The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DORAMECTIN (Pigs and Sheep)

SUMMARY REPORT (3)

1. Doramectin is an avermectin produced by fermentation of a selected strain of *Streptomyces avermitilis* in the presence of cyclohexanecarboxylic acid. An ADI of 0-0.5 µg/kg bw per day was calculated by applying a safety factor of 200 to the NOEL of 0.1 mg/kg bw per day, based on mydriasis in a 3-month repeated-dose toxicity study in dogs. EU MRLs for cattle were set as follows:

   Fat - 150 µg/kg; liver - 100 µg/kg; kidney - 30 µg/kg; muscle - 10 µg/kg.

   Doramectin is now being developed for use in sheep, as a single subcutaneous 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.

2. In pigs, up to 10 times the recommended intramuscular dose of doramectin was well tolerated. Clinical signs of toxicity (ataxia and depression) were observed at 25 times the recommended dose. Transient tissue discoloration with mild inflammation was the only effect observed at injection sites. Three times the recommended dose had no effect on reproductive parameters in either male or female pigs.

3. In sheep, no signs of toxixcity, no injection site reactions, and no effects on male or female reproductive parameters were observed following subcutaneous administration of 3 times the recommended dose.

4. Absorption, distribution, metabolism and excretion were studied in pigs given a single intramuscular injection of 300 µg/kg ^{3}H-doramectin. Mean peak plasma concentrations (C_{max}) were observed 3-4 days after treatment and were 19 ng/ml and 28 ng/ml for doramectin and total residues respectively. The mean terminal elimination half-lives in plasma were 6.4 and 7.7 days for doramectin and total residues respectively. Almost all of the administered dose was excreted in faeces with only approximately 1% recovered from urine. Significant amounts of radioactivity were excreted in bile. Over the 21-day collection period, 22-39% of the radioactivity in faeces was unchanged doramectin and 31% was the metabolite 3''-O-desmethyldoramectin. Small amounts of 24-hydroxymethyl-doramectin were also present in faeces.

5. Swine were given an intramuscular injection of 300 µg/kg ^{3}H-doramectin. Groups of 2 males and 2 females were slaughtered 7, 14, 21 and 28 days after treatment. Total residues were determined using scintillation counting. Residues of doramectin were determined using HPLC; the limit of quantification was 10 µg/kg for fat and 2.5 µg/kg for other tissues. Total residues in fat declined from 412 ± 54 µg/kg 7 days after treatment, to 255 ± 42 µg/kg 14 days after treatment and 90 ± 18 µg/kg 21 days after treatment. Over the same time period residues of unchanged doramectin declined from 242 ± 22 µg/kg, to 113 ± 26 µg/kg to 42 ± 14 µg/kg. The percentage of unchanged drug in these fat samples was therefore 59%, 44% and 45% respectively. Total residues in kidney declined from 79 ± 19 µg/kg, 7 days after treatment, to 17 ± 4 µg/kg 21 days after treatment and the residues of doramectin in kidney declined from 23 ± 7 µg/kg 7 days after treatment to 6 ± 2 µg/kg 21 days after treatment; the percentage of doramectin in kidney at these 2 time points was 30 and 35% respectively. Total residues in muscle declined from 35 ± 7 µg/kg 7 days after treatment to 19 ± 6 µg/kg 14 days after treatment and to 6 ± 1 µg/kg 21 days after treatment. Residues of unchanged doramectin in muscle were 7 ± 1 µg/kg 7 days after treatment, corresponding to 20% of

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Switchboard (44-20-7) 418 8400 Fax (44-20-7) 418 8447
E-mail: mail@emea.eudra.org http://www.eudra.org/emea.html

the total residues. Residues of unchanged doramectin in some later samples were below the limit of quantification and so the ratio of doramectin to total residues could not be determined reliably at subsequent time points. Residues were much higher in injection site muscle; total residues declined from 5132 ± 4431 µg/kg 7 days after treatment to 2512 ± 1802 µg/kg 14 days after treatment and 1078 ± 721 µg/kg 21 days after treatment and residues of doramectin declined from 3660 ± 3037 µg/kg to 2553 ± 1944 µg/kg to 1032 ± 850 µg/kg, over the same time period. Total residues in liver declined from 186 ± 47 µg/kg 7 days after treatment, to 111 ± 20 µg/kg 14 days after treatment, to 46 ± 10 µg/kg 21 days after treatment. Over the same time period, residues of unchanged doramectin declined from 66 ± 15 µg/kg to 37 ± 11 µg/kg and to 11 ± 3 µg/kg. The extraction of unchanged doramectin from the liver samples was found to be unreliable and the mean proportion of total residues present as doramectin was disproportionately low (around 30%). The day 7 liver samples were therefore re-assayed using an improved procedure and the percentage of residues present as doramectin was shown to be 71 ± 4%. 20 ± 2% of the residues in the day 7 liver samples were present as 3"-O-desmethyldoramectin. The faecal metabolites 24-hydroxymethyl-3"-O-desmethyldoramectin and 24-hydroxymethyldoramectin were not detectable in tissues.

6. Suffolk crossbred sheep were given a single subcutaneous injection of 300 µg/kg ^{3}H-doramectin. Around 30% of the residues in all sheep tissues were unextractable. Fourteen days after treatment, unmetabolised doramectin comprised 92%, 75%, 73% and 67% of the radioactivity present in muscle, liver, kidney and fat respectively. Concentrations of doramectin at subsequent time points were too low for the ratios of doramectin to total residues in these tissues to be calculated. The metabolite 3"-desmethyldoramectin was a minor component in all edible tissues of sheep except for muscle. A metabolite tentatively identified as the 2-epimer of doramectin was a minor component in sheep fat. The metabolites 24-hydroxymethyl-3"-O-desmethyldoramectin and 24-hydroxymethyl-doramectin were found in sheep faeces but were not detectable in tissues.

7. The depletion of residues of doramectin in sheep was investigated as part of the metabolism study described in paragraph 6, above. Suffolk crossbred sheep were given a single subcutaneous injection of 300 µg/kg ^{3}H-doramectin, formulated as the commercial product (150% of the recommended dose). The sheep were killed in groups of 4 (2 males and 2 females), 14, 35, 42, 49 and 56 days after treatment. Residues of doramectin in tissues were determined using HPLC with fluorescence detection; the limit of quantification (LOQ) was 2.5 µg/kg for all tissues. Mean residues in pooled renal and omental fat declined from 62.8 µg/kg, 14 days after treatment, to less than 4.48 µg/kg, 35 days after treatment. Mean residues in liver were 47.5 µg/kg, 14 days after treatment, and were below the limit of quantification in 3 out of 4 sheep killed 35 days after treatment. Residues in kidney and muscle were 17.9 µg/kg and 13.5 µg/kg respectively, 14 days after treatment, and were below the limit of quantification in all samples 35 days after treatment. Residues at the injection site were very variable and declined from a mean of 2554 µg/kg 14 days after treatment, to 365 µg/kg 35 days after treatment. Fifty-six days after treatment mean residues at the injection site were below 113 µg/kg.

8. A residue depletion study was carried out in which crossbred swine were given a single intramuscular injection of a 1% doramectin formulation at a dose of 375 µg/kg bw (125% of the recommended dose). The swine were slaughtered in groups of 6 (3 females and 3 castrated males), 7, 14, 21, 28 and 35 days after treatment. Residues of doramectin in tissues were determined using HPLC with fluorescence detection. The limits of quantification were claimed to be 5 µg/kg for fat and 2.5 µg/kg for other tissues. Residues were always highest at the injection site and declined from 9000 ± 6000 µg/kg, 7 days after treatment to 70 ± 70 µg/kg, 35 days after treatment. Residues in back fat declined from 470 ± 120 µg/kg, 7 days after treatment, to 50 ± 20 µg/kg, 35 days after treatment. No samples of skin were taken for analysis. Residues in liver declined from 160 ± 30 µg/kg, 7 days after treatment, to 18 ± 8 µg/kg, 35 days after treatment. Residues in kidney and muscle declined from 80 ± 20 µg/kg and 40 ± 9 µg/kg, 7 days after treatment, to 18 ± 7 µg/kg and 11 ± 5 µg/kg, 21 days after treatment.

9. The proposed routine analytical method for determination of residues of doramectin in tissues was based on HPLC with fluorescence detection. The limits of quantification were 2.5 µg/kg for ovine liver, kidney, muscle and fat and 10 µg/kg for porcine fat, liver, kidney and muscle.

Conclusions and recommendation

Having considered that:

- an ADI of 0 - 0.5 µg/kg bw (i.e. 30 µg/person) had been calculated for doramectin,
- doramectin was shown to be the marker residue in tissues of pigs and sheep,
- the pattern of distribution and residue depletion in the target species;

the Committee recommends the inclusion of doramectin 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
Doramectin	Doramectin	Porcine, ovine	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.

It was previously estimated that consumer intake of residues fom bovine tissues would result in the entire ADI being used up. Because lower MRLs were proposed for porcine and ovine tissues, it was considered that the intake of residues from these species would not present a greater risk to consumers.

The CVMP noted that residues at the injection site were persistent following parenteral administration to sheep and pigs which should be take into account when calculating withdrawal periods for individual products.