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Veterinary Medicines and Information Technology

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

MOXIDECTIN
(Modification of the ADI and Extension to bovine milk)

SUMMARY REPORT (3)

1. Moxidectin is used for the treatment of endo- and ecto-parasites in cattle, sheep and horses at the recommended dosages of 0.2 mg/kg bw administered by oral or subcutaneous routes for sheep and cattle, 0.5 mg/kg bw by dermal route for cattle and of 0.4 mg/kg bw by oral route for horses.

   A toxicological ADI of 0.0015 mg/kg bw was prevoiusly established by the Committee for Veterinary Medicinal Products (CVMP) based on the NOEL of 0.3 mg/kg bw from the 90-day toxicity study performed in dogs and applying a safety factor of 200.

   Currently, moxidectin is 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 tissues	Other provisions
Moxidectin	Moxidectin	Bovine, ovine, Equidae	50 µg/kg 500 µg/kg 100 µg/kg 50 µg/kg	Muscle Fat Liver Kidney	

Modification of the ADI

2. Moxidectin was first assessed by CVMP in 1993. At that time, the most appropriate end-point for setting an ADI was identified as the reduced pup survival, which was observed in the 2-generation rat study. Based on a NOEL of 0.4 mg/kg bw and using a safety factor of 500 to compensate for the absence of data on the mice CF1 strain, an ADI of 0.0008 mg/kg bw was established, providing an adequate margin of safety for the effects seen in the repeat dose toxicity studies (NOEL: 0.3 mg/kg bw).

   In 1996, new scientific data showed that the hypersensitivity of CF-1 mouse compared with the CD-1 mouse for ivermectin was due to individuals with a mutation at the MDR1a locus which resulted in a deficiency of P-glycoprotein, a protein affecting drug transport. In this sensitive strain, the concentrations of ivermectin were 90 times higher in brain and 3 to 4 times higher in other tissues than those measured in CF-1 mice presenting no deficiency of P-glycoprotein.

   In addition, over ten million people world-wide had been treated with oral doses of ivermectin of up to 200 µg/kg bw. No major side effects had been reported in humans, except those resulting from the effects of the parasite itself (the Mazzotti reaction). It was reasonable to suppose that the same conclusions could be drawn with moxidectin, which had been developed for veterinary use. So, the value of the safety factor and the animal species retained for the first assessment were reconsidered. The NOEL of 0.3 mg/kg bw from the 90-day toxicity study performed in dogs was retained to establish the ADI of 0.0015 mg/kg bw applying a safety factor of 200 to take into account the absence of data on mice CF1 strain and the uncertain sensitivity of the test system used to assess the neurotoxicity of moxidectin.

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A request for the modification of the ADI based on results obtained in mice CF1 strain has now been submitted.

3. Similar data to those provided for the ivermectin family have now been provided. A new embryo/teratogenicity study was carried out in mice CF1strain. Four groups of 30 CF-1 pregnant female mice received by oral gavage moxidectin in corn oil at doses of 0, 1.5, 3 and 8 mg/kg bw/day, from day 6 to day 15 of gestation. Two other groups received moxidectin at doses of 0 and 6 mg/kg bw/day from day 6 to day 15 of gestation, because of a high level of mortality in the 8 mg/kg bw/day dose group.

   In dams, only significant adverse effects were reported at the two highest dosages. In the 8 mg/kg bw/day dosed group, 14 out of 30 females died after having exhibited neurological signs (decreased activity, ataxia) and bradypnea. A loss of weight and a reduced feed consumption were noted. Because of a high degree of toxicity, no further investigations were performed in this group. At a dose of 6 mg/kg bw/day, 4 out of 30 animals died or were sacrificed because of their moribund condition, the maternal body weight was transitorily significantly reduced from day 6 to day 9 of gestation and on day 9 and day 10 and a significant decreased feed consumption was recorded. A NOEL of 3 mg/kg bw/day was retained for maternotoxicity

   In foetuses, a significant increased percentage of malformed foetuses was reported after oral administrations at the two highest doses 96.9%, 53.9 % at 3 and 6 mg/kg bw/day respectively versus 7.2 and 6.3 % in the lowest dosed and control groups. The malformations included manubrium fused, cleft palate and skull palate incompletely ossified. A significant increase of foetuses presenting manubrium fused was only reported at 6 mg/kg bw (3% versus 0% in controls). In the 3 and 6 mg/kg bw/day dosed groups, a significant increase in the percentages of cleft palate were reported (95.9% and 47.7%, respectively) whereas in the control and 1.5 mg/kg dosed groups, no significant increase was reported; only 0.7 to 1.2% and 2.8 % of the foetuses presented cleft palate. In the 3 and 6 mg/kg bw/day dosed groups, the percentages of foetuses presenting skull palate incompletely ossified were significantly increased and were 95.2% and 49.1%, respectively whereas in the control and 1.5 mg/kg dosed groups, no significant differences were reported. As at the lowest dose, no compound related adverse effects were recorded in foetuses, 1.5 mg/kg bw/day was retained as NOEL for foetoxicity.

4. The new data provided did not highlight particular hypersensitivity, e.g. maternotoxicity and/or of the mice CF1 strain with regard to moxidectin. This test system used to assess the neurotoxicity of moxidectin was considered appropriate and therefore the safety factor could be decreased from 200 to 100. Considering that the most relevant NOEL to establish the acceptable daily intake derived from the 90-day toxicity study performed in dogs was 0.3 mg/kg bw/day and applying a safety factor of 100, a toxicological ADI of 0.0030 mg/kg bw was established.

5. This new ADI value is no longer in accordance with that established at the 45th Joint Experts Committee for Food Additives (JECFA) meeting. JECFA experts retained the same NOEL to derive the ADI and applied a safety factor of 200 to account for the uncertain sensitivity of the test system used to assess the neurotoxicity of moxidectin as when the assessment was done the results of the new study conducted on CF-1 mice were not available.

Extension to bovine milk

6. An application for the extension of the current MRLs for bovine species to include bovine milk has now been submitted.

   Moxidectin is intended to be used in lactating cows as a pour-on formulation at a single dermal recommended dose of 0.5 mg/kg bw.

7. In a radiolabelled study carried out in 6 lactating Holstein cows (3 in the 1st trimester and 3 in the 2d trimester of lactation), a single dose of 0.750 mg ^{14}C-moxidectin/kg bw (specific activity: 9.49 µCi/mg) was applied dermally. Milk samples were collected at 12 hour intervals up to 10 days after treatment. The total radioactivity was measured by direct liquid scintillation counting (limit of quantification: 4 µg/kg). The extractable ^{14}C-moxidectin derived residues were analysed by an HPLC method using UV detection to determine the nature of the different metabolites.

The radioactivity levels ranged from 4 to 31 µg equivalent moxidectin in milk collected from the 4th milking post treatment to the 20th milking after treatment. Large individual variations were reported. In milk collected at the 20th milking, significant amounts of radioactivity could still be quantified in 5 of 6 animals and were in the range of 6 to 15 µg equivalent moxidectin/kg.

Moxidectin represented the major residue and accounted for approximately 70% of the total radioactivity, whereas the two monohydroxylated metabolites (C29/C30 hydroxymethyl and ^{14}C-hydroxymethyl metabolites) accounted for 3.8 and 2.7%, respectively.

8. Three milk residue depletion studies were carried out in lactating cows at the recommended dosage.

In a first study, 8 Holstein lactating cows received a single dermal dose of 0.5 mg/kg bw of moxidectin as a pour-on formulation. Milk samples were collected before treatment and twice daily for 28 days after treatment at 12 hours apart. The analysis of moxidectin in whole milk was performed using an HPLC method based on fluorometric detection with a limit of quantification of 10 µg/kg and a limit of detection of 1 µg/kg. Moxidectin could be quantified in the milk of the 3rd milking post treatment in 5 animals. The individual moxidectin concentrations in milk ranged from 10 to 26 µg/kg and were mainly quantified in milk collected from the 2nd to the 9th milking after treatment. From the 13th milking and onwards, moxidectin concentrations in milk were below the limit of quantification (10 µg/kg).

Moxidectin residues in milk fat were also measured using an HPLC method based on fluorometric detection with a limit of quantification of 100 µg/kg. Residues of moxidectin in milk fat samples of the 10th and 11th milkings ranged from 260 µg/kg 110 µg/kg in 7 of the 8 animals treated whereas they were below the limit of quantification in one animal. In samples collected at the 20th and 21st milkings after treatment, moxidectin concentrations in milk fat were below the limit of quantification (100 µg/kg) in all samples.

In the second study, 3 lactating Holstein cows (daily milk production: 29 to 49 kg) received a single dermal dose of 0.5 mg moxidectin/kg bw as pour-on formulation. Milk samples were collected immediately before the treatment and twice daily (10 and 14 h apart) for 7 days after treatment and then only once daily (afternoon) up to 14 days. Moxidectin residues were quantified in milk of the third milking (13 and 18 µg/kg) in 2 animals. The highest moxidectin concentrations were found in milk of the 11th milking and were 25, 30 and 34 µg/kg respectively. In milk collected after the 21st milking e.g. 10 days after treatment, no moxidectin residues could be quantified (less than 10 µg/kg), except in 1 cow (10 µg/kg).

In the third study, six Friesian lactating cows received a single dermal dose of 0.5 mg moxidectin/kg bw as a pour-on formulation. The dose was applied along the back mid-line and retained at this site without run-off being observed. Milk samples were collected twice daily (10 and 14 hours apart) up to 21 days after treatment. Moxidectin concentrations were determined with an HPLC method using fluorescence detector. The limits of quantification and detection, defined as minimum proficiency limit and as lowest detectable level were 0.4 and 0.2 µg/kg, respectively.

The mean moxidectin concentration in milk quantified in the first milking after treatment was 1.37 µg/kg. Concentrations were 16, 15.0 and 7.4 µg/kg in milk collected at the 3rd, 5th and 7th milking, respectively. Moxidectin individual concentrations in milk ranged from 0.4 to 33.9 µg/kg during the experimental study, the significant amounts being quantified in milk of the 2nd milking to the 20th milking.

In milk fat, 15.98, 9.55, 2.33 and 1.72 µg/kg of moxidectin were quantified in samples collected at the 5th, 6th, 19th and 20th milkings after treatment.

9. An HPLC method, based on with fluorescence detection was provided for monitoring residues of moxidectin in milk. This method was fully validated according to the recommendations of Volume VI of the Rules Governing Medicinal Products in the European Community and was presented according to format ISO 78/2. The limits of quantification and detection were 10 and 0.14 µg/kg, respectively.

Conclusions and recommendations

Having considered that:

- a toxicological ADI of 0.003 mg/kg bw (i.e. 180 µg/person) has been established for moxidectin,
- moxidectin is the marker residue in milk and accounts for 70% of the total radioactivity,
- a fully validated routine analytical method for monitoring the residues in milk is available;

the Committee for Veterinary Medicinal Products recommends the inclusion of moxidectin for bovine milk 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 tissues	Other provisions
Moxidectin	Moxidectin	Bovine	40 µg/kg	Milk	

Taking into account the MRLs established for edible tissues of bovine, ovine and Equidae, and for bovine milk, the amount of residues likely to be ingested by the consumer represents about 95% of the ADI.