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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ALTRENOGEST

SUMMARY REPORT (2)

1. Altrenogest (or allyltrenbolone) is a synthetic trienic C21 steroidal progestomimetic, belonging to the 19-nor-testosterone series. It is an orally active (pro)gestagen. Like all steroids, altrenogest acts by its liposolubility by penetrating the target cells where it binds to specific receptors. In veterinary medicine, altrenogest is used in gilts and mares for zootechnical purposes (oestrus synchronization). The recommended dose for gilts is 20 mg/animal/day given orally for 18 consecutive days, and for mares is 0.044 mg/kg bw/day given orally for 10 to 15 days.

   A pharmacological ADI of 0.04 µg/kg bw, based on the absence of hormonal effects in monkeys and pigs, had previously been established by the Committee for Veterinary Medical Products.

   Altrenogest is currently included in Annex III 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
Altrenogest	Altrenogest	Porcine, *Equidae*	3 µg/kg 3 µg/kg 3 µg/kg	Fat Liver Kidney	For zootechnical purposes only. Provisional MRLs expire on 1.1.2003

   Additional data were provided in response to the list of questions further to the recommendation for the establishment of provisional MRLs.

2. The pharmacodynamic activity of altrenogest has been demonstrated in a number of animal models. The most important effects are the progestomimetic and anti-gonadotrophic effects. Altrenogest also has weak oestrogenic, anabolic and androgenic effects, but has no corticoid or anti-inflammatory effects. An overall no-hormonal-effect level of 4 µg/kg bw/day can be established in monkeys receiving altrenogest during three menstrual cycles (effects on menstrual cycle length and serum hormonal concentrations).

3. There is only one limited study available on the pharmacokinetics of altrenogest in laboratory animals. After receiving a single oral dose of altrenogest, rats excreted altrenogest mainly via bile (60%) with the faeces. Excretion in urine, which was largely completed within 24 hours, amounted to approximately 20% of the administered dose.

4. There are few data available on the acute toxicity of altrenogest. In rats and mice, the intraperitoneal LD_{50} values were 176 and 233 mg/kg bw, respectively. In dogs, oral doses up to 400 mg/kg bw are well tolerated.

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5. Several repeated dose toxicity studies are available after oral administration of altrenogest. In rats, a 2-month study (tested doses 0, 0.5, 2 mg/kg bw/day), a 13-week study (0, 1, 10, 100 mg/kg feed, equal to 0.06 to 7.82 mg/kg bw/day), and a 1-year study (0, 2, 10, 50 mg/kg feed, equal to 0.15 to 4.58 mg/kg bw/day) were carried out, and in dogs a 1-year study (0, 0.04, 0.2, 1 mg/kg bw/day). In these studies, effects were found which are directly related to the pharmacological activity of altrenogest (decreased weights/histopathology in the hormone dependent organs), resulting in an overall oral LOEL of 0.04 mg/kg bw/day.

6. In several tolerance studies with pigs, the main effects observed were directly related to the hormonal activity of altrenogest (decreased weights/histopathology in ovaries, uterus, mammary glands, prostate, testes, seminal vesicles). A no hormonal effect level of 4 µg/kg bw/day can be established from a tolerance study in which sexually mature pigs received 4, 40 or 200 µg altrenogest/kg bw/day orally for 3 months.

7. A 1- and a 2-generation reproduction study in rats at doses of 25, 50, 100 and 0.4, 4, 40 mg altrenogest/kg feed, respectively, are available. In these studies, effects on the reproduction were found (reduced pregnancy rate, depression of spermatogenesis, decreased litter size and weight, decreased weight of hormone dependent organs), resulting in an oral NOEL of 0.4 mg/kg feed (equal to 0.03 mg/kg bw/day). No indications for teratogenic effects were found in the teratology phase of the 2-generation reproduction study in rats and in a tolerance study with pigs receiving 20 mg altrenogest/day on days 28 to 112 of pregnancy.

8. Long-term toxicity/carcinogenicity experiments have not been performed. These data are not deemed necessary, because in an adequate set of mutagenicity tests (*in vitro*: Ames test, forward mutation tests, chromosome aberration test, DNA repair tests; *in vivo*: chromosome aberration test in rats), altrenogest did not show a genotoxic potential.

9. Between 1997 and 1999, new data became available on the genotoxicity and carcinogenicity of steroid hormones, although not including altrenogest. 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.

10. As the no hormonal effect level of 4 µg/kg bw/day (observed in monkeys and pigs) is lower than the toxicological NOEL of 0.03 mg/kg bw/day (observed in the 2-generation reproduction study with rats), it is most appropriate to use the former as basis for the ADI. Based on the no hormonal effect level, and a safety factor of 100, a pharmacological ADI of 0.04 µg/kg bw (equivalent to 2.4 µg for a 60 kg person) can be established for altrenogest.

11. After oral administration of radiolabelled altrenogest at the recommended dose to pigs and horses, altrenogest is readily absorbed, reaching peak levels after 3 to 6 hours. During prolonged treatment, accumulation in plasma is found in pigs. Plasma concentrations decline biphasically in both species, with an elimination half-life of about 10 days in pigs. The radioactivity is in both species mainly distributed to the liver, and to a lesser extent to kidney, muscle and fat. Excretion data are limited. In pigs, the major route of elimination is via the bile in the faeces, and about 20% of the administered dose is excreted with the urine. In horses, in 24 hours approximately 44% of the administered dose is excreted in urine and approximately 53% in faeces.

    Although only a small fraction of the metabolites in plasma, urine and tissues is extractable and identifiable, the data indicate that, in line with all steroids, the major metabolic pathway for altrenogest is conjugation. Dealkylation (rendering trenbolone) does not occur.

12. Residue experiments with pigs have only been carried out with radiolabelled altrenogest. After oral treatment with the recommended dose (20 mg/day for 18 consecutive days), pigs were slaughtered after withdrawal times of 6 hours and 5, 10, 15, 30, 60 and 179 days. The highest total residue levels were found in liver (476 µg/kg at 6 hours, declining from 105 µg/kg at 5 days, via 54 µg/kg at 15 days to less than 30 µg/kg at 30 days and thereafter) and to a lesser extent in kidney (210 µg/kg at 6 hours, and declining to 23 µg/kg at 5 days and less than 15 µg/kg at 15 days and thereafter). In muscle and fat, the total residue levels were at or below 2 µg/kg at all time points.

    Following extraction, the 15- and 30-day liver and kidney samples were analysed for altrenogest. Although altrenogest could not be measured specifically, as part of the fraction that rendered any parent compound present as well as other non-polar metabolites, it represented less than 5% of the total liver radioactivity (corresponding to less than 2 µg/kg) and approximately 20% of the total kidney radioactivity (corresponding to less than 2 µg/kg).

13. Residue experiments with horses have been carried out with radiolabelled and non-labelled altrenogest at the recommended dose (0.044 mg/kg bw/day for 10 consecutive days). In the radiolabel study, horses were slaughtered after withdrawal times of 4 hours and 15 days. At 4 hours, the highest total residues were found in the liver (1062 µg/kg) and to a lesser extent in kidney (84.1 µg/kg), muscle (12.4 µg/kg) and fat (63.9 µg/kg). These levels declined to 17.8, 1.1, 0.2 and 0.5 µg/kg, respectively, at 15 days withdrawal.

    Following extraction, the 15-day liver sample was analysed for altrenogest. As part of the fraction that rendered parent compound plus other non-polar metabolites, it represented less than 5% of the total liver radioactivity (corresponding to less than 1 µg/kg). In fact, the 15-day liver contained less than 0.12 µg/kg parent compound (including the isobaric form of altrenogest).

    In the study with non-labelled altrenogest, horses were slaughtered after withdrawal times of 4 hours, 2 and 14 days. Only at 4 hours, detectable amounts of altrenogest were measured in liver (5.5 to 17 µg/kg), kidney (4.3 to 7.5 µg/kg), muscle (1.6 to 5.8 µg/kg) and fat (6.7 to 63.6 µg/kg). At later time points, altrenogest residues were at or below the limits of quantification (1 µg/kg for muscle and 2 µg/kg for liver, kidney and fat).

14. No information is provided on the hormonal activity of the metabolites of altrenogest. In line with the metabolism of other steroids, a reduced hormonal activity of the metabolites as compared to altrenogest is expected, because metabolism will probably result in metabolites with an increased polarity. These polar metabolites are less lipid soluble and have probably less affinity for the receptor. However, the hormonal activity of conjugates of altrenogest are expected to have a hormonal activity after deconjugation in the gut. Also, the hormonal activity of the isobaric form of altrenogest, a major metabolite is unknown. Therefore, no ratio of the hormonal activity between altrenogest and the metabolites of altrenogest can be determined.

15. From the metabolism and residue data in pigs and horses it becomes clear that the parent compound altrenogest is the only possible marker residue: altrenogest is extractable, can be detected and quantified, and represents the structure with the highest hormonal activity. The ratio marker/total residues has been determined at 15- and 30-day withdrawal for liver of both pigs and horses, and for kidney of pigs.

    In liver of both pigs and horses, 80% of the total residues is irreversibly bound to tissue macromolecules and therefore inactive. Hence, only 20% of the total residues in liver is unbound and potentially active, with altrenogest constituting maximally 25% of these potentially active residues. In liver of horses on day 15, altrenogest constitutes minimally 0.5% of the unbound and potentially active residues. No minimal percentage can be determined for liver of pigs.

    In the kidney of pigs, 20% of the total residues is bound, leaving 80% of the total residues unbound and potentially active. In kidney altrenogest constitutes maximally about 25% of these potentially active residues. No minimal percentage can be determined for kidney of pigs and horses.

For muscle and fat no ratio marker to total potentially active residues could be determined because total residues were too low to allow metabolite identification. Therefore, it is assumed that all of the total residues is unbound and is altrenogest. As the residues in muscle and fat are so low at all time points, in fact no MRLs are required for these tissues. However, for residue surveillance purposes, it is necessary to establish an MRL for at least one of these tissues. In the case of altrenogest, fat is most suitable, as altrenogest is a lipophilic compound, and residues in fat are higher than in muscle.

16. Fully validated routine analytical HPLC-methods with UV detection are available for the determination of residues of altrenogest in liver, kidney, skin plus fat and muscle of pig and in kidney, liver and fat of horse. The methods are described according to ISO standard 78/2. The limits of quantification are 1.0 µg/kg in all tissues of both species. These methods did however not include a step to hydrolyse conjugates of altrenogest and the the quantified residue did not include the isobaric form.

Conclusions and recommendation

Having considered that:

- a pharmacological ADI of 0.04 µg/kg bw (2.4 µg/person) has been established,
- altrenogest was retained as the marker residue,
- the marker residue was only a small part of the total residue in the target tissue liver,
- no ratio of the hormonal activity between altrenogest and the metabolites of altrenogest could be determined,
- the extractable metabolites were assumed to have equal hormonal activity equivalent to the parent compound (worst case) and assuming that the ratio of marker to total residues of 0.25 (not worst case),
- fully validated routine analytical methods are available but do not determine the conjugates of altrenogest nor its isobaric form,
- the Applicant has committed to address the outstanding issues;

the Committee recommends, in accordance with Article 4 of Council Regulation (EEC) No 2377/90 as amended, a 2-year extension of the provisional MRLs for altrenogest, in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Altrenogest	Altrenogest	Porcine	3 µg/kg 3 µg/kg 3 µg/kg	Skin + fat Liver Kidney	For zootechnical purposes only. Provisional MRLs expire on 1.1.2005
		Equidae	3 µg/kg 3 µg/kg 3 µg/kg	Fat Liver Kidney	

Based on these MRLs values, the theoretical maximum daily intake will be equivalent to about 81% of the pharmacological ADI.

Before the Committee for Veterinary Medicinal Products can consider the inclusion of altrenogest in Annex I of Council Regulation (EEC) No. 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant should provide reports on tests showing the possible hormonal activity of the main non-bound metabolites (after deconjugation) of altrenogest in tissues of pigs and horses. If these test show that one or more metabolites are hormonally active then the applicant should provide information to determine ratio's between the marker residue and the hormonally active metabolites or include the hormonally active metabolites in the marker residue definition and adjust the routine analytical method accordingly.

2. The applicant should improve the routine analytical method for the determination of the marker residue in tissues of pigs and horses by incorporating a step in the sample preparation to hydrolyse conjugates of altrenogest. Hydrolysis of conjugates of the parent compound renders not only altrenogest but also the isobaric form of altrenogest. Therefore, this isobaric form should be part of the residue that is quantified with the method. The applicant should provide validation data for this altered method.