} was 287.0 ng/ml·h and the apparent serum half-life was around 22 hours. In another study, a product containing betamethasone sodium phosphate together with 2 antibiotics was administered by intramuscular injection to 5 male and 5 female cattle at a dose of 0.02 mg/kg bw and the residues in serum were determined by radioimmunoassay. Mean peak serum concentrations of 4.9 ng/ml were attained around 1.2 hours after dosing. The AUC_{0-∞} was 72.6 ng/ml·h and the apparent serum half-life was around 15 hours.
20. The serum kinetics of 2 medicinal products containing betamethasone were investigated in pigs. A product containing betamethasone (alcohol) was administered by intramuscular injection to 5 male and 5 female pigs at the recommended dose of 0.08 mg/kg bw and residues in serum were determined by radioimmunoassay. Mean peak serum concentrations of 12.0 ng/ml were attained around 3.2 hours after dosing. The AUC_{0-∞} was 196.2 ng/ml·h and the apparent serum half-life was 11.5 hours. In another study, a product containing betamethasone sodium phosphate together with 2 antibiotics was administered by intramuscular injection to 10 boars at a dose of 0.02 g/kg bw and the residues in serum were determined by radioimmunoassay. Mean peak serum concentrations of 5.3 ng/ml were attained around 0.5 hours after dosing. The AUC_{0-∞} was 26.2 ng/ml·h and the apparent serum half-life was 4.75 hours.

- Four male and 7 female cattle were given a single intramuscular injection of 0.08 mg betamethasone/kg bw. The animals were slaughtered (2 or 3 per time point) 5, 8, 10, 12 or 15 days after treatment. Residues in tissues were determined using a radioimmunoassay with claimed limits of detection of 3.4 µg/kg, 2.3 µg/kg, 3.9 µg/kg and 4.4 µg/kg for liver, kidney, muscle and fat respectively. Residues of 5.4 µg/kg and 7.8 µg/kg were found in the liver of the 2 animals killed 2 days after dosing. Residues in the liver from one of the animals slaughtered 8 days after dosing were 10.9 µg/kg. Residues in all other tissues were below the limit of detection. The radioimmunoassay method was insufficiently sensitive and not validated.
21. Six male and 6 female cattle were given 3 daily intramuscular injections of a combined preparation of betamethasone sodium phosphate, dihydrostreptomycin and procaine benzylpenicillin. The dose of betamethasone corresponded to 0.038 mg/kg bw/day. The cattle were slaughtered (2 males and 2 females per time point) at 3, 28 or 42 days after the last dose. Residues of betamethasone in tissues were determined using the proposed routine analytical method based on liquid chromatography-mass spectrometry (LC-MS). Three days after the last dose, mean residues in liver, kidney, and the last injection site were 9.03, 3.10 and 0.43 µg/kg, respectively. At the same time point, residues in 2 samples of muscle were 0.17 and 0.20 µg/kg and were below the limit of detection (0.1 µg/kg) in the other 2 samples. Also at 3 days after the last dose, residues in 3 samples of fat were in the range 0.14 to 0.18 µg/kg and residues in the fourth sample were below the limit of detection. Twenty-eight days after the last dose, detectable residues (limits of detection: 0.25 µg/kg in liver and 0.1 µg/kg in the other tissues) were found only in one sample of liver (2.2 µg/kg) and one sample of fat (0.13 µg/kg).
 22. Seven lactating cattle were given a single intramuscular injection of 0.04 mg betamethasone, equivalent to approximately 0.001 mg/kg bw. This was lower than the recommended dose. Residues in milk were determined using a radioimmunoassay with a claimed limit of detection of 1.6 µg/l. At the first milking after treatment, residues in milk were in the range 3.82 to 38.22 nmol/l. Residues in all samples taken at the 7th milking after treatment were below the limits of detection. The Committee considered that the radioimmunoassay method was not acceptable because it was insufficiently sensitive and was not validated.
 23. Eight dairy cows (4 high milk yielders, 4 low milk yielders) were given 3 daily intramuscular injections of a combined preparation of betamethasone sodium phosphate, dihydrostreptomycin and procaine benzylpenicillin. The dose of betamethasone corresponded to 0.038 mg/kg bw/day. The cows were milked twice daily. Milk was taken from the 4th to 8th milking after the last dose and analysed for betamethasone using the proposed routine analytical method based on LC-MS. At the 4th milking after the last dose, the residues in the milk samples taken from 3 cows were below the limit of detection and residues in milk from the remaining 5 cows were in the range 0.1 to 2.4 µg/kg. By the 7th milking after the last dose, residues in 7 out of 8 milk samples were below the limit of detection (0.05 µg/kg).
 24. Three male and 5 female pigs were given a single intramuscular injection of 0.08 mg betamethasone/kg bw. The pigs were killed (2 or 3 per time point), 4, 5 or 8 days after dosing. Residues in tissues were determined using a radioimmunoassay with claimed limits of detection of 3.7 µg/kg in liver, 1.3 µg/kg in kidney, 1.9 µg/kg in muscle and 4.2 µg/kg in fat, respectively. Residues were found only in one muscle sample (3.9 µg/kg) and 2 injection site samples (6.9 and 13.8 µg/kg) from the animals killed 4 days after dosing; by mistake, these animals had received only half the target dose. Residues in all other samples were below the limits of detection. The analytical method employed in this study was not validated.
 25. Six male and 6 female pigs were given 3 daily intramuscular injections of a combined preparation of betamethasone sodium phosphate, dihydrostreptomycin and procaine benzylpenicillin. The dose of betamethasone corresponded to 0.038 mg/kg bw/day. The pigs were slaughtered (2 males and 2 females per time point) at 3, 28 or 42 days after the last dose. Residues of betamethasone in tissues were determined using the proposed routine analytical method based on LC-MS. Residues of betamethasone (0.21 µg/kg) were detectable only in 1 out of 4 skin samples taken 3 days after the last dose. Residues in all other samples were below the respective limits of detection (limits of detection: 0.25 µg/kg in liver, and 0.1 µg/kg in the other tissues).

26. There were no data on absorption, distribution, metabolism and excretion in the target species and no information concerning total residues or the ratio of marker residue to total residues. In humans, both betamethasone and dexamethasone were well absorbed after oral administration and had similar volumes of distribution. Both substances were extensively bound to plasma proteins in humans, dogs, cows and rats; neither substance was bound to corticosteroid-binding globulin nor displaced hydrocortisone from its binding site. Both substances were rapidly eliminated. The metabolism of betamethasone had been studied only in humans; the metabolic pathways were similar to those of the other corticosteroids including dexamethasone. The metabolism of dexamethasone was known to follow the same metabolic pathways in humans and the target species. The absence of total residue depletion studies was justified by the evidence that in humans the metabolism of betamethasone followed pathways similar to those of dexamethasone resulting in a large decrease in corticosteroid activity. The parent drug, betamethasone, was therefore proposed as the marker residue.
27. The analytical method for determination of residues of betamethasone in edible tissues of cattle and pigs and in cows' milk was based on LC-MS and was described in the ISO 78/2 format. The method was fully validated and the limits of quantification were 0.25 µg/kg for muscle, kidney and fat and 1.25 µg/kg in liver for bovine and porcine species, and 0.15 µg/kg in bovine milk. It was shown that residues of other corticosteroids did not interfere in the analysis.

Conclusions and recommendation

Having considered that:

- the chemical structure of betamethasone was the same as that of dexamethasone, except for the conformation of the 16-methyl group which projects above the plane of the steroid moiety in betamethasone and below the plane in dexamethasone,
- an ADI of 0.000015 mg/kg bw (i.e. 0.0009 mg/person) was retained for betamethasone, the same as previously established for dexamethasone,
- betamethasone was considered to be the marker residue,
- for the sake of consistency, the numerical values of the MRLs for betamethasone should be the same as previously established for dexamethasone,
- a fully validated analytical method was available for the determination of residues of betamethasone in edible tissues of pigs and cattle including milk;

the Committee on Veterinary Medicinal Products recommends the inclusion of betamethasone 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
Betamethasone	Betamethasone	Bovine	0.75 µg/kg 2.0 µg/kg 0.75 µg/kg 0.3 µg/kg	Muscle Liver Kidney Milk	
		Porcine	0.75 µg/kg 2.0 µg/kg 0.75 µg/kg	Muscle Liver Kidney	

Based on these MRLs, the consumer intake was estimated to be 0.0009125 mg/day. Although this exceeded the ADI of 0.0009 mg/person by a small amount, it was considered that this would not present a risk to human health because the substance was used only occasionally in individual animals.