European Medicines Agency
Veterinary Medicines and Inspections

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FLUGESTONE ACETATE

SUMMARY REPORT (4)

1. Flugestone acetate (synonym: cronolone) (CAS: 2529-45-5) is a synthetic progesterone, with a progestational action higher than that of progesterone itself. It is used for intravaginal use in sheep and goats to induce oestrus synchronisation. The dosage is one sponge, impregnated with 30 or 40 mg of flugestone acetate for sheep and 45 mg flugestone acetate for goats, which is to be removed after 12 to 14 days from ewes and after 17 to 21 days from goats.

   Flugestone acetate is not used in human medicine.

   Currently flugestone acetate is included in Annex I of Council Regulation (EEC) No 2377/90 for ovine and caprine milk 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.

   and in Annex III for ovine and caprine tissues as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Flugestone acetate	Flugestone acetate	Ovine, caprine	0.5 µg/kg 0.5 µg/kg 0.5 µg/kg 0.5 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.1.2008 For therapeutic and zootechnical purposes only.

   Additional data on the validation of the routine analytical method for tissues of ovine and caprine species were provided at the request of the Commission further to the establisment of provisional MRLs for flugestone acetate in ovine and caprine tissues.

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 was metabolised mainly into hydroxylated products, which are thought to possess less pharmacological activity as compared to the parent compound.

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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 $t_{½}$ 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 $t_{½}$ of 28.7 hours.

   Plasma concentrations of flugestone acetate in intravaginally treated goats formed two plateaus, a 0 to 2 days and a 3 to 9 days plateau with mean levels of 0.77 and 0.53 µg/l. Thereafter, plasma concentrations decreased gradually to 0.15 µg/l at removal on day 17 after treatment. Within 24 hours after removal of the sponges the plasma concentrations were below the limit of detection (0.01 µg/l).

4. An *in vitro* metabolism study with ovine hepatocytes showed that flugestone acetate was 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 body weight gain, decreased adrenal weight and some histological changes in the adrenals.

6. The reproductive toxicity was extensively investigated in rabbits. In a one-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 for reproductive toxicity was 0.003 mg/kg bw/day, based on reduced fertility, and the NOEL for embryotoxicity was also 0.003 mg/kg bw/day, based on intrauterine mortality. The NOEL for pup toxicity was 0.010 mg/kg bw/day, based on perinatal mortality.

7. Teratogenicity studies were carried out in rats and rabbits. Rats were orally dosed at 0 (vehicle), 0.1, 1.0 or 10.0 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), 0.010, 0.040, or 0.160 mg/kg bw/day on gestation days 7 through 19. The NOEL for maternal toxicity was 0.010 mg/kg bw/day, based on body weight changes. The NOEL for embryotoxicity/foetotoxicity was 0.040 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. Investigations of the pharmacodynamic action and 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 was established, i.e. 1.8 µg for a 60 kg person.

11. As it was indicated that flugestone acetate was metabolised following the normal pathways for steroids, and that such breakdown products generally possess less hormonal activity, the parent compound was considered the most suitable marker residue. No radiometric studies in target animals were carried out, and, as a consequence, no information was available on the ratio marker residue to total residues. However, milk residues declined to undetectable residues very shortly after treatment (within two days), and tissue residues at one 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 considered not to be necessary.

12. Tissue residues were only investigated in sheep at 1, 3 and 5 days after a 14 days 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 a withdrawal period of 5 days. Tissue residue data in goats were not available. However, taking into account the Note for Guidance on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL) these data were not required.

13. Milk residues of flugestone acetate were investigated in sheep during and after a 14 days 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.

14. Flugestone acetate residues in goat's 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 2 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 0.10 µg/l at 10 hours withdrawal, 0.03 µg/l at 1 day withdrawal, and to undetectable levels thereafter.

15. 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 described in an ISO 78/2 format. The method was sufficiently validated in tissues and milk from sheep and goats, with limits of quantification of 0.25 µg/kg for muscle, fat, liver, and kidney and 0.5 µg/kg for milk.

Conclusions and recommendation:

Having considered that:

- an ADI of 0.03 µg/kg bw, i.e. 1.8 µg/person was established,
- flugestone acetate was considered the marker residue,
- flugestone acetate is used only for zootechnical purposes (synchronisation of oestrus),
- a fully validated routine analytical HPLC-MS-MS method for the determination of flugestone acetate residues is available for sheep and goats' tissues;

the Committee recommends the inclusion of flugestone acetate in Annex I to Council Regulation (EEC) No 2377/90 for tissues 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	0.5 µg/kg 0.5 µg/kg 0.5 µg/kg 0.5 µg/kg	Muscle Fat Liver Kidney	For therapeutic and zootechnical purposes only

These MRLs result in a maximum theoretical intake from tissues of 0.25 µg of flugestone acetate per day. Together with the maximum theoretical intake from milk, the total maximum theoretical intake is 1.75 µg, i.e. 97% of the ADI of 1.8 µg for a 60 kg person.