



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### PROGESTERONE

#### SUMMARY REPORT

1. Progesterone is a naturally occurring steroid hormone. In veterinary medicine, progesterone is used in cows and mares for therapeutically (disorders of the reproductive system, including termination of an unwanted pregnancy) and zootechnical (oestrus synchronization and preparation of donor- and receptor animals in the case of embryo transfer) purposes. It is intended for intravenous or intramuscular administration (simultaneously with human chorionic gonadotrophin, at a dose of 125 mg progesterone/cow) or for intravaginal treatment (simultaneously with 17 $\beta$ -oestradiol, at a dose of 1.55 g progesterone/cow or mare).
2. Progesterone is a hormone with different actions dependent on the period in the oestrous cycle. Progesterone regulates maturation of the oocytes, ovulation, myometrial quiescence, mammary gland growth and endometrial enzymes. Furthermore, progesterone exerts other wide-ranged effects, e.g. on metabolism, respiratory system and central nervous system.
3. The production rate of endogenous progesterone and hence the natural progesterone levels in plasma show a large variation, depending on the species, sex, age and physiological status. In humans, the production rate of progesterone amounts to 0.15 mg/day in prepubertal boys, 0.416 to 0.75 mg/day in men, 0.253 mg/day in prepubertal girls, 0.418 to 50 mg/day in women (depending on phase in the menstrual cycle), and 92 to 563 mg/day in pregnant women (depending on trimester of pregnancy). The physiological plasma concentration of progesterone in men is approximately 0.3 ng/ml, in women this ranges from 0.23 to 1.2 ng/ml (follicular phase), via 8.3 to 25 ng/ml (luteal phase) to 21 to 200 ng/ml (pregnancy).  
Physiological plasma concentrations in cattle and horses during oestrus are less than 0.2 to 8 ng/ml and less than 0.3 to 22 ng/ml, respectively, and during pregnancy less than 8 to 12 ng/ml and less than 7 to 25 ng/ml, respectively.

4. Pharmacokinetic studies with the commercial products at the recommended dosages show that after intravenous, intramuscular or intravaginal treatment of cattle, the plasma progesterone concentrations are elevated only within the first few hours after treatment, but never higher than the values observed under physiological conditions.

From literature data the following can be extracted. Due to rapid absorption from the gut and an extensive (first-pass) metabolism in the liver and gastro-intestinal tract, the oral bioavailability of exogenously administered progesterone is less than 10%. Circulating progesterone is bound to proteins in blood. In the body, progesterone accumulates in fatty tissue due to its lipophylic properties, and in tissues/organs containing progesterone-receptors. Metabolism of exogenous and endogenous progesterone takes place by reduction of the double bonds and hydroxylation at the C16 and C21 atoms. The metabolites are conjugated to sulphates and glucuronides and excreted as such. Following intravenous injection of radiolabelled progesterone to humans, reported half-lives range from 3 to 90 minutes, and excretion is predominantly via urine (50 to 60%) and to a lesser extent via bile and faeces (10%). Following intravenous injection of

radiolabelled progesterone to cows, within 24 to 48 hours 50% of the dose was recovered in bile/faeces and only 3% in urine.

5. There are little data available on the acute toxicity of progesterone. In rabbits, the intravenous LD<sub>50</sub> is 26.5 mg/kg bw. For the neonate mouse the subcutaneous LD<sub>50</sub> progressed with age from 70 mg/kg bw in 0 to 24 hours old mice to 2700 mg/kg bw in 121 to 168 hours old mice.

6. In a 26-week toxicity study, rats were dosed orally with 40 or 160 mg progesterone/kg bw/day or subcutaneously with 4 or 16 mg progesterone/kg bw/day. Oral administration of progesterone led to virtually no effects (NOEL = 160 mg/kg bw/day). Subcutaneous administration revealed effects only at the highest dose of 16 mg/kg bw/day: in females and males the endocrine target organs (gonads, uterus, prostate) were atrophied and in males the pituitary weight was increased.

In a 13-week dog study, an oestradiol/progesterone combination (150 µg/kg bw oestradiol plus 6.25 mg/kg bw progesterone) administered subcutaneously on alternate days to immature ovariectomised dogs, induced severe anaemia, cystic endometrial hyperplasia, accelerated activity of the pituitary gland, and secretory epithelial cell proliferation in addition to stromal and ductal cell proliferation in the mammary gland.

Treatment of dogs for 1 to 1.5 years with progesterone containing subcutaneous implants (approximately 225, 375, 1125 or 1650 mg progesterone/kg bw) resulted in a slight degree of mammary enlargement, glandular activity and nodule development in the 3 highest dose groups.

Treatment of monkeys for 1 year with vaginal rings releasing 235 or 1770 µg progesterone/day showed effects on organs of the reproductive system for both dosages.

7. Although limited data were provided on the reproductive toxicity of progesterone, it is clear that progesterone exerts effects on the reproductive system. A Clauberg-McPhail test in rabbits was performed to assess the progestational activity of progesterone after oral administration compared to subcutaneous administration. From this study an oral hormonal NOEL of 3.2 mg/kg bw/day can be established, while the subcutaneous hormonal NOEL is 0.025 mg/kg bw/day.

8. Data on teratogenicity/embryotoxicity reveal that no congenital disorders are found after treatment with natural progesterone. Progesterone administered intramuscularly to rats at a dose of 5 mg/day on days 16 to 19 of gestation had no effect, but the same dosage on days 20 to 23 of gestation caused fetal death, which was probably related to the prolonged delay of parturition due to progesterone administration.

9. According to the International Agency for the Research on Cancer (IARC), progesterone does not exhibit mutagenic activity in most *in vitro* and *in vivo* tests performed, but is known to increase the tumour incidence in endocrine target tissues (ovaries, uterus, mammae) after continuous (parenteral) doses clearly above the physiological levels. Progesterone is not carcinogenic per se, but acts via an epigenetic mechanism associated with its endocrine activity, i.e. its ability to cause a hyperproliferative effect at cellular levels mediated by steroid-hormone receptor interaction. Hence, tumours will not result from ingestion of progesterone at levels that do not produce any hormonal effects.

10. Between 1997 and 1999, new data became available on the genotoxicity and carcinogenicity of steroid hormones, although not including progesterone (apart from some carcinogenicity data). These data were also reviewed and discussed by the Joint FAO/WHO Committee on Food Additives (JECFA) in 1999, by the Scientific Committee on Veterinary Measures Relating to Public Health (SCVPH) of the European Commission in 1999 and by the International Agency for Research on Cancer (IARC) in 1999. Upon evaluation of these data, mainly concerning 17β-oestradiol, the CVMP concluded that steroid hormones are devoid of genotoxic activity *in vivo* and that these compounds exert their carcinogenic action only after prolonged exposure and at levels considerably higher than those required for a physiological (hormonal) response. Hence, the previous conclusions with respect to genotoxicity and carcinogenicity could be endorsed.

11. From tolerance studies with target animals it appears that progesterone containing formulations are generally well tolerated.

12. Due to the low oral bioavailability, the oral toxicity of progesterone is low (the overall oral NOEL is 160 mg/kg bw/day). Therefore, the pharmacological effects are more relevant for the safety evaluation of progesterone. The most sensitive pharmacological parameter is the lowest daily endogenous production rate of 150 µg as found in prepubertal boys. Assuming that a daily intake corresponding to 10% of this quantity presents no risk after consumption, and correcting for the low oral bioavailability (less than 10%), the maximum intake of exogenous progesterone should not exceed 150 µg/day.

13. As progesterone is an endogenous hormone, it must be regarded as a natural constituent of food of animal origin (dairy products and tissues/organs). In cattle the highest average physiological progesterone concentrations are found in fat (2.5 µg/kg in steer, 5.8 µg/kg in veal calves, 16.7 µg/kg in heifers and 239 to 360.2 µg/kg in pregnant cattle). Lower concentrations are found in liver, kidney and muscle (0.12 to 0.46 µg/kg in veal calves and steer, and 3.4, 6.2 and 10.1 µg/kg, respectively, in pregnant cattle).

In milk and milk products progesterone levels are correlated with the percentage of fat: average physiological levels up to 4.6, 6.5, 72.7 µg/l and 300 µg/kg are found in skim milk, buttermilk, cream and butter, respectively. In milk itself physiological progesterone levels relate to the stage of oestrus cycle, with minimal concentrations at oestrus (less than 0.2 to 0.92 µg/l) and highest levels during the luteal phase (0.2 to 30 µg/l) and pregnancy (20 to 35.7 µg/l).

14. Milk residue experiments with the commercial products at the recommended dosages show that after intravenous, intramuscular or intravaginal treatment of cattle, the milk progesterone levels are highly correlated with the plasma progesterone levels: the milk progesterone concentrations are elevated only in the first few milkings after treatment, but never higher than the values observed under physiological conditions.

15. As the administration of the commercial products containing progesterone failed to induce progesterone blood concentrations exceeding the physiological levels, no specific residue studies in edible tissues other than fat and injection site were carried out.

After intravenous administration of 125 mg progesterone (commercial product) to synchronized cows, progesterone levels were determined in renal fat after a withdrawal time of 1 hour. The mean level in fat of treated animals (260 µg/kg; range 65 to 607 µg/kg) was higher than that of control animals (30.5 µg/kg; range 12 to 56 µg/kg), but still within the physiological range.

After intramuscular administration of 125 mg progesterone (commercial product) into the neck of synchronized cows, progesterone levels were determined in the injection site after withdrawal times of 7, 14 and 21 days. Residues higher than the physiological levels were observed: the mean levels in the injection site of treated animals depleted from 1472 µg/kg at 7 days (range 5 to 4400 µg/kg), via 283 µg/kg at 14 days (range 4 to 830 µg/kg) to 6 µg/kg at 21 days (range 3 to 10 µg/kg). Control levels in neck muscle at these withdrawal times were 2, 36 and 5 µg/kg, respectively.

16. For progesterone as growth-promoter (subcutaneous implant), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered the establishment of an ADI and Acceptable Residue Levels unnecessary for a hormone that is produced endogenously in human beings and shows marked variation in levels according to age and sex. JECFA concluded that, compared to normal human daily production rates of progesterone and the amounts of progesterone that are normally present in dairy products and tissues of untreated animals, the amount of exogenous progesterone that humans will be exposed to through ingestion of tissue from treated animals is biologically insignificant, and will be incapable of exerting a hormonal effect in human beings.

17. As the residue studies showed that the milk, tissue and plasma levels after the recommended treatment with progesterone were at or within physiological limits, the conclusion of the JECFA that no ADI and MRLs for progesterone need to be established can also be adopted for the therapeutic and zootechnical use of progesterone.

## 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 that:

- Progesterone is of endogenous origin, and is a natural constituent of food of animal origin,
- The oral bioavailability of progesterone is less than 10%,
- The animals are unlikely to be sent for slaughter during or immediately after treatment,
- Milk, tissue and plasma levels after treatment with progesterone have shown to be at or within physiological limits;

the Committee considers that there is no need to establish an MRL for progesterone and recommends its inclusion into Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance	Animal species	Other provisions
Progesterone	Bovine, equidae	For therapeutic and zootechnical uses only

For injection site residues after parenteral administration of progesterone, it may be necessary to establish a withdrawal time at national marketing authorization.