



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Altrenogest (equidae and porcine species)

On 1 February 2012 the European Commission adopted a Regulation¹ modifying the maximum residue limits for altrenogest in equidae and porcine species, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Altrenogest is used in equidae and porcine species for oestrus synchronisation by oral administration.

Maximum residue limits were initially established² in 2004 for altrenogest in equidae and porcine species.

The Veterinary Medicines Directorate (United Kingdom) submitted a request for the modification of the maximum residue limits for altrenogest to the European Medicines Agency, on 6 June 2011.

Based on the information available, the Committee for Medicinal Products for Veterinary Use recommended, on 13 October 2011, the modification of the maximum residue limits for altrenogest in equidae and porcine species.

Subsequently the Commission recommended, on 16 December 2011, the modification of the maximum residue limits in equidae and porcine species. This recommendation was confirmed on 6 January 2012 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 1 February 2012.

¹ Commission Regulation (EU) No 85/2012, O.J. L30, of 02.02.2012

² Commission Regulation (EU) No 85/2012, O.J. L30, of 02.02.2012



Summary of the scientific discussion for the establishment of MRLs

Substance name:	Altrenogest
Therapeutic class:	Agents acting on the reproductive system
Procedure number:	EU/ART11/11/188/VMD
Applicant:	Veterinary Medicines Directorate
Target species:	Porcine, <i>Equidae</i>
Intended therapeutic indication:	Oestrus synchronisation
Route(s) of administration:	Oral

1. Introduction

Altrenogest (synonym: 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, its liposolubility allows it to penetrate 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.

Altrenogest was previously assessed by the CVMP and the pharmacological ADI of 0.04 µg/kg bw, i.e 2.4 µg/person was established as the overall ADI.

Currently altrenogest is included in table 1 of the Annex to Commission Regulation (EU) No 37/2010 of 22 December 2009 in accordance with the following table:

Pharmaco- logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Altrenogest	Altrenogest	Porcine	1 µg/kg 0,4 µg/kg	Skin and fat Liver	Only for zootechnical use and in accordance with the provisions of Directive 96/22/EC.	Agents acting on the reproductive system
		<i>Equidae</i>	1 µg/kg 0,9 µg/kg	Fat Liver		

On 6 June 2011 the United Kingdom submitted to the European Medicines Agency a request for the Committee for Medicinal Products for Veterinary Use (CVMP) to review its opinion on the maximum residue limits for altrenogest in pigs and horses as a result of new information.

In support of the request the UK indicated that, since the establishment of MRLs for altrenogest, the scientific community has accepted new approaches in the way that uncertainty factors may be derived and that this could influence the ADI for altrenogest, allowing the modification of MRLs. In addition, pharmacovigilance data, residue monitoring results, and residue data not previously evaluated by the Committee were provided for consideration.

2. Scientific risk assessment

2.1. Safety assessment

In response to the request from the UK the CVMP reviewed its previous safety evaluation, focusing on the derivation of the ADI and on the uncertainty factors used for this purpose.

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties including mode of action

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. A no-hormonal-effect level of 4 µg/kg bw/day was established in monkeys receiving altrenogest during three menstrual cycles (effects on menstrual cycle length and serum hormonal concentrations).

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

Pharmacokinetic properties

There is a 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.

A review of residue data in pigs showed limited variation in plasma radioactivity profiles of individual animals following oral administration of tritiated altrenogest. After 7 days of oral administration with 20 mg altrenogest per day to 7 pigs the average standard deviation was approximately 22% of the mean; after 18 days of oral administration with 20 mg altrenogest per day to 12 pigs the average standard deviation was approximately 25% of the mean; after 18 days of oral administration with 20 mg altrenogest per day to 14 pigs the average standard deviation was approximately 23% of the mean.

2.1.2. Calculation of pharmacological ADI, if relevant

The basis for the pharmacological ADI is the NOAEL of 4.0 µg/kg bw/day observed in monkeys and pigs. The NOAEL for pharmacological effects was concluded to be the same as the NOAEL for toxicological effects. The derivation of the ADI and a discussion of the uncertainty factors used in the derivation of the ADI is provided in section 2.1.4.

2.1.3. Overview of toxicology

Single-dose toxicity

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.

Repeated dose toxicity

Several repeated dose toxicity studies using oral administration of altrenogest are available. In rats, a 2-month study using doses of 0, 0.5 and 2 mg/kg bw/day led to a NOAEL of 0.5 mg/kg bw/day, a 13-week study using doses of 0, 1, 10 and 100 mg/kg feed, equal to 0.06 to 7.82 mg/kg bw/day, led to a NOAEL of 0.06 mg/kg bw/day and a 1-year study using doses of 0, 2, 10 and 50 mg/kg feed, equal to 0.15 to 4.58 mg/kg bw/day, led to a NOAEL of 4.3 mg/kg bw/day was established.

In dogs a 1-year study using doses of 0, 0.04, 0.2, 1 mg/kg bw/day led to a LOAEL of 0.04 mg/kg bw/day.

In these studies, effects were found which were directly related to the pharmacological activity of altrenogest (decreased weights/histopathology in the hormone dependent organs), resulting in an overall oral LOAEL of 0.04 mg/kg bw/day.

Tolerance in the target species

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.

Reproductive toxicity, including developmental toxicity

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 NOAEL of 0.4 mg/kg feed (equal to 0.03 mg/kg bw/day). No indications of teratogenic effects were found in the teratology phase of the 2-generation reproduction study in rats or in a tolerance study in pigs receiving 20 mg altrenogest/day on days 28 to 112 of pregnancy.

Genotoxicity and carcinogenicity

A battery of genotoxicity tests have been undertaken using altrenogest. In vitro tests include Ames tests (at concentrations of 10 to 10,000 µg/plate, with and without metabolic activation), assays of forward mutation at the TK locus in mouse lymphoma L5178Y cells (at concentrations of 30 to 100 µg/ml with metabolic activation and 22 to 70 µg/ml without metabolic activation), assays of forward mutation at the HGPRT locus in Chinese hamster ovary cells (at concentrations of 25 to 50 µg/ml with metabolic activation and 1 to 10 µg/ml without metabolic activation), chromosome aberration assays in Chinese Hamster ovary cells (at concentrations of 5 to 50 µg/ml with metabolic activation and 1 to 10 µg/ml without metabolic activation), DNA repair tests in HeLa S3 cells (at concentrations of 12.5 to 200 µg/ml with and without metabolic activation) and in primary rat hepatocyte cultures (at concentrations of 0.01 to 2 µg/ml). Altrenogest was tested in an in vivo chromosome aberration assay in rats after both single administration (one intragastric dose of 100 mg/kg bw) and repeated administration (4 daily doses of 25 or 50 mg/kg bw).

Although positive results were seen in the forward mutation assay in mouse lymphoma L5178Y cells with metabolic activation, all other tests produced negative results. Overall it was concluded that altrenogest does not show genotoxic potential. Consequently, long-term toxicity/carcinogenicity studies were not required.

Between 1997 and 1999, new data became available on the genotoxicity and carcinogenicity of steroid hormones, although not including altrenogest. These data, mainly concerning 17 β -oestradiol, were reviewed by the Joint FAO/WHO Committee on Food Additives (JECFA), by the Scientific Committee on Veterinary Measures Relating to Public Health (SCVPH) of the European Commission and by the International Agency for Research on Cancer (IARC), as well as by the CVMP (see section 2.1.8). The CVMP concluded, in 1999, 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. Based on the conclusion that the tumourigenic effect of steroid hormones was not a result of direct genotoxic activity, the mechanism of action was considered to be a threshold based effect and not to represent a barrier to the establishment of an ADI.

Studies of other effects including immunotoxicity and neurotoxicity

No specific studies of immunotoxicity or neurotoxicity were performed but as there were no relevant effects were seen in standard toxicity studies this is acceptable.

2.1.4. Calculation of the toxicological ADI or alternative limit

The most sensitive effects from pharmacology and toxicology studies were related to the same mechanism of action with the same overall NOAEL. Therefore, the pharmacological and the toxicological ADI are indistinguishable.

The previous ADI was established using a standard uncertainty factor of 100, representing a factor of 10 to account for possible interspecies variation and a factor of 10 to account for possible intraspecies variation. However, since establishment of the original ADI there have been developments in the way uncertainty factors may be established (specific reference is made to the IPCS/WHO publication of 2005 on the issue of uncertainty assessment), with the possibility of deviating from the standard inter- and intra-species uncertainty factors based on chemical specific adjustment factors. According to this approach the intra- and inter-species uncertainty factors are each divided into two components, one representing pharmacodynamic sources of variation and the other representing pharmacokinetic sources of variation. Chemical specific information can then be used to justify deviation from the standard uncertainty sub-factors. The IPCS recommends standard sub-factors of 2.5 for pharmacodynamics and 4 for pharmacokinetics when considering between (inter-)species variation, and sub-factors of 3.16 and 3.16 when considering within (intra-)species variation. Multiplied, these sub-factors equate to the standard uncertainty factor of 100.

For altrenogest the following observations may contribute to justification for refinement of the uncertainty factor:

- A. Interspecies – dynamics: Quite often in risk assessment, the underlying mechanism of the toxic effect is not known. In the case of altrenogest, the mechanism is related to the hormonal activity, which requires binding to progesterone receptors. This is a well known process and conserved across all mammalian (and many other) species. This fact provides for less uncertainty with regard to the (pharmaco)dynamic aspects when compared to many other chemical substances.
- B. Interspecies – kinetics: Exogenous chemical substances may undergo different metabolism depending on the animal species. However, the pathways of production and metabolism of (natural) hormones is quite common across mammalian species, therefore also the metabolism of hormone analogues (such as altrenogest) can be expected to follow similar pathways in

these species. This fact provides for less uncertainty with regard to (pharmaco)kinetic aspects when compared to many other chemicals.

C. Interspecies – kinetics: The pharmacokinetic behaviour of altrenogest appears to be similar in pigs and horses (as described in section 2.1.1). After oral administration in these species, the peak concentration is reached within 3 to 6 hours, and the plasma concentration shows a biphasical decline, with relatively long terminal plasma elimination half-life. These data are supportive of a limited interspecies variation with regard to kinetics.

D. Interspecies – kinetics: Normally, the ADI is derived from studies in a laboratory species while the consumer will be exposed to residues formed in the food producing target species. To ensure that the metabolites formed in the target species have been (intrinsically) tested in the laboratory species, a comparative metabolism study is conducted in the laboratory species, and a qualitative (not quantitative!) comparison of the metabolite patterns is made. In the case of altrenogest however, the ADI was based on studies with monkeys and pigs, while the pig is the major target species for altrenogest. Therefore, the effects of residues of altrenogest formed in the pig are directly covered in the ADI, without extrapolation. Moreover, the NOAEL of altrenogest in pigs was confirmed by a study in monkeys and was considered the overall NOAEL to be used for the ADI. This is quite a unique situation in MRL assessments, and provides for less extrapolation hence less uncertainty.

E. Interspecies – dynamics+kinetics: The overall NOAEL is considered to be robust, because (1) studies of 90 days or longer were conducted in four different animal species (the standard is two) (2) consistent effects were observed in all four species (3) the overall NOAEL was derived from two species, the monkey and the pig, and (4) the overall NOAEL is both the pharmacological NOAEL and the toxicological NOAEL. The robustness of the overall NOAEL for altrenogest in itself provides for less uncertainty.

F. Interspecies – dynamics+kinetics: Despite the different choices of dose levels in the various studies, the threshold for hormonal effects appeared to be very similar in the different species. The monkey and the pig had the same NOAEL of 0.004 mg/kg bw/day. For the dog no NOAEL could be established but the LOAEL in dogs was equal to the LOAEL in pigs. The rat appeared to be slightly less sensitive, however the NOAEL in rats (0.030 mg/kg bw/day) was in between the NOAEL and the LOAEL for pigs. These data from four species support a low variation between species and confirm the more mechanistic considerations given above.

G. Interspecies – dynamics+kinetics: Generally, the ADI is based on studies in small rodents or dogs. In the case of altrenogest however, the overall NOAEL was derived from primates, which are much closer related to humans than e.g., rodents. A closer species relationship means a smaller extrapolation step, hence less uncertainty.

H. Interspecies – dynamics+kinetics: Human studies with norgestomet, another synthetic progestagen with the same mode of action as altrenogest, indicated that humans are not more sensitive to its hormonal effects than monkeys (CVMP MRL Summary report EMEA/CVMP/208625/2004-FINAL, published in 2005).

I. Intraspecies – dynamics+kinetics: The data on intraspecies variation are limited. For the monkey study, no individual data are presented. However, given the relatively low standard deviations of the mean cycle length, and the clear cut-off for that effect between 0.004 and 0.0086 mg/kg bw/day, it can be concluded that the intraindividual variation was quite small.

J. Intraspecies – dynamics+kinetics: Also in the pig 90-days study, none of the pigs showed effects at the lowest dose, while all animals at the next dose up showed effects, albeit to various extents. Other studies in pigs showed limited variation in plasma kinetics between individuals. From the data from both pigs and monkeys (H), it is concluded that although animals may react to a different extent following altrenogest exposure, there appears to be very little variation in the actual thresholds for expressing effects.

In summary, ten observations have been made that suggest a lower than standard level of uncertainty.

Observations A, B, and G are of a theoretical nature, and are supported by the other observations, which are of an empirical nature.

Observation A through H are related to the interspecies part of uncertainty, whereas observations I and J deal with the intraspecies part of it. Both dynamic and kinetic parts of uncertainty are addressed.

It is clear that the standard subfactors are merely a further subdivision of the arbitrary 10x10 factors, rather than a robust quantification of uncertainty. Likewise, it will be quite difficult to actually quantify the uncertainty for a specific substance. Even in the case of altrenogest, where a number of observations clearly indicate a lower level of uncertainty compared to average cases, a precise quantification cannot be achieved. However, a relative reduction of the uncertainty factors can be considered.

The interspecies factor

Given the observations A through H, and in particular considering that the effects were studied in four species, including primates, showing similar effects at similar thresholds, it is agreed to reduce the standard interspecies factor of 10 down to 2. This still represents a cautious approach because with regard to the most sensitive effect, the average human is still considered to be two times more sensitive than the most sensitive species studied.

The intraspecies factor

While observations I and J do indicate a lower uncertainty regarding intraspecies variation than the average case, the data are less robust than those related to interspecies variation and data on the use of altrenogest in humans are not available. Consequently, although there is some evidence to suggest that intraspecies variation may be limited, the available data were not considered robust enough to justify the use of a reduced uncertainty factor. The standard uncertainty factor of 10 is retained to account for intraspecies variation.

The overall uncertainty factor for altrenogest

Considering inter- and intraspecies uncertainty factors of 2 and 10 respectively, the overall uncertainty factor is 20. This would result in an ADI that is twenty times lower than the level that causes no effects on the most sensitive endpoints in the most sensitive and relevant species. The overall uncertainty factor of 20 is believed to be representative for all observations made on the pharmacology and toxicology of altrenogest.

Applying an overall uncertainty factor of 20 to the overall NOAEL of 4 µg/kg bw/day, an ADI of 0.20 µg/kg bw is established (equivalent to 12 µg for a 60 kg person).

2.1.5. Overview of microbiological properties of residues

No data were provided but as microbiological effects are not expected for this type of substance this is acceptable.

2.1.6. Calculation of microbiological ADI

No microbiological ADI was calculated as microbiological effects are not expected for this type of substance.

2.1.7. Observations in humans

No data on the effects of altrenogest in humans were provided.

2.1.8. Findings of EU or international scientific bodies

The data on the genotoxicity and carcinogenicity of steroid hormones (although not altrenogest in particular) were 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. JECFA and IARC concluded that the tumourigenic action of steroid hormones, in particular 17 β -oestradiol, were the consequence of the receptor-mediated cell division stimulating activity of these compounds in somatic target cells and that the potential genotoxic properties of the compounds would not be expressed *in vivo* and would not play a role in the tumourigenic activity. These conclusions are consistent with those of the CVMP. The SCVPH, which did not specifically consider altrenogest, considered that no threshold level could be identified for the toxicological effects of the six hormones it evaluated (17 β -oestradiol, testosterone, progesterone, trenbolone, zeranol and melengestrol) and that consequently no ADI could be established. The SCVPH reviewed its original opinion in 2002 and concluded that it was still valid.

2.1.9. Overall conclusions on the ADI

Both pharmacological studies and toxicological studies showed that the most sensitive effects of altrenogest were related to its hormonal activity, with an overall NOAEL of 4 μ g/kg bw/day. Using a refined uncertainty factor of 20, an overall ADI of 0.2 μ g/kg bw can be established. No microbiological ADI was established as no microbiological effects are expected for this substance.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

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 in the urine. In a second experiment in pigs, the urine was the main route of elimination (60%). In horses, approximately 44% of the administered dose is excreted in urine and approximately 53% in faeces within 24 hours.

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 oxidation and conjugation. Dealkylation (rendering trenbolone) does not occur.

Information was also 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, glucuronic acid conjugates of altrenogest are expected to have 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 tumour virus promotor 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 isomeric 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.

2.2.2. Residue depletion studies

Several residue depletion studies in pigs have been carried out using radiolabelled and non-labelled 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 to 105 µg/kg at 5 days, to 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 from 15 and 30 days after administration.

In a second radiolabel residue study, pigs were administered the recommended dose by the oral route (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 to 122 µg/kg after 7 days and to 62 µg/kg on day 15) and to a lesser extent in the kidney (372 µg/kg at 4.5 hours, declining to 75 µg/kg on day 7 and to 11.7 µg/kg on day 15). The total residue level in muscle declined from 30 µg/kg at 4.5 hours, to 7.1 µg/kg at 7 days and to 3.6 µg/kg at 15 days. In skin plus fat, the total residue level declined from 91 µg/kg at 4.5 hours, to 3.6 µg/kg at 7 days and to 1.6 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 to 0.74 µg/kg at 7 days and to 0.25 µg/kg

at day 15. The level in kidney declined 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 58.7 µg/kg, respectively.

Two non-radiolabeled residue studies in pigs, not previously evaluated by the CVMP, were provided.

In one study, pigs were administered the recommended dose by the oral route (20 mg/day for 18 consecutive days), and slaughtered after withdrawal times of 1, 7, 14, and 21 days. On day 1, the average altrenogest concentrations were 4.70 µg/kg in muscle, 55.27 µg/kg in skin and fat, 85.37 µg/kg in liver, and 9.16 µg/kg in kidney. At all subsequent slaughter times, the residue concentrations of altrenogest were below the LOQ of 1.25 µg/kg in all tissue samples.

The second cold residue study in pigs also employed the recommended dose, after which the animals were slaughtered at 7, 14, and 21 days. In this study, only liver samples were analysed, using an analytical method with a lower limit of quantification of 0.125 µg/kg. The altrenogest concentrations ranged from 1.045 to 2.519 µg/kg at day 7, from 0.639 to 1.471 µg/kg at day 14, and from below limit of quantification to 0.229 µg/kg at day 21.

Residue studies in 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 a study using radiolabelled altrenogest, 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 the parent compound plus other non-polar metabolites, altrenogest 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. 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.

In a study with non-labelled altrenogest, horses were slaughtered after withdrawal times of 4 hours, 2 and 14 days. Detectable amounts of altrenogest were measured at 4 hours only 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).

In a non-radiolabelled residue study in horses that had not previously been evaluated by the CVMP, animals were treated orally at the recommended dose and for the recommended duration and slaughtered at 7, 14, and 21 days. The altrenogest concentrations in all tissue samples taken from liver, kidney and perirenal fat were below the LOQ of 1 µg/kg at all time points, with the exception of one fat sample, from a horse slaughtered at 7 days, which contained a concentration of 2.81 µg/kg.

Selection of marker residue and target tissues

The marker residue was previously established as the parent compound, altrenogest, although this substance makes up only a small part of total residues in the target tissue liver and is not present at

measurable levels in kidney, muscle or fat. Evaluation of the new data provides no reason to change the marker residue, which remains altrenogest.

The marker to total residues ratio in liver was established based on residue data at day 15 after drug administration. Levels of altrenogest residues were quantified in different liver HPLC fractions based on radioactivity (expressed as µg equivalents of [¹⁴C]altrenogest). The progestagenic activity of the fractions was then determined (based on activation of the human progesterone receptor B in an *in vitro* cell based assay) and compared to the activity of parent altrenogest. This allowed the concentration of hormonally active residues in the fractions to be expressed as µg equivalents of altrenogest per kg. In this way it was possible to establish the total hormonal activity of residues in the liver (expressed as µg equivalents of altrenogest). The marker to total ratio was then established by comparing the concentration of altrenogest to the concentration of total hormonally active residues.

In this way the marker to total residues (with hormonal activity) ratio was established as 0.046 for pig liver and 0.083 for horse liver.

No ratio of marker to total residues could be determined for kidney, fat and muscle in either species due to the low amount of residues present.

As the residues in muscle and fat are very low at all time points, no MRLs were established 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 more suitable than muscle, as altrenogest is a lipophilic compound, and residues in fat are higher than in muscle.

2.2.3. Monitoring or exposure data

Pharmacovigilance data (Periodic Safety Update Reports for a number of altrenogest-containing products covering the use of these products up to 2010) indicate that there have been no suspected adverse reactions reported which involve the detection of residues in food.

Residues surveillance data held in the EU database indicate that there have been no non-compliant results for altrenogest in the past five years.

2.2.4. Analytical method for monitoring of residues

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 and 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 and 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 limits of quantification are acceptable, given the the MRL values proposed in this report. No further validation is considered necessary. The method has been reviewed by the relevant European Reference Laboratory, which confirmed the overall suitability of the method.

2.2.5. Findings of EU or international scientific bodies

No evaluations by other international committees were available.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

No data were available for review but in view of the nature of the substance such data are not considered necessary.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

No such considerations were identified.

3.3. Elaboration of MRLs

Further to the review of the safety assessment the ADI has been increased by five-fold. Applying the existing ratios of marker residue to total residues with hormonal activity, it can be concluded that there is scope to increase the MRL by up to five-fold. However, in order to leave a part of the ADI free for unexpected or changed situations, it is recommended to increase the MRLs by only four fold.

In its previous evaluation the CVMP did not recommend MRLs for kidney and muscle, because these tissues contained very low levels of residues and were therefore considered not suitable for residue surveillance. The establishment of MRLs in these tissues would require new residue depletion studies for already authorised products and would not further contribute to the protection of consumer safety. Consequently the establishment of MRLs in kidney and muscle is not recommended. MRLs for fat were previously recommended based on the limit of quantification.

Further to the above considerations the CVMP recalculated the MRLs for porcine species to be 4 µg/kg for skin and fat, and 2 µg/kg for liver. The MRLs for *equidae* were recalculated to be 4 µg/kg for both fat and for liver. These values have been rounded.

Calculation of the Theoretical Maximum Daily Intake

While altrenogest could not be quantified in kidney, muscle or fat, it was considered important to take account of possible hormonally active residues in these tissues when calculating the theoretical maximum daily intake. To do this, the daily intake of residues resulting from ingestion of liver has first been derived in the normal way. Total residue levels in kidney, muscle and fat have then been compared to those in liver in order to establish the relative distribution of total residues. It is assumed that the distribution of hormonally active residues parallels the distribution of total residues, and on this basis levels of hormonally active residues in kidney, muscle and fat were estimated based on the distribution of residues between liver and other tissues.

Calculation of daily intake of hormonally active residues from ingestion of pig liver:

Tissue	MRL (µg/kg)	M:T (altrenogest : hormonally active residues)	Total hormonally active residues (µg/kg)	Consumption (kg)	Total intake (µg)
Liver	2	0.046	43.5	0.1	4.4

Calculation of daily intake of hormonally active residues from ingestion of pig tissues:

Tissue	Total residues on day 15 (µg/kg)	Fraction of total residues compared to liver	Hormonally active residues (µg/kg)	Consumption (kg)	Daily intake of hormonally active residues (µg)	% ADI
Liver	6.5	1.0	43.5	0.1	4.4	37
Kidney	3.8	0.58	25.2	0.05	1.3	11
Muscle	2.0	0.31	13.5	0.3	4.0	33
fat	1.3	0.2	8.7	0.05	0.4	3
Total % ADI						84

Calculation of daily intake of hormonally active residues from ingestion of horse liver:

Tissue	MRL (µg/kg)	M:T (altrenogest : hormonally active residues)	Total hormonally active residues (µg/kg)	Consumption (kg)	Total intake (µg)
Liver	4	0.083	48.2	0.1	4.8

Calculation of daily intake of hormonally active residues from ingestion of horse tissues:

Tissue	Total residues on day 15 (µg/kg)	Fraction of total residues compared to liver	Hormonally active residues (µg/kg)	Consumption (kg)	Daily intake of hormonally active residues (µg)	% ADI
Liver	0.96	1.0	48.2	0.1	4.8	40
Kidney	0.55	0.57	27.5	0.05	1.4	12
Muscle	0.2	0.21	10.1	0.3	3.0	25
fat	0.5	0.52	25.1	0.05	1.3	11
Total % ADI						88

Based on these MRLs, the worst case theoretical daily intake is 84% for pigs and 88% for horses. This calculated intake covers the intake of hormonally active residues from kidney, muscle and fat.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EU) No 470/09, the Committee considered the possibility of further extrapolating the existing MRLs to other species and foodstuffs with a view to ensuring the availability of veterinary medicinal products for conditions affecting food-producing animals while ensuring a high level of protection of human health.

Other food producing species where oestrus synchronisation is applied are ruminants (cattle, sheep and goats). However, no pharmacokinetic or residue data were available for ruminants. In the absence of these data and considering the particularities of the gastrointestinal anatomy and physiology of ruminants, no scientific grounds were identified on which to base a conclusion that the pharmacokinetic behaviour of altrenogest, an orally administered substance, will be similar in ruminants to that seen in species for which data are available. Therefore, extrapolation of the MRLs to ruminants is not recommended.

Given that the reproductive cycle is significantly different in birds and fish compared to mammals, altrenogest is not considered to represent a useful zootechnical tool in birds or fish. Extrapolation to birds and fish is, in any case, not warranted from an availability point of view.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- an ADI of 0.2 µg/kg bw (i.e. 1.2 µg/person) was established as the overall ADI for altrenogest, using a refined uncertainty factor of 20;
- the parent compound altrenogest was retained as the marker residue;
- the ratios of marker to total hormonal active residues calculated in liver at 15 days were 0.046 for pigs and 0.083 for horses;
- residue concentrations were persistently low in kidney, fat and muscle in both species but fat was chosen as the target tissue for monitoring of residues in the carcass;
- residues of hormonally active residues in other tissues were quantified relative to the levels in liver;
- a validated routine analytical method for monitoring of residues of altrenogest in edible porcine and equine liver and fat is available;
- in the absence of appropriate data extrapolation of the MRLs to species other than pigs and horses was not recommended;

the Committee recommends the modification of the maximum residue limits for altrenogest in accordance with the following table:

Pharmaco- logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Altrenogest	Altrenogest	Porcine	4 µg/kg 2 µg/kg	Skin and fat Liver	Only for zootechnical use and in accordance with the provisions of Directive 96/22/EC.	Agents acting on the reproductive system
		<i>Equidae</i>	4 µg/kg 4 µg/kg	Fat Liver		

4. Background information on the procedure

Submission of the request	6 June 2011
Steps taken for assessment of the substance	
Clock started:	7 June 2011
CVMP opinion adopted:	12 October 2011