European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

DORAMECTIN
(Modification of the MRLs)

SUMMARY REPORT (6)

1. Doramectin is an avermectin (CAS number 117704-25-3) produced by fermentation of a selected strain of *Streptomyces avermitilis* in the presence of cyclohexanecarboxylic acid. Doramectin is administered by subcutaneous injection to cattle at a dose of 200 µg/kg bw for the treatment and control of gastrointenstinal nematodes, lungworms, eyeworms, warbles, lice and mange mites. Doramectin has also been developed for use in sheep as a single subcutaneous or intramuscular injection at a dose of 200 µg/kg bw and in pigs, as a single intramuscular injection at a dose of 300 µg/kg bw. In reindeer doramectin is used against nematodes and throat bot as a single treatment to the neck using subcutaneous injection at the dose of 200 µg/kg bw.

   The Committee for Veterinary Medicinal Products (CVMP) previously established an ADI of 0.5 µg/kg bw (i.e. 30 µg/person) for doramectin, based on the NOEL of 0.1 mg/kg bw for mydriasis identified in a 3-month repeated dose toxicity study in dogs and applying an uncertainty factor of 200.

   Currently, doramectin is included in Annex I of Council Regulation (EEC) No 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Doramectin	Doramectin	Bovine	10 µg/kg 150 µg/kg 100 µg/kg 30 µg/kg	Muscle Fat Liver Kidney	Not for use in bovines producing milk for human consumption
		Porcine, ovine, deer, including reindeer	20 µg/kg 100 µg/kg 50 µg/kg 30 µg/kg	Muscle Fat Liver Kidney	Not for use in ovines producing milk for human consumption

   An application was submitted for the revision of the toxicological ADI to take into account similar modifications of the ADIs for abamectin, ivermectin and moxidectin following re-consideration of the relevance of certain toxicological data derived from rodent toxicity studies and a modification of the MRLs for bovine, porcine and ovine species and deer.

   Further to the assessment of the application, a CVMP opinion on doramectin was adopted in April 2006. The Opinion agreed the increase of the ADI and recommended the modification of the maximum residue MRLs tissues with harmonised values in the different animal species and extension to all mammalian food producing species. The Committee did not agree with the applicant's proposal to eliminate the muscle MRL for doramectin.

   An intention to appeal against the opinion and grounds for appeal were subsequently submitted to the EMEA.

   The applicant also provided oral explanations to the Committee.

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2. Avermectins are significantly more toxic to CF-1 mice than to CD-1 mice. The increased sensitivity of the CF-1 strain is due to animals with a mutation of the *mdr1a* locus, resulting in a deficiency of P-glycoprotein. Deficiencies of this transporter protein affect the intestinal absorption and brain penetration of its substrates. Brain concentrations of ivermectin were 90 times higher and concentrations in other tissues 3 to 4 times higher in CF-1 mice with the *mdra1a* deletion than those without it.

3. The genetic basis of avermectin sensitivity in collies was studied in 13 clinically normal collies. Seven were identified as sensitive to ivermectin with typical signs of neurotoxicity including depression, ataxia, mydriasis and salivation following a single oral dose of 120 mg/kg. The level of gene expression was similar in sensitive and insensitive animals. Sequence analysis of the *mdr1* gene by reverse transcriptase polymerase chain reaction on RNA from isolated leucocytes from the collies and four dogs of other breeds were conducted. Sequence analysis revealed a four-base pair deletion that causes a frame-shift mutation, which results in production of a truncated non-functional protein in all the sensitive collies, which were also homozygous for the deletion. Insensitive collies and the other breeds had heterozygous genotypes with one mutant allele and one wild-type allele. It was concluded that the sensitivity to ivermectin resulted from this frame-shift mutation.

4. Sensitivity to avermectin B_1 was reported in Murray Grey cattle on an Australian farm. Three heifers from a group of 50 aged 18 to 26 months died within two days of receiving a parenteral dose of 175 to 200 µg/kg bw and three steers from a group of 144 cattle aged 4 to 18 months developed severe neurological signs within 48 hours of an estimated dose of 120 to 200 µg/kg bw. A fourth steer showed mild signs. A field trial was conducted on the farm with 90 steers previously treated with avermectin B_1 and 118 cattle that had not been treated before. Animals received 0 (the vehicle) or the recommended dose of 200 µg/kg bw. One steer that had not been treated previously developed neurological signs within 42 hours of administration. No pathological changes that would account for the severe effects observed could be found in tissues from the four initial cases or the animal from the field trial. The average brain concentration of avermectin was 56 µg/ml in the affected animals and 4 µg/ml in the normal animal. Two additional trials on Murray Grey cattle on other farms found no adverse reactions in a total of 83 cattle treated with at least twice the therapeutic dose of avermectin B_1. It was noted that the farm on which the reactions occurred had maintained a virtually "closed" herd (no breeding animals imported) for about 15 years, suggesting a genetic causality.

5. Information has become available that polymorphisms of the *mdr1* gene exist within the human population, these appear to include functional variations that result in over-expression of P-glycoprotein as well as under-expression. Eleven variants of this gene were found in a sample population of 461 caucasian volunteers in Germany. One of the variants was correlated with decreased levels of P-glycoprotein expression in the duodenum. Volunteers with this variant showed enhanced bioavailability of an oral dose of digoxin, with a steady-state concentration that was 38% higher (statistically significant) than those without the variant. Whether this variant could result in enhanced bioavailability of orally administered avermectins is unknown. Studies of variations in P-glycoprotein genes in other ethnic groups have not been reported. The data that are currently available suggest that the 10-fold factor for inter-individual variability within the overall 100-fold standard uncertainty factor used to establish ADIs is sufficient to account for any increased sensitivity to abamectin as a result of these polymorphisms. However, should other variations resulting in greater effects of P-glycoprotein expression be identified in future, it may be necessary to revise this value accordingly.

6. The revised ADI established by JEFCA in 1998 was based on the NOEL for mydriasis of 0.1 mg/kg bw from the 90-day toxicity study in dogs, with an uncertainty factor of 100. This gave a value of 1.0 µg/kg bw (60 µg/person, for a 60 kg person).

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7. As indicated in the CVMP summary report concerning the modification of the ADI for abamectin (EMEA/MRL/838/02-FINAL), the CF-1 mouse is not directly relevant for human risk assessment due to the genetic predisposition of this strain to avermectin toxicity. The previous CVMP ADI for doramectin from 1997 was based on the NOEL for mydriasis of 0.1 mg/kg bw from the 90-day toxicity study in dogs, with an uncertainty factor of 200. The additional two-fold factor was then considered necessary due to the uncertainty of the sensitivity of this endpoint in the absence of a study in CF-1 mice.

   As studies in the CF-1 strain of mouse are no longer considered to be of relevance or value to the risk assessment of consumer exposure to avermectin residues, the additional uncertainty factor is not necessary, and a revised ADI using an uncertainty factor of 100, giving a value of 1.0 µg/kg bw (60 µg/person, for a 60 kg adult) has now been established for doramectin.

8. Although new residue depletion data were submitted, the ratio of marker to total residues and residue depletion profile for doramectin remains unchanged for the purpose of modification of the existing MRLs. MRLs in all four edible tissues (muscle, fat, liver and kidney) have previously been established and are considered necessary for the residue control for doramectin.

9. Codex established MRLs for doramectin as follows: for cattle: 10 µg/kg in muscle, 100 µg/kg in liver, 30 µg/kg in kidney and 150 µg/kg in fat and milk; for pigs: 5 µg/kg in muscle, 100 µg/kg in liver, 30 µg/kg in kidney and 150 µg/kg in fat.

10. The previously established MRLs for bovine, porcine, ovine and deer (including reindeer) are identical or slightly different. Therefore, in accordance with the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL) it was possible to extrapolate the MRLs to all mammalian tissues.

11. An HPLC method with fluorescence detection was previously assessed by the CVMP and considered validated for the monitoring of residues of doramectin, with limits of quantification of 2.5 µg/kg for bovine and ovine muscle, liver and kidney and ovine fat, 10 µg/kg for bovine fat and porcine muscle, fat, liver, and kidney. The method validation previously submitted is considered to be valid for the proposed new MRLs. Therefore, a validated routine analytical method for the determination of the marker residue in edible tissues is available. This method should also be applicable to other mammals.

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Conclusions and recommendation

Having considered that:

- Sensitivity to avermectin toxicity is linked to the expression of P-glycoprotein that plays a critical role in the development of the blood-brain barrier,
- CF-1 mice show mutations that result in deficient/absent P-glycoprotein in the endothelial cells of brain capillaries and sensitivity to avermectin toxicity shows a direct link to P-glycoprotein genotype. Similar mutations have been reported in collie dogs and one herd of Murray Grey cattle but not in CD-1 mice, rats or non-human primates,
- The data available on P-glycoprotein expression polymorphisms in humans indicates that a 10-fold factor for inter-individual variability is sufficient to account for any resultant increased sensitivity to abamectin,
- The increased sensitivity of CF-1 mice to avermectin toxicity is not directly relevant for human risk assessment due to the genetic predisposition of this strain to avermectin toxicity and, consequently it is not necessary to include an additional uncertainty factor in the determination of the ADI for doramectin because of the absence of such data,
- A revised toxicological ADI of 1.0 µg/kg bw (60 µg/person per day) is now established,
- MRLs had previously been established in bovine, porcine, ovine species and deer (including reindeer); these MRLs are identical or slightly different,
- Given the increase in the ADI, the same MRLs can now be established in bovine, ovine and porcine species and deer and these can be extrapolated to all mammalian species,
- MRLs in all 4 tissues (muscle, fat, liver and kidney) are considered necessary for residue control for doramectin,
- A validated routine analytical method for the determination of the marker residue in edible tissues is available;

the CVMP, further to the consideration of the grounds for appeal and the argumentation presented during the oral explanation, recommends the revision of the Annex I entry of Council Regulation (EEC) No 2377/90 for doramectin in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissue	Other provisions
Doramectin	Doramectin	All mammalian food producing species	40 µg/kg 150 µg/kg 100 µg/kg 60 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption

Based on these MRLs, it was calculated that the consumer intake of total residues from the consumption of the above tissues would account up to 90% of the ADI.

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