The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FLUGESTONE ACETATE

SUMMARY REPORT (1)

1. Flugestone acetate (synonym: cronolone) is a synthetic progesterone, with a progestational action higher than that of progesterone itself. It is intended for intravaginal use in sheep and goats to induce oestrus synchronisation. The proposed dosage is 1 sponge, impregnated with 30, 40 (for sheep) or 45 mg (for goats) flugestone acetate, which is to be removed after 12 to 14 days from ewes and after 17 to 21 days from goats. Flugestone acetate is not indicated for use in humans.

2. The pharmacodynamic studies indicated that the main action of flugestone acetate is progestational, which was most evident in rabbits. A pharmacological hormonal NOEL of 0.003 mg/kg bw/day was observed for endometrial proliferation in the uterus of female juvenile, oestrogen pre-treated rabbits. Rats were less susceptible to flugestone acetate, due to a lower affinity of receptor sites to flugestone acetate. Flugestone acetate also showed glucocorticoid effects in some studies. These studies were however inadequate for the determination of a NOEL. Flugestone acetate was devoid of estrogenic and androgenic activity. Flugestone acetate is metabolised mainly into hydroxylated products, which are thought to possess less pharmacological activity as compared to the parent compound.

3. No pharmacokinetic studies in laboratory animals were provided. Plasma kinetic studies with non-radiolabelled flugestone acetate in sheep and goats used the intravaginal route of administration at the recommended dose, and only the parent compound was measured. After intravaginal insertion of flugestone acetate impregnated sponges to sheep, flugestone acetate concentrations in plasma reached a plateau level of approximately 1.2 µg/l within 10 hours, and these concentrations remained at the same level as long as the sponge was in place. After removal of the sponge at 14 days after insertion the elimination from plasma was biphasic: a rapid phase with a half-life of 1.6 hours during which the concentrations declined to 0.04 to 0.32 µg/l within 12 hours, followed by a slower phase with a half-life of 28.7 hours.

4. An *in vitro* metabolism study with ovine hepatocytes showed that flugestone acetate is metabolised to a number of (mostly) hydroxylated products, indicating that flugestone acetate follows the normal breakdown pathway for progestagens.

5. Single dose toxicity studies were not performed. One 90 day oral toxicity study in rats, in which flugestone acetate was administered in the diet at doses of 0.2, 1.0, and 5.0 mg/kg bw/day revealed a NOEL of 0.2 mg/kg bw/day, based on decreased bodyweight gain, decreased adrenal weight and some histological changes in the adrenals.

6. The reproductive toxicity was extensively investigated in rabbits. In a 1-generation reproductive toxicity study, flugestone acetate was administered to rabbits by gavage at oral doses of 0 (vehicle), 0.001, 0.003, 0.010 or 0.045 mg/kg bw/day. A NOEL for parental toxicity of 0.003 mg/kg bw/day was established, based on effects on the adrenals and the liver. The NOEL was also 0.003 mg/kg bw/day for reproductive toxicity based on reduced fertility, and for embryotoxicity based on intrauterine mortality. The NOEL for pup toxicity was 0.010 mg/kg bw/day, based on perinatal mortality.

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7. Teratogenicity studies were carried out in rats and rabbits. Rats were orally dosed at 0 (vehicle), 0.1, 1 or 10 mg/kg bw/day on gestation days 7 through 16. The NOEL for maternal toxicity was 0.1 mg/kg bw/day, based on reduced bodyweight gain and decreased absolute spleen weights. No signs of embryotoxicity or teratogenicity were observed.

   Rabbits received flugestone acetate at oral doses of 0 (vehicle), 0.01, 0.04, or 0.16 mg/kg bw/day on gestation days 7 through 19. The NOEL for maternal toxicity was 0.01 mg/kg bw/day, based on bodyweight changes. The NOEL for embryotoxicity/foetotoxicity was 0.04 mg/kg bw/day, based on post-implantation loss retarded growth and delayed ossification of the skull. Flugestone acetate is considered not teratogenic in rats and rabbits.

8. Flugestone acetate tested negative in *in vitro* tests for gene mutations in bacteria and mouse lymphoma cells, and for chromosomal aberrations in human lymphocytes. It was concluded that flugestone acetate, like other progestagens, can be considered a non-genotoxic compound.

9. Carcinogenicity studies were not performed. This was considered not necessary as flugestone acetate belongs to a class of non-genotoxic compounds and tested negative in *in vitro* mutagenicity tests. The possible tumourigenic effects of progestagens are related to epigenetic mechanisms that are secondary to the progestational effects of these compounds.

10. Between 1997 and 1999, new data became available on the genotoxicity and carcinogenicity of steroid hormones, although not including flugestone acetate. 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 17ß-oestradiol, the CVMP concluded that steroid hormones are devoid of genotoxic activity *in vivo* and that these compounds exert their (possible) 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. Investigations on the pharmacodynamic action and on the reproductive toxicity of flugestone acetate demonstrated that the rabbit was the most sensitive species. Progestational, glucocorticoid, and reproductive effects were the most sensitive parameters observed, resulting in an oral NOEL of 0.003 mg/kg bw/day, based on endometrium proliferation in the uterus of female juvenile, oestrogen pre-treated rabbits and on effects on adrenals, fertility and on embryotoxicity in the one-generation reproductive toxicity test. Although repeated dose toxicity data were limited, it can be concluded that the most sensitive parameters were investigated in the most sensitive species. Therefore, the 0.003 mg/kg bw/day can be regarded as the overall NOEL.

    Based on this overall NOEL, and using a safety factor of 100, an ADI of 0.03 µg/kg bw can be derived (i.e. 1.8 µg for a 60 kg person).

12. As it was indicated that flugestone acetate is metabolised following the normal pathways for steroids, and that such breakdown products generally possess less hormonal activity, the parent compound is considered the most suitable marker residue. No radiometric studies in target animals were carried out, and, as a consequence, no information is available on the ratio marker residue to total residues. However, milk residues declined to undetectable residues very shortly after treatment (within 2 days), and tissue residues at 1 day after treatment represent a theoretical total intake (using the standard consumption figures) of 0.625 µg per day, i.e. approximately 35% of the ADI of 1.8 µg for a 60 kg person. Therefore, radiometric studies were not considered to be necessary.

13. Tissue residues were only investigated in sheep at 1, 3 and 5 days after a 14-day intravaginal treatment with sponges that were impregnated with 40 mg flugestone acetate. At one day after removal of the sponges, highest mean flugestone acetate concentrations were found in muscle (1.84 µg/kg). In fat and liver mean concentrations of 0.45 and 0.44 µg/kg were found, respectively, and kidney had the lowest flugestone acetate concentration (0.17 µg/kg). These levels declined rapidly to 0.06, 0.03, 0.03 and 0.02 µg/kg in muscle, fat, liver and kidney, respectively, at 5 days withdrawal.

14. Tissue residue data in goats were not available.

15. Milk residues of flugestone acetate were investigated in sheep during and after a 14-day intravaginal treatment with 40 mg flugestone acetate sponges. Concentrations in milk followed the plasma kinetics, reaching a plateau level of 1.33 µg/l at 10 hours after insertion, and declining quickly after removal of the sponges to 0.22 µg/l at 10 hours withdrawal, 0.08 µg/l at 1 day withdrawal, and undetectable thereafter.

16. Flugestone acetate residues in goat milk were investigated after a 17-day intravaginal treatment with sponges that were impregnated with 45 mg flugestone acetate. As in sheep, the concentrations of flugestone acetate in milk reflected the plasma kinetics. During treatment two plateaus were formed, a 1 to 4 day and a 5 to 11 day plateau with mean levels of 0.82 and 0.64 µg/l, respectively. Thereafter, concentrations in milk decreased gradually to 0.17 µg/l at removal. Flugestone acetate residue concentrations had declined to 0.1 µg/l at 10 hours withdrawal, 0.03 µg/l at 1 day withdrawal, and to undetectable levels thereafter.

17. For the routine determination of flugestone acetate residues in tissues and milk from sheep and goats, an HPLC-MS-MS method was proposed. The method was well described, although not in an internationally recognised format. Validation data were only provided for ovine tissues and milk. The validation was not sufficient with respect to accuracy and precision, as these were calculated from 3 instead of 6 determinations per concentration level. As a consequence, the limit of quantification of 0.5 µg/kg in all tissues and in milk was not substantiated properly. Furthermore, substantiated limits of detection, information on sample throughput, and representative chromatograms were lacking.

Conclusions and recommendation:

Having considered that:

- an ADI of 0.03 µg/kg bw (i.e. 1.8 µg for a 60 kg person) was established, based on the lowest NOEL of 0.003 mg/kg bw/day and using a safety factor of 100,
- flugestone acetate residues in milk from sheep and in tissues from sheep are rapidly depleted after treatment,
- flugestone acetate is used only for zootechnical purposes (synchronisation of oestrus), in the breeding season,
- the animals are unlikely to be sent for slaughter during or immediately after treatment,
- during treatment residues of flugestone acetate can be detected in milk,
- a routine analytical HPLC-MS-MS method for the determination of flugestone acetate residues was available for milk, but was not fully validated;

the Committee recommends the inclusion of flugestone acetate in Annex III to Council Regulation (EEC) No 2377/90 for milk of ovine and caprine species in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Flugestone acetate	Flugestone acetate	Ovine, caprine	1 µg/kg	Milk	For intravaginal use for zootechnical purposes only. Provisional MRLs expire on 1.1.2002

and for all tissues except milk of ovine and caprine species the inclusion of flugestone acetate in Annex II to Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Flugestone acetate	Ovine, caprine	For intravaginal use for zootechnical purposes only and for all tissues except milk

Based on these MRLs values, the maximum daily intake will represent about 83% of the ADI.

LIST OF QUESTIONS

1. The proposed HPLC-MS-MS method for the determination of flugestone acetate in milk of sheep and goats should be completely validated according to the requirements laid down in Volume VI of the Rules Governing Medicinal Products in the European Community, taking into account the CVMP Note for Guidance on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL). Special attention should be paid to the practicability (sample throughput) of the method. The method should be described in an internationally recognised format (e.g. ISO 78/2).