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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FLUNIXIN
(Extension to horses)

SUMMARY REPORT (2)

1. Flunixin is a non-steroidal anti-inflammatory drug (NSAID), and a non-narcotic analgesic with antipyretic activities. In veterinary medicine, it is used with meglumine as solubilizer as flunixin meglumine. Flunixin meglumine is used in the alleviation of inflammation and pain associated with musculo-skeletal disorders and colic in horses; the control of acute inflammation associated with infectious diseases in cattle and as an aid in the treatment of mastitis metritis agalactia syndrome (MMA) in sows. A dose of 2.2 mg/kg bw by the intravenous route once a day for up to 3 days is indicated for bovines, whilst 2.2 mg/kg bw intramuscularly (up to 2 injections, 12 hours apart) is recommended for sows affected by MMA syndrome. Flunixin meglumine is also used in veterinary medicine in combination with oxytetracycline for the treatment of bovine pneumonia at a dose of 2 mg/kg bw (once a day for 3 to 5 days by the intravenous or the intramuscular routes).

   The Committee for Veterinary Medicinal Products (CVMP) previously retained an ADI of 6 µg/kg bw (i.e. 360 µg/person) for flunixin, by applying a safety factor of 100 to the NOEL of 0.6 mg/kg bw/day which was established in a 90-day repeated dose gavage study in the dog.

   Flunixin is currently included in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissue	Other provisions
Flunixin	Flunixin	Bovine	20 µg/kg 30 µg/kg 300 µg/kg 100 µg/kg	Muscle Fat Liver Kidney	
	5-Hydroxy flunixin	Bovine	40 µg/kg	Milk	
	Flunixin	Porcine	50 µg/kg 10 µg/kg 200 µg/kg 30 µg/kg	Muscle Skin + fat Liver Kidney	

   An application has now been received for the extention of the MRLs for flunixin to edible tissues of horses. Flunixin meglumine is available in injectable, granular and paste formulations for the horse and can be administered by the intravenous, intramuscular and oral routes in this species at the recommended therapeutic dose of 1.1 mg/kg bw once a day for up to 5 days.

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2. A radiolabelled GLP compliant metabolism study was performed in the rat in which ^{14}C-flunixin meglumine was administered orally at a daily dose rate of 10 mg flunixin free acid/kg bw for 7 days. Approximately 33% and 30% of the administered radioactivity was excreted in urine and faeces, respectively. Parent compound was the dominant fraction in urine and faces. Metabolism of flunixin did occur at several sites on the molecule, including hydroxylation steps to form 5-hydroxy flunixin, 4-hydroxy flunixin and 2'-hydroxymethyl flunixin. Approximately 80% of a one-day dose was excreted in urine and faeces within 24 hours of dosing.

3. In a non-radiometric residue depletion study performed in horses, 3 male and 3 female animals were sacrificed 10 days following the oral administration of flunixin meglumine at a dose of 1 mg/kg bw daily for 5 consecutive days. Flunixin was assayed in this study. The limit of detection of the analytical method (HPLC) was 50 µg/kg. No residues could be detected above the limit of detection in all tissues at the single sacrifice time point, with the exception of kidney where values ranged between 100 to 291 µg/kg. The single, late, time-point of sacrifice was a deficiency in this study.

   Three groups of 3 horses per time point were treated with flunixin meglumine by the intramuscular route at a dose level of 1.1 mg/kg once daily for 5 days. Flunixin concentrations were assayed by an HPLC method, with a limit of detection of 50 µg/kg. Two days after the last dose, highest levels of flunixin were detected at the injection site (range of 49000 to 60000 µg/kg). By 7 days after the last dose, all tissue residue levels were less than 100 µg/kg. The number of animals used per slaughter time point did not meet the requirements of Volume VI of The Rules Governing Medicinal Products in the European Community.

   Flunixin meglumine was administered orally via stomach tube to 3 groups of horses (3 animals/group) at a dose of 1.1 mg flunixin/kg bw once daily for 5 consecutive days. Animals were sacrificed at 2, 7 and 14 days after treatment. A single horse served as a control. Residues of flunixin in edible tissues were assayed by HPLC, with a limit of quantification of 50 µg/kg. All samples were negative at all time points employed in this study.

   The absence of good quality depletion data in the above three studies following the oral or intramuscular use of flunixin in the horse was identified as a limitation. In addition, no information on the ratio of marker to total residues following the oral or intramuscular administration of flunixin to horses was available from the results of the above studies.

   A GLP compliant residue depletion study was performed in the horse in which ^{14}C-flunixin meglumine was administered intravenously at a dose rate of 1.1 mg of flunixin free acid/kg bw, once daily for 5 consecutive days. Groups of 4 animals per time point were slaughtered at 1 day and 4 days post final treatment. Concentrations of total radioactive residues were assayed by radiocombustion and liquid scintillation counting, with a limit of detection of 8 to 9 µg flunixin equivalents/kg. The mean concentrations of total radioactive residues were 363, 205, less than the limit of detection and 15 µg flunixin equivalents/kg in liver, kidney, muscle and fat at the 1 day time point, respectively. The corresponding values at the day 4 time point were 123, 46, less than the limit of detection and 9 µg flunixin equivalents/kg for liver, kidney, muscle and fat respectively.

   A GLP compliant non-radiolabelled study was performed in horses treated intravenously with 1.1 mg flunixin free acid/kg bw, once daily for 5 consecutive days. Groups of 4 animals per time point were slaughtered at 1, 3 and 6 days post last dose. Concentrations of flunixin in tissues were assayed by an LC-MS/MS analytical technique, with a limit of quantification of 1 µg/kg for all tissue matrices. The ratios of marker to total residues utilizing the LC-MS/MS technique were 0.08, 0.24, 0.41 and 0.26 for liver, kidney, muscle and fat respectively at day 1 post-dose. The mean concentrations of flunixin residues at the day 1 time point post last dose were 31, 88, 3 and 8 µg/kg for liver, kidney, muscle and fat respectively. The corresponding values for the day 3 time point were 23, 46, 2 and 6 µg/kg for liver, kidney, muscle and fat respectively. At the day 6 time point post last treatment, the mean flunixin concentrations had declined further to 9, 13, less than the limit of quantification and 2 µg/kg for liver, kidney, muscle and fat respectively.

4. The proposed routine analytical method presented was based on liquid chromatography with MS/MS detection (LC - MS/MS). The method was designed to monitor residues of flunixin in porcine, bovine and equine tissues, and residues of 5-hydroxy flunixin in bovine milk. The limit of quantification of the analytical technique for all edible porcine, bovine and equine tissues and bovine milk was 1 µg/kg. The method was validated in accordance with the requirements of Volume VI and was presented in the ISO 78/2 format.

Conclusions and recommendation

Having considered that:

- a toxicological ADI of 6 µg/kg bw (i.e. 360 µg/person) was established for flunixin,
- flunixin was identified as the marker residue,
- the ratios of marker to total residues were 0.08, 0.24, 0.41 and 0.26 in liver, kidney, muscle and fat of equine (1 day after treatment),
- a validated routine analytical method is available for monitoring residues of flunixin in equine tissues,
- given the different ratios of marker to total residues for the different animal species the MRLs already allocated for bovine and porcine tissues could not be retained, as this would have led to the ADI being exceeded.

the Committee recommends the inclusion of flunixin in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissue	Other provisions
Flunixin	Flunixin	Equidae	10 µg/kg 20 µg/kg 100 µg/kg 200 µg/kg	Muscle Fat Liver Kidney	

Based on the above MRL values, the daily intake will represent approximately 90% of the ADI.