



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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EMA/CVMP/339062/2014
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Methylprednisolone (*Equidae*)

On 30 January 2015 the European Commission adopted a Regulation¹ establishing maximum residue limits for methylprednisolone in *Equidae*, 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.

Methylprednisolone is used in cattle for the treatment of respiratory disease and urogenital infections and in *Equidae*, is intended for the treatment of pain and lameness associated with arthritic conditions.

Methylprednisolone had maximum residue limits already established² for bovine species.

Zoetis Belgium SA submitted the application for the extension of maximum residue limits to the European Medicines Agency, on 22 January 2014.

Based on the original data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 10 July 2014 the extension of maximum residue limits for methylprednisolone to *Equidae*.

Subsequently the Commission recommended on 15 November 2014 that maximum residue limits in *Equidae* are established. This recommendation was confirmed on 6 December 2014 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 30 January 2015.

¹ Commission Implementing Regulation (EU) No 2015/149, O.J. L26 , of 31.01.2015

² Commission Regulation (EU) No 77/2002, O.J. L 16, of 18.01.2002



Summary of the scientific discussion for the establishment of MRLs

Substance name:	Methylprednisolone
Therapeutic class:	Corticoids / Glucocorticoids
Procedure number:	EMA/V/MRL/002964/EXTN/0004
Applicant:	Zoetis Belgium SA
Target species:	Equidae
Intended therapeutic indication:	Treatment of pain and lameness associated with arthritic conditions
Route(s) of administration:	Intra-articular

1. Introduction

Methylprednisolone, a 6 α -methyl derivative of prednisolone, is a synthetic corticosteroid, which is used in veterinary medicine as a free alcohol, and as various esters. As a free alcohol methylprednisolone is used in the treatment of respiratory disease and urogenital infections in cattle by intramuscular injection combination products with antibiotics.

Methylprednisolone is also used in the treatment of respiratory disease and urogenital infections in cattle by intramuscular injection in combination with antibiotics.

The substance is also used in human medicine (as a free alcohol, acetate, aceponate and as the sodium succinate).

Methylprednisolone was previously assessed by the CVMP and an ADI of 0.16 $\mu\text{g}/\text{kg}$ bw, i.e 9.6 $\mu\text{g}/\text{person}$, established.

Currently, methylprednisolone is included in Table 1 of the Annex to Commission Regulation (EU) No 37/2010 in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Methylprednisolone	Methyl-prednisolone	Bovine	10 $\mu\text{g}/\text{kg}$ 10 $\mu\text{g}/\text{kg}$ 10 $\mu\text{g}/\text{kg}$ 10 $\mu\text{g}/\text{kg}$ 2 $\mu\text{g}/\text{kg}$	Muscle Fat Liver Kidney Milk	NO ENTRY	Corticoids / Glucocorticoids

On 22 January 2014 Zoetis Belgium SA submitted to the European Medicines Agency an application for the extension of maximum residue limits for methylprednisolone to horses.

In horses the intended use concerns methylprednisolone acetate for the treatment of pain and lameness associated with arthritic conditions. The proposed dosing regimen consists of up to two intra-articular doses of 120 mg within a 2 week interval.

2. Scientific risk assessment

2.1. Safety assessment

The consumer safety of methylprednisolone was previously assessed by the CVMP and a pharmacological ADI of 0.16 µg/kg bw, i.e. 9.6 µg/person established. This was based on the no observed effect level (NOEL) of 16 µg/kg bw/day, which was established in an oral study investigating tyrosine aminotransferase activity in rats, and applying a safety factor of 100.

No further assessment regarding the consumer safety of the substance is required for the purpose of this extension application.

2.2. Residues assessment

A reduced data package was submitted for evaluation taking into account that horses are considered a minor species and therefore the requirements laid down in the guideline on safety and residue data requirements for veterinary medicinal products intended for minor uses or minor species (EMA/CVMP/SWP/66781/2005) and the note for guidance on the risk analysis approach for residues of veterinary medicinal products in food of animal origin (EMA/CVMP/187/00-FINAL) apply.

2.2.1. Pharmacokinetics in target species

No proprietary data on the pharmacokinetics of methylprednisolone acetate in horses have been presented; however, several published studies have been provided, which demonstrate that methylprednisolone acetate is readily transformed to methylprednisolone in horses.

2.2.2. Residue depletion studies

A non-radiolabel residue depletion study, conducted according to GLP, was provided. Twenty horses were administered a formulation of methylprednisolone acetate at a dose of 120 mg per horse (irrespective of bodyweight) directly into the femorotibial joint (i.e. intra-articularly) as a single injection. This dose was repeated 14 days after the first administration, into the same site.

Samples of liver, kidney, muscle and peri-renal fat were taken at 12, 24, 72, 120 and 168 hours after the final treatment, from four horses per timepoint. The highest concentrations of residues were measured at the 12-hour timepoint in all tissue types, and depleted thereafter.

Mean residues at 12 hours were 31.33 µg/kg in liver, 11.04 µg/kg in kidney, 2.47 µg/kg in fat and 5.98 µg/kg in muscle. The limit of quantification of the analytical method used was 2.00 µg/kg for all tissues. Residue concentrations in all samples were below the limit of quantification by 72 hours after the final treatment, except in liver, where residues persisted at levels above the limit of quantification, but less than 3 times the limit of quantification (i.e. between 2 – 6 µg/kg) from 72 hours after treatment until the final timepoint at 168 hours after treatment.

Selection of marker residue and ratio of marker to total residues

The marker residue established for bovine species was the parent compound, methylprednisolone. The residue depletion study described above demonstrates that methylprednisolone is present in horse tissues and consequently, in line with the CVMP guideline on safety and residue data requirements for

veterinary medicinal products for minor uses and minor species (EMA/CVMP/SWP/66781/2005), methylprednisolone is considered an appropriate marker for monitoring of residues in food commodities produced from treated horses. In its MRL evaluation for methylprednisolone in bovine species, the CVMP concluded that the metabolites of methylprednisolone are biologically inert and that consequently a marker to total residues ratio of 1 could be accepted. This is also considered to be the case for residues in horses.

2.2.3. Monitoring or exposure data

No monitoring or exposure data other than that described elsewhere in this report were available.

2.2.4. Analytical method for monitoring of residues

An analytical method, using reversed-phase high performance liquid chromatography coupled with mass spectrometry detection (LC-MS/MS), for the assay of methylprednisolone in equine edible tissues (liver, kidney, muscle and fat) was validated in line with the requirements described in the CVMP guideline on safety and residue data requirements for veterinary medicinal products for minor uses and minor species (EMA/CVMP/SWP/66781/2005).

Tissue samples were homogenised with a sodium acetate/methanol mixture. Methylprednisolone and flumethasone (used as internal standard) were extracted from equine tissues by solid/liquid extraction followed by solid phase extraction and were then separated on a C18 analytical column with binary gradient elution using acetic acid in purified water and methanol. Multiple reaction monitoring (MRM) experiments with electrospray ionisation (ESI) source were performed to detect ion transitions of $[M+CH_3COOH]^-$ in negative ion mode. The limit of quantification in liver, kidney, muscle and fat was 2.00 µg/kg. The reported limits of detection in liver, kidney, muscle and fat were 0.173, 0.260, 0.185 and 0.413 µg/kg respectively.

The relevant European Reference laboratory has reviewed the proposed analytical method and agrees that it has been validated in line with Volume 8 of the Notice to Applicants.

2.2.5. Findings of EU or international scientific bodies

No relevant reports relating to residues of methylprednisolone in horses were identified.

3. Risk management considerations

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

Microbiological effects are not expected for this type of substance therefore no data were required.

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

Horses are considered a minor species and therefore the guideline on safety and residue data requirements for veterinary medicinal products intended for minor uses or minor species

(EMA/CVMP/SWP/66781/2005) and the note for guidance on the risk analysis approach for residues of veterinary medicinal products in food of animal origin (EMA/CVMP/187/00-FINAL) were taken into account for the evaluation of the reduced data package which complied with the requirements of the guidelines mentioned above.

3.3. Elaboration of MRLs

Methylprednisolone, the marker residue established for monitoring of residues in bovine tissues, has been demonstrated to be an appropriate marker for monitoring of residues in equine tissues, and an analytical method appropriate for monitoring of residues in equine tissues has been provided. Consequently, in line with the CVMP guideline on safety and residue data requirements for veterinary medicinal products for minor uses and minor species (EMA/CVMP/SWP/66781/2005), the MRLs established in bovine tissues can be extended to equine tissues and are as follows:

Muscle: 10 µg/kg

Fat: 10 µg/kg

Liver: 10 µg/kg

Kidney: 10 µg/kg

It is noted that in the residue depletion study conducted in horses, the route of administration was intra-articular, which is unusual for an MRL application. However, since MRLs are not route-specific, and are generally based on systemic distribution of the substance to each of the edible tissues, this is not considered to have an impact on the proposed MRLs.

The MRLs for bovine species were established after consideration of a study conducted using the intramuscular route of administration, and relatively persistent residues at the site of injection were seen. Relatively persistent residues may also be seen following intramuscular injection in horses. National authorities should take this into account for the establishment of the withdrawal period in case of an intended intramuscular administration, in order to ensure that residues at the injection site do not exceed the muscle MRL.

Calculation of theoretical daily intake of residues

Tissue	Consumption per day (kg)	Proposed MRLs (µg/kg)	Ratio of marker to total residues	Daily intake of residues (µg)
Muscle	0.3	10	1	3.0
Fat	0.05	10	1	0.5
Liver	0.1	10	1	1.0
Kidney	0.05	10	1	0.5
Milk	1.5	2	1	3.0
Total daily intake of residues (µg)				8.0
TMDI as % pharmacological ADI (9.6 µg/person) used				83.3%

Based on the proposed MRLs for equine tissues and the existing MRL for bovine milk, the theoretical maximum daily intake is calculated to be 83.3% of the ADI.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EC) No 470/2009, the CVMP considered the possibility of extrapolating its recommendation on maximum residue limits for methylprednisolone in horses to other food producing species and commodities. However, the horse is considered a minor species in the EU with regards to food production and consequently the residue depletion data provided were based on reduced data requirements for a minor species, in line with the CVMP Guideline on safety and residues data requirements for veterinary medicinal products intended for minor uses or minor species (EMA/CVMP/SWP/66781/2005). The CVMP does not consider it appropriate to extrapolate MRLs derived using a reduced data package to other species.

An MRL has been established for milk of bovine species. Considering that it has been demonstrated that methylprednisolone is a suitable marker residue for both species' tissues, it is likely that this would also be the case for milk. In addition, it can be considered that the analytical method validated for monitoring of residues in bovine milk would also be expected to be applicable to horse milk. Consequently the bovine milk MRL can be extrapolated to horse milk.

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

Having considered that:

- the pharmacological ADI of 0.16 µg/kg bw (i.e. 9.6 µg/person) was established as the overall ADI for methylprednisolone,
- the horse is considered a minor species and therefore the data requirements are those defined in the Note for guidance on the risk assessment analysis approach for residues of veterinary medicinal products in food of animal origin (EMA/CVMP/187/00-FINAL),
- the marker residues, methylprednisolone, established for cattle also exists in horse tissues and can be retained as the marker residue for horses as well,
- an analytical method for the monitoring of residues of methylprednisolone in edible equine tissues is available,
- extrapolation of the maximum residue limit established for bovine milk to horse milk is considered appropriate;

the CVMP recommends the establishment of maximum residue limits for methylprednisolone in horse tissues and, furthermore, with reference to Article 5 of Regulation (EC) No 470/2009, recommends the extrapolation of the MRL established in bovine milk to horse milk as follows:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Methyl-prednisolone	Methyl-prednisolone	<i>Equidae</i>	10 µg/kg 10 µg/kg 10 µg/kg 10 µg/kg 2 µg/kg	Muscle Fat Liver Kidney Milk	NO ENTRY	Corticoides / Glucocorticoides

4. Background information on the procedure

Submission of the dossier	22 January 2014
Steps taken for assessment of the substance	
Application validated:	5 February 2014
Clock started:	6 February 2014
CVMP opinion adopted:	10 July 2014