COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

ALTRENOGEST

SUMMARY REPORT (3)

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:

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

Additional data regarding the hormonal activity of metabolites and validation of the analytical method 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 LD₅₀ values were 176 and 233 mg/kg bw, respectively. In dogs, oral doses up to 400 mg/kg bw are well tolerated.
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 a second experiment in pigs, the urine was the main route of elimination (60 %). 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 oxydation and 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. Altrenogest could not be determined in the liver and kidney samples of day 15 and 30.

In a second radiolabel residue study, pigs were orally treated with the recommended dose (20 mg/day for 18 consecutive days), and slaughtered after withdrawal times of 4.5 hours, 7 days and 15 days. The highest total residue levels were found in the liver (1444 µg/kg at 4.5 hours, declining from 122 µg/kg after 7 days to 62 µg/kg on day 15) and to a lesser extent in the kidney (372 µg/kg at 4.5 hours, declining from 75 µg/kg on day 7 to 11.7 µg/kg on day 15). The total residue level in muscle declined from 30 µg/kg at 4.5 hours, via 7.1 µg/kg at 7 days to 3.6 µg/kg at 15 days. The main non-bound metabolites in the tissues were determined. The metabolites in the liver at 4.5 hours were partly identified using mass spectrometry (MS). The polar metabolites consisted mainly of glutathione and glucuronide conjugates of altrenogest, the isomeric form of altrenogest and hydroxylated forms of altrenogest. The non-ionic relatively polar metabolites consisted of altrenogest, isomeric altrenogest and hydroxylated forms of altrenogest. The metabolites in the non-polar fraction could not be determined. The isomeric forms of altrenogest and its conjugates can be formed from altrenogest and its conjugates under the influence of light. The altrenogest concentration in liver declined from 196 µg/kg at 4.5 hours via 0.74 µg/kg at 7 days to 0.25 µg/kg at day 15. The level in kidney reduced from 11.6 µg/kg at 4.5 hours to 0.26 µg/kg at 7 days. The levels in muscle and fat plus skin at 4.5 hours were 6.7 and 38.7 µg/kg, respectively.

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).

Additional research on these fractions and metabolites confirmed the presence of glutathione and hydrolysable conjugates of altrenogest in the polar fraction. Also some additional metabolites were partly identified as glutathione conjugates. The amount of altrenogest in kidney, muscle and fat from the mare slaughtered on day 15 could not be determined.

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. Additional information was 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 results in hydroxylated metabolites and conjugates with an increased polarity. These polar metabolites are less lipid soluble and have probably less affinity for the receptor. 6-Hydroxylation of other steroids leads to loss (less than 1 %) of the hormonal activity. However, the hormonal activity of glucuronic acid conjugates of altrenogest are expected to have a hormonal activity after deconjugation in the gut. The progestagenic activity of the polar and non-ionic relatively polar fractions of the liver of the sow slaughtered at 4.5 hours was determined using Chinese hamster ovary cells containing the human progesterone receptor B, the mouse mammary tumor virus promoter and a luciferase reporter gene. The non-hydrolysed polar fraction showed only a very low activity compared to altrenogest. After enzymatic hydrolysis of the glucuronic conjugates, the activity increased to approximately 14 % of the activity of altrenogest. The non-ionic relatively polar fraction showed an activity of approximately 21 % of altrenogest. This is below the activity expected based on the altrenogest content. The hormonal activity of the imesic form of altrenogest was very low. No information on the hormonal activity of individual metabolites could be provided with this test due to the limited available amount of each metabolite.

No information on the hormonal activity in the fractions and peaks of the liver of the mare slaughtered on day 1 and 15 could be obtained due to the low amount of residues and a matrix effect.

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. A worst case assumption of the ratio between the marker residue and the total hormonal active residue of 0.046 can be determined for the liver in sows based on the data on day 15. No ratio can be determined for kidney of pigs.

A ratio of 0.083 can be determined for the liver of horses based on the same worst case assumptions. No ratio can be determined for the kidney of horses. Because of the low amount of residues and altrenogest on day 15 and the low amount of altrenogest in the kidney compared to the liver, no MRL for the kidney is necessary.

For muscle and fat no ratio marker to total potentially active residues could be determined for both species because total residues were too low to allow metabolite identification and determination of the altrenogest concentration. 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.

15. Routine analytical HPLC-methods with MS detection described according to ISO standard 78/2 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 were validated in both species with limits of quantification of 1.0 µg/kg in muscle, fat plus skin and kidney and 0.2 µg/kg in liver of pigs and 1.0 µg/kg in fat, liver and kidney of horses. The limit of quantification of the method in horse liver is slightly above the MRL for this target tissue in horses. However considering that the same method was validated in pig liver at 0.2 µg/kg, and horses is a minor species, no further validation was considered necessary for this animal species.
Conclusion 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,
- a ratio between altrenogest and the total hormonal active residue was determined for the liver of pigs of 0.046 and for the liver of horses of 0.083,
- as no ratio between the marker and total residue can be determined for fat and muscle in both species due to the low amount of residues, only an MRL for fat at the limit of quantification is proposed in both species for monitoring purposes,
- a validated routine analytical method is available for pigs,
- a routine analytical method is available for horses validated for fat; although the limit of quantification of the method was not considered fully validated at the level of the MRL for liver, the validation data for pigs was accepted for horses,

the Committee for Medicinal Products for Veterinary Use recommends the inclusion of altrenogest for pigs and horses in Annex I of Council Regulation (EEC) No. 2377/90, in accordance with the following table:

<table>
<thead>
<tr>
<th>Pharmacologically active substance(s)</th>
<th>Marker residue</th>
<th>Animal species</th>
<th>MRLs</th>
<th>Target tissues</th>
<th>Other provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altrenogest</td>
<td>Altrenogest</td>
<td>Porcine</td>
<td>1 µg/kg 0.4 µg/kg</td>
<td>Skin + fat Liver</td>
<td>Only for zootechnical use and in accordance with the provisions of Directive 96/22/EC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Equidae</td>
<td>1 µg/kg 0.9 µg/kg</td>
<td>Fat Liver</td>
<td></td>
</tr>
</tbody>
</table>

Based on these MRLs values, the theoretical maximum daily intake will be equivalent to about 98 % of the pharmacological ADI in horses and 93 % in pigs.