DORAMECTIN

Draft prepared by

Dr. Jose Luis Rojas Martinez, Alajuela, Costa Rica

Dr. Richard Ellis. Rockville, Maryland, USA

ADDENDUM

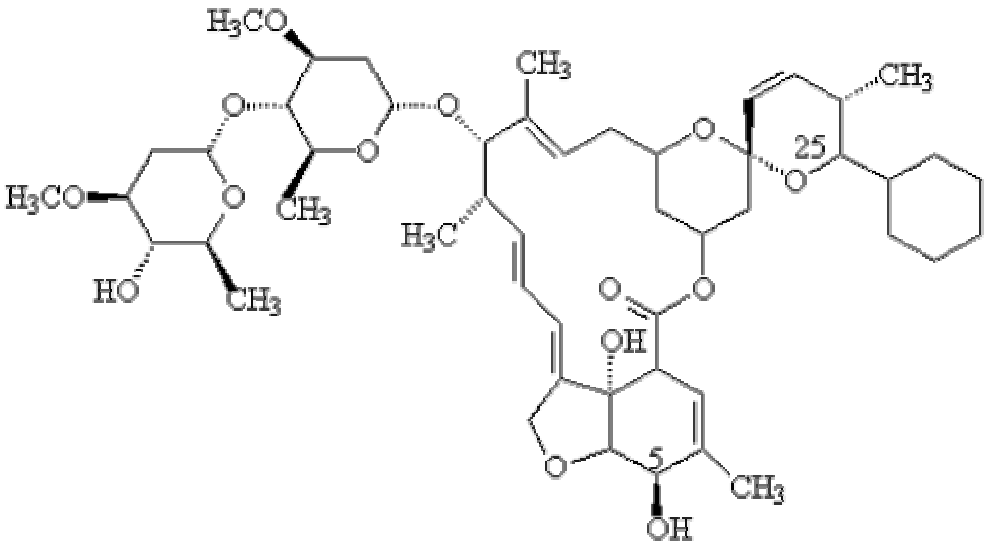
to the doramectin residue monograph prepared by the 45th meeting of the Committee in 1995, published in FAO Food and Nutrition Paper 41/8, and the 52nd meeting of the Committee in 1999, published in FAO Food and Nutrition Paper 41/12

IDENTITY

Chemical Name; 25-cyclohexyl-5-O-demethyl-25-de(1-methylpropyl)avermectin A_{1a}

Synonyms: Doramectin; Dectomax; UK-67,994

Structural formula:



Molecular formula: $C_{50}H_{74}O_{14}$

Molecular weight: 899.14

INTRODUCTION

Doramectin is an ecto- and endoparasiticide for use in cattle and pigs. It is a semi synthetic member of the avermectin class, structurally similar to abamectin and ivermectin. Previous evaluations by the Committee did not consider use in lactating cattle. The 14th Session of the Codex Committee on Residues of Veterinary Drugs in Foods in 2002 requested the Committee to evaluate its use in lactating dairy cows.

Doramectin was first reviewed by the 45th Committee that established an ADI of 0-0.5 µg/kg bw on the basis of a NOEL of 0.1 mg/kg per day for mydriasis in a 3-month study in dogs treated with doramectin by gavage, using a safety factor of 200. The additional safety factor of 2 was applied because doramectin was not tested in CF-1 mice, the animal test considered at the time as the most sensitive to the neurotoxic effect of this class of drugs.. The Committee at its fiftieth meeting accepted the conclusions of the 1997 Joint FAO/WHO Meeting on Pesticide Residues (JMPR) that determined it was no longer necessary to apply an additional safety factor of 2 for the avermectins and milbemycins that had not been tested in CF-1 mice. On the basis of the decision at the fiftieth meeting of the Committee, the 58th Committee established an ADI of 0-1.0µg/kg bw.

The 58th Committee recommended the following MRLs for cattle: 10 µg/kg for muscle, 100 µg/kg for liver, 30 µg/kg for kidney and 150 µg/kg for fat, expressed as parent drug. Based on these values for the MRLs, the maximum theoretical intake would be 33 µg per day for a 60 kg person using the JECFA model diet.

In the dossier provided by the sponsor to the present Committee, information was presented for use of doramectin pour-on and injectable solution in lactating cattle in three new residue depletion studies. In addition, method performance data were provided for the analytical method to determine residues of doramectin in milk from lactating dairy cattle.

MILK RESIDUE STUDIES

Doramectin pour-on

These studies were designed to determine the profile of depletion of doramectin residues in milk following the administration of a 5 mg/ml doramectin pour on formulation when administered according to maximum labeled treatment regime to lactating dairy cattle

The first study using a pour-on treatment was conducted with twelve dairy Holstein cows. One animal with a medium level of production was randomly selected as a negative control, while the remaining eleven cows (one retained as a replacement animal) were treated with the test formulation. The cattle used in this trial were 3.5 to 9.5 years old weighing from 345 to 618 kg (mean 480 kg) at the time of treatment. The mean yield of milk was 13.28 liters and the mean milk fat was 4.91%

The treatment of the eleven animals with doramectin pour on formulation by topical application was at a dose rate of 0.58 mg/kg (1.0 ml/8.6 kg) doramectin and re-treatment with the same dose at 56 days later. The treatment was in accordance with Veterinary Health Research Standard Operating Procedure.

The sample collection during the initial phase (day 0 to 49) was by triplicate individual milk samples (collected immediately prior to treatment from all trial cattle on day 0), at both the morning and evening milking on days 0 to 7 days post treatment and at the morning milking on days 10, 16, 22, 28, 36 and 49 post treatment and at the evening milking on days 13, 19, 25, 32, and 40 On days 1, 4 and 10 an additional 100 ml sample was collected, refrigerated for 24 hours, separated into skim milk and milk fat with the individual portions subsequently stored frozen. During the second treatment from days 56 to 66 using the same topical dose; triplicate individual milk samples were collected from all trial cattle. at both the morning and evening milking on days 56, 57, 58, 59, 60, 61, 62 and 63 post treatment and at the morning milking on day 66.

Results –Study 1

The doramectin milk residue and milk/fat residue analysis were determined using a High Performance Liquid Chromatography validated method and fluorescence detection (LOQ; 3.12 µg/kg. The doramectin concentrations in milk increased from non-detectable concentrations at pre-treatment to a maximum mean value of 22.1 µg/kg at 72 hours post-dose. Between 60 and 120 hours post-dose, mean concentration were between 19.5 µg/kg and 22.1 µg/kg. The highest individual value (37.0 µg/kg) was observed at 72 hours post-dose. Mean doramectin residues decreased to a concentration below the limit of quantitation on day 16 (384 hours post-dose). Results are presented in Table 1a.

After re-treatment on day 56 using the same treatment as on day 0, doramectin residues increased gradually to a maximum mean value of 12.3 µg/ kg at 48 hours post-dose. The residues were constant between 48 and 96 hr post-dose, ranging between 10.1µg/ kg and 12.3 µg/kg and then decreasing to <LOQ at 240 hr post-dose. Results from re-treatment are summarized in Table 1b.

The milk/fat analyses were conducted at 1, 4, and 10 days post-dosing. Mean doramectin residues in the milk fat at these time points were 170.9 µg/kg, 501.4 µg/kg and 114.1 µg/kg, respectively. The concentration increases observed in the milk fat were consistent with the increases in doramectin residues in whole milk. The doramectin ratios in milk fat versus milk were calculated by dividing the measured concentration of each at the corresponding sampling times. Mean ratios at 1, 4 and 10 days were 29.6, 32.2 and 24.7, respectively.

The second study using the same pour-on treatment trial was conducted with twelve lactating cows, mean weight of 523.2 kilograms (392 - 620 kg) and mean milk production of 27.8 liters per day (23.2 - 32.1 liters) were selected. One animal was randomly selected to remain untreated as a negative control group and the remaining eleven cows were treated with the test formulation and one of the eleven treated cows was allocated to be a replacement animal and was sampled according to the trial schedule, however, the samples from this animal were not analyzed as no replacement of the initial study animals were required. These animals were treated with doramectin by topical route) and the dose rate of 0.58 mg/kg (1.0 ml/8.6 kg of pour on formulation) and re-treatment with the same dose 56 days later. The sample collection during the initial phase (day 0 to 49) was made by triplicate individual milk samples collected from all trial cattle at both the morning and evening milking on days 0 to 7 days post treatment and at the morning milking on days 10, 16, 22, 28, 36 and 49 post treatment and at the evening milking on days 13, 19, 25, 32, and 40. During the second treatment from days 56 to 66, triplicate individual milk samples were collected from all trial cattle at both the morning and evening milking on days 56, 57, 58, 59, 60, 61, 62 and 63 post treatment and at the morning milking on day 66. On days 1, 4 and 10 following re-treatment, an additional 100 ml sample was collected, refrigerated for 24 hours, separated into skim milk and milk fat with the individual portions subsequently stored frozen.

Results-Study 2

doramectin concentrations in milk increased gradually from non-detectable at pre-treatment to a mean of 8.9µg/kg. Individual maximum values were observed at day 1 and day 5 with the highest replicate value (15.8 µg/kg) observed at 33 hours post-treatment. Between 21 and 129 hours post-treatment, the group means milk doramectin residue concentrations were fairly constant, ranging between 6.1 and 8.9 µg/kg. Subsequently, doramectin residues decreased to concentrations below the limit of quantitation in all animals by the evening milking on day 19

Following re-treatment on day 56 using the same topical dose, residues increased to a mean maximum value of 8.2±5.2 µg/kg. at 93 hours. Individual maximum values were observed between the evening milking on day 58 and day 61 with the highest replicate value (21.7µg/kg) observed at 57 hours post-treatment. As seen the first treatment, residues were fairly constant between 33 and 141 hours post- re-treatment, with group mean values between 6.2 and 8.2 µg/kg before decreasing to <LOQ at 237 hours post re-treatment. Compared to the first treatment, doramectin milk residues were similar following re-treatment. Results are summarized in Table 2a and 2b

Milk fat analysis were conducted on samples collected on days 1 (21 hours), 4 (93hours) and 10 (237 hours) post treatment respectively. Mean doramectin residues in the milk fat at these times points were 90.8 µg/kg, 142.0 µg/kg and 55.1µg/kg respectively. The concentration increases observed in the milk fat were consistent with the increases in doramectin residues in whole milk. The highest individual milk fat concentration observed was 233.2 µg/kg. at 93 hours post treatment. The doramectin ratios in milk fat versus whole milk were calculated by dividing the measured concentrated of each at the corresponding sampling times. The mean ratios at 21, 93 and 237 hours post treatment were 14.2, 20.9 and 14.1 The highest calculated ratio was 48.5 at day 4.

Doramectin injectable formulation

This study was designed to determine the residue depletion profile of doramectin in milk following the administration of a 10 mg/ml doramectin injectable formulation to lactating cattle. Trial animals were lactating Holstein-Friesian.

Twenty four lactating cows were selected from al larger herd of 450 Holstein milking cows. Trial animals were selected to give a range of production levels representative of those in the larger herd. Two animals with milk production levels approximating the group mean were randomly selected to remain untreated as a negative control (treated with 0.9 % sodium chloride by subcutaneous injection). This study was conducted utilizing internationally acceptable standard operating procedures.

Trial cattle were individually weighed (on days 0 and 56) using calibrated electronic stock scales and animals treated by subcutaneous injection (on days 0 and 56) with the test formulation Injectable product at mean dose rates of 0.234 and 0.233 mg/kg individual body weight, respectively. These dose rates were equivalent to a dose volume of 1 mL per 42.65 body weight and 1 mL per 42.84 body weight.

During the initial phase (days 0 to 49), triplicate individual milk samples (replicates 1, 2 and 3) were collected from all trial cattle, at both the morning and evening milking on days 0, 1, 2, 3, 4, 5, 6, and 7 post treatment; at the morning milking on days 10, 16, 22, 28, 36, and 49 post treatment and at the evening milking on days 13, 19, 25, 32 and 40. An additional milk sample was collected at the morning milking on days 1, 4 and 10, refrigerated for 24 hours, separated into milk fat and skim milk portions and then stored frozen. During the second phase (days 56 to 66) triplicate individual milk samples were collected from all trial cattle, at both the morning and evening milking on days 56, 57, 58, 59, 60, 61, 62 and 63 post treatment and at the morning milking on day 66. Replicate 1 and 2 samples were frozen following collection, while replicate 3 samples were refrigerated. Replicate 1 samples were subsequently frozen on dry ice and forwarded to the analytical laboratory for doramectin milk residue analysis, Refrigerated replicate 3 samples were forwarded to a herd testing laboratory for milk fat content analysis and replicate 2 samples were retained frozen as back up samples, for analysis if required. Following separation into milk fat and skim milk portions, samples were forwarded frozen on dry ice to the designated analytical laboratory for milk fat doramectin residue analysis

Table 1a. Concentration of doramectin in milk (µg/kg) after treatment with a topical dose of 0.58 mg/kg in Southern Australia

	Hours	Concentration of doramectin (µg/kg) / cow number												
Day Time	Post-Dose	284	2236	2322	2643	2812	2967	3176	3394	3467	3516	Max	Mean	Std dev
Day 0 am	0													
Day 0 pm	12	1.6	1.6	1.6	3.7	1.6	3.9	3.2	4.0	1.6	1.6	4.0	2.4	1.1
Day 1 am	24	6.0	5.0	1.6	6.1	6.3	11.7	8.7	3.9	5.5	3.6	11.7	5.8	2.8
Day 1 pm	36	10.6	12.5	13.8	9.8	13.2	17.3	22.7	16.1	12.8	8.9	22.7	13.8	4.1
Day 2 am	48	15.8	13.3	18.1	11.8	18.2	19.2	25.0	11.5	17.7	8.9	25.0	15.9	4.7
Day 2 pm	60	17.6	16.7	20.5	15.2	23.7	17.4	22.8	27.3	21.5	12.0	27.3	19.5	4.5
Day 3 am	72	19.8	18.8	18.1	21.1	18.6	20.3	37.0	27.6	25.3	13.9	37.0	22.1	6.5
Day 3 pm	84	18.2	16.9	21.5	19.4	24.0	18.3	30.7	22.4	26.6	12.8	30.7	21.1	5.2
Day 4 am	96	14.7	15.2	10.0	10.6	21.9	19.3	23.2	14.5	29.6	10.6	29.6	17.0	6.4
Day 4 pm	108	20.8	15.2	23.8	16.3	21.5	17.5	34.4	24.9	24.1	9.2	34.4	20.8	6.8
Day 5 am	120	23.5	13.8	21.5	13.3	25.8	13.4	31.4	19.4	20.6	11.0	31.4	19.4	6.5
Day 5 pm	132	15.3	14.2	12.5	15.3	19.0	10.3	22.5	18.3	19.5	5.1	22.5	15.2	5.1
Day 6 am	144	13.8	10.4	8.9	11.2	15.4	8.5	27.3	17.5	16.1	4.1	27.3	13.3	6.4
Day 6 pm	156	9.4	11.0	7.5	8.2	12.3	8.1	14.1	13.9	12.2	3.5	14.1	10.0	3.3
Day 7 am	168	10.2	13.5	8.4	8.4	13.3	7.6	21.7	14.2	11.9	1.6	21.7	11.1	5.3
Day 7 pm	180	8.9	9.6	9.2	6.4	9.9	6.2	25.1	11.3	9.0	1.6	25.1	9.7	6.1
Day 10 am	240	3.3	4.7	3.4	1.6	6.7	5.6	10.8	1.6	4.5	1.6	10.8	4.4	2.9
Day 13 am	324	1.6	6.8	1.6	1.6	5.0	3.3	5.9	1.6	1.6		6.8	3.2	2.1
Day 16 am	384	1.6	4.3	1.6	1.6	3.4	1.6	1.6	1.6	1.6		4.3	2.1	1.0
Day 19 pm	468	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	
Day 22 am	528	1.6	1.6		1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	
Day 25 pm	612		1.6			1.6								
Day 28 am	672		1.6											
Day 32 pm	780		1.6											
Day 36 am	864													
Day 40 pm	972						1.6							
Day 49 am	1176													

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 1b. Concentration of doramectin in milk(µg/kg) after retreatment with a topical dose of 0.58 mg/kg in Southern Australia

	Hours	Retreatment with doramectin at a dose rate of 0.581 mg/kg												
Day Time	Post-Dose	284	2236	2322	2643	2812	2967	3176	3394	3467	3516	Max	Mean	Std dev
Day 56 am	0													
Day 56 pm	12	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	0.0
Day 57 am	24	7.3	4.3	1.6	3.2	4.4	4.2	5.5	3.3	7.5	6.6	7.5	4.8	1.9
Day 57 pm	36	3.3	6.0	1.6	4.6	5.7	8.2	9.1	3.7	14.4	9.9	14.4	6.6	3.8
Day 58 am	48	16.6	8.3	7.9	6.2	11.6	14.6	20.8	8.8	18.0	9.8	20.8	12.3	4.9
Day 58 pm	60	10.4	8.4	11.9	6.1	7.8	9.6	14.4	10.5	14.3	7.2	14.4	10.1	2.8
Day 59 am	72	13.5	9.8	11.1	4.8	11.5	13.0	20.3	4.4	16.6	7.3	20.3	11.2	5.0
Day 59 pm	84	13.7	10.2	11.1	6.1	10.2	11.9	16.9	10.9	15.9	7.5	16.9	11.4	3.4
Day 60 am	96	13.1	10.5	11.2	5.6	8.8	14.9	14.8	8.0	15.6	6.2	15.6	10.9	3.7
Day 60 pm	108	10.0	8.9	5.2	5.3	5.6	12.1	14.5	4.9	13.7	5.7	14.5	8.6	3.8
Day 61 am	120	10.9	9.2	9.6	5.3	8.7	10.9	15.2	7.5	12.4	3.5	15.2	9.3	3.4
Day 61 pm	132	9.3	9.5	7.8	6.2	6.1	9.4	12.7	5.3	11.3	5.9	12.7	8.3	2.5
Day 62 am	144	8.3	12.2	8.1	4.0	7.9	10.4	10.7	5.3	11.1	3.3	12.2	8.1	3.1
Day 62 pm	156	9.0	7.5	8.1	3.4	8.2	5.9	9.6	7.7	8.7	5.3	9.6	7.3	1.9
Day 63 am	168	7.6	8.0	7.6	4.5	5.0	7.1	11.3	7.7	8.6	4.6	11.3	7.2	2.1
Day 63 pm	180	4.7	5.9	5.7	3.9	4.6	5.9	7.9	1.6	6.8	1.6	7.9	4.8	2.1
Day 66 am	240	1.6	5.6	1.6	1.6	3.8	1.6	4.0	1.6	4.3	1.6	5.6	2.7	1.6

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 2a. Concentration of doramectin in milk (µg/kg) after treatment with a topical dose of 0.58 mg/kg in Northern Australia

	Hours	Concentration of doramectin (µg/kg) / cow number												
Day Time	Post-Dose	7	56	59	75	1408	1413	1623	1717	1754	1807	Max	Mean	Std dev
Day 0 am	0													
Day 0 pm	9	1.6	4.9	1.6	1.6	1.6	1.6	1.6		1.6	1.6	4.9	1.9	1.1
Day 1 am	21	4.8	14.1	6.4	10.0	1.6	4.3	13.8	6.1	1.6	1.6	14.1	6.4	4.8
Day 1 pm	33	6.7	15.8	6.7	7.1	1.6	8.3	13.1	3.7	4.2	4.8	15.8	7.2	4.3
Day 2 am	45	6.3	15.6	11.5	7.7	1.6	12.2	13.0	8.4	4.7	7.8	15.6	8.9	4.2
Day 2 pm	57	6.0	13.1	8.9	8.6	1.6	12.7	11.0	5.8	4.7	8.8	13.1	8.1	3.6
Day 3 am	69	5.6	11.3	7.9	4.8	1.6	11.2	10.0	6.0	4.2	9.2	11.3	7.2	3.3
Day 3 pm	81	5.0	6.2	10.0	4.7	1.6	7.5	9.4	3.6	4.1	9.0	10.0	6.1	2.8
Day 4 am	93	4.7	10.6	9.9	4.8	4.7	9.1	8.4	6.4	3.9	8.3	10.6	7.1	2.5
day 4 pm	105	4.9	12.9	9.4	7.8	5.9	10.4	7.3	5.9	4.4	7.9	12.9	7.7	2.6
Day 5 am	117	5.3	8.6	7.0	6.1	5.8	7.7	5.9	6.1	3.9	6.5	8.6	6.3	1.3
Day 5 pm	129	5.8	7.9	5.9	6.9	4.6	7.3	9.5	5.2	7.7	6.5	9.5	6.7	1.4
Day 6 am	142	4.1	5.9	5.4	4.5	4.7	6.7	5.6	4.6	5.8	5.4	6.7	5.3	0.8
Day 6 pm	153	3.7	5.1	5.3	3.4	4.8	5.2	6.4	4.2	4.8	4.7	6.4	4.7	0.9
Day 7 am	165	1.6	6.0	4.0	1.6	4.4	5.0	5.4	5.1	4.5	4.7	6.0	4.2	1.5
Day 7 pm	177	1.6	3.7	1.6	3.5	4.0	3.7	6.8	4.1	4.7	5.4	6.8	3.9	1.6
Day 10 am	237	1.6	3.2	3.6	1.6	1.6	1.6	3.6	4.5	4.5	5.3	5.3	3.1	1.4
Day 13 am	320	1.6	1.6	1.6	1.6	1.6	1.6	1.6	3.6	1.6	3.6	3.6	2.0	0.8
Day 16 am	381	1.6	1.6	1.6	3.1	1.6	1.6	1.6	1.6	1.6	1.6	3.1	1.7	0.5
Day 19 pm	464	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6		1.6	
Day 22 am	525	1.6	1.6	1.6	1.6	1.6		1.6	1.6	1.6	1.6		1.6	
Day 25 pm	608	1.6	1.6	1.6	1.6	1.6		1.6	1.6	1.6	1.6		1.6	
Day 28 am	669		1.6	1.6	1.6	1.6		1.6	1.6	1.6	1.6		1.6	
Day 32 pm	777		1.6		1.6					1.6	1.6			
Day 36 am	862		1.6							1.6				
Day 40 pm	968					1.6				1.6				
Day 49 am	1173					1.6				1.6				

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 2b. Concentration of doramectin in milk (µg/kg) after retreatment with a topical dose of 0.58 mg/kg in Northern Australia

	Hours	Retreatment with doramectin at 0.581 mg/kg												
Day Time	Post-Dose	7	56	59	75	1408	1413	1623	1717	1754	1807	Max	Mean	Std dev
Day 56 am	0						1.6			1.6				
Day 56 pm	9	5.5	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	5.5	2.0	1.2
Day 57 am	21	9.9	3.8	1.6	5.8	1.6	1.6	1.6	3.4	4.4	1.6	9.9	3.5	2.7
Day 57 pm	33	11.1	5.5	3.4	13.8	1.6	1.6	6.0	12.1	5.3	1.6	13.8	6.2	4.6
Day 58 am	45	10.7	5.4	1.6	10.0	1.6	1.6	5.9	14.0	7.1	4.8	14.0	6.3	4.3
Day 58 pm	57	12.8	6.0	1.6	12.7	3.2	1.6	6.8	21.7	5.8	9.3	21.7	8.1	6.2
Day 59 am	69	7.2	5.0	4.0	6.8	1.6	1.6	5.8	18.1	5.8	6.7	18.1	6.3	4.6
Day 59 pm	81	5.7	6.0	5.0	11.7	4.4	3.9	4.9	15.9	5.1	8.8	15.9	7.1	3.9
Day 60 am	93	8.8	9.2	6.1	18.2	1.6	3.9	4.5	15.8	5.5	8.4	18.2	8.2	5.2
Day 60 pm	105	9.2	9.8	5.4	12.5	1.6	3.2	4.4	7.7	6.4	8.0	12.5	6.8	3.3
Day 61 am	117	7.4	8.7	7.6	13.9	1.6	3.7	4.4	16.6	7.1	8.1	16.6	7.9	4.5
Day 61 pm	129	8.0	9.1	5.5	10.7	1.6	1.6	4.5	10.6	6.4	6.9	10.7	6.5	3.3
Day 62 am	141	7.0	6.8	3.9	11.6	1.6	1.6	4.8	11.4	6.6	7.9	11.6	6.3	3.5
Day 62 pm	153	1.6	5.3	1.6	6.6	1.6	1.6	4.6	11.4	5.1	4.7	11.4	4.4	3.1
Day 63 am	165	7.8	5.2	5.7	7.8	1.6	3.7	4.7	10.9	5.0	6.0	10.9	5.8	2.6
Day 63 pm	177	6.5	4.4	4.9	5.9	1.6	1.6	3.5	6.1	4.5	3.9	6.5	4.3	1.7
Day 66 am	237	1.6	1.6	1.6	5.3	1.6	1.6	1.6	4.3	3.5	3.7	5.3	2.6	1.4

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 3a. Concentration of doramectin in milk (µg/kg) after injectable treatment of 0.23 mg/kg in Northern Australia – Group 1

	Hours	Concentration of doramectin (µg/kg) / cow number											Max	Mean	Std dev
Day Time	Post-Dose	29	497	551	552	563	575	646	656	673	685	445			
Day 0 am	0														
Day 0 pm	7	7.4	3.7	4.0	9.4	6.2	9.4	8.9	8.5	7.1	15.4	12.2	15.4	8.4	3.4
Day 1 am	19	12.3	11.7	17.2	20.8	36.3	10.6	36.6	21.7	21.0	44.8	29.0	44.8	23.8	11.4
Day 1 pm	31	22.2	18.0	21.8	36.2	57.1	15.4	56.5	30.7	28.9	50.4	46.61	57.1	34.9	15.5
Day 2 am	43	28.1	18.7	23.4	42.2	56.7	16.4	57.7	37.9	30.1	67.5	51.0	67.5	39.1	17.3
Day 2 pm	55	31.5	24.5	33.3	1.6	71.4	16.1	60.8	33.7	41.1	76.3	54.39	76.3	40.4	23.2
Day 3 am	67	36.3	26.3	33.0	50.9	64.2	39.5	54.7	34.5	39.7	76.3	54.73	76.3	46.4	15.1
Day 3 pm	78	35.3	22.1	35.3	58.8	35.5	32.5	53.7	23.8	23.1	77.7	32.64	77.7	39.1	17.3
Day 4 am	91	37.0	26.6	50.2	46.5	77.4	41.5	47.2	43.8	46.4	75.9	53.42	77.4	49.6	15.1
day 4 pm	102	33.9	26.7	33.9	44.1	58.4	28.8	45.2	25.0	50.0	65.2	49.49	65.2	41.9	13.3
Day 5 am	116	33.4	26.6	46.1	43.3	52.2	43.1	36.4	42.0	41.7	60.5	48.73	60.5	43.1	9.2
Day 5 pm	127	32.7	36.9	46.2	37.1	52.4	45.9	37.2	32.4	40.7	56.2	43.5	56.2	41.9	7.7
Day 6 am	139	31.7	31.2	31.5	35.9	49.0	42.7	30.9	28.6	38.8	40.1	39.34	49.0	36.3	6.2
Day 6 pm	151	28.7	26.7	30.5	26.2	43.0	37.9	30.0	21.6	32.2	31.5	36.55	43.0	31.3	6.0
Day 7 am	163	24.0	22.7	26.2	25.7	40.8	46.1	25.4	18.0	29.4	23.0	39.52	46.1	29.2	8.9
Day 7 pm	175	27.2	25.5	30.8	22.7	36.0	31.6	21.3	16.7	30.4	27.4	31.11	36.0	27.3	5.5
Day 10 am	236	15.2	15.5	14.0	14.7	19.0	21.0	13.9	8.9	25.9	10.9	25.13	25.9	16.7	5.4
Day 13 pm	318	8.7	11.8	9.5	8.5	10.4	13.0	3.9	4.2	16.7	4.1	20.56	20.6	10.1	5.3
Day 16 am	380	4.1	6.7	4.5	4.5	5.6	7.4	1.6	1.6	11.1	1.6	11.24	11.2	5.4	3.5
Day 19 pm	462	3.2	7.9	3.5	1.6	3.3	3.9	1.6	1.6	7.2	1.6	10.06	10.1	4.1	3.0
Day 22 am	524	1.6	1.6	3.8	1.6	1.6	1.6	1.6	1.6	5.5	1.6	8.2	8.2	2.7	2.2
Day 25 pm	606	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	3.8	1.6	4.7	4.7	2.1	1.1
Day 28 am	668	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6		1.6		1.6	
Day 32 pm	780	1.6	1.6	1.6	1.6	1.6	1.6			1.6	1.6	1.6			
Day 36 am	860	1.6	1.6	1.6	1.6		1.6			1.6					
Day 40 pm	966						1.6								
Day 49 am	1172														
Day 56 am	1340														

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 3b. Concentration of doramectin in milk (µg/kg) after a second injectable treatment of 0.23 mg/kg in Northern Australia - Group 1

	Hours	Retreatment Concentration of doramectin (µg/kg) / cow number											Max	Mean	Std dev
Day Time	Post-Dose	29	497	551	552	563	575	646	656	673	685	445			
Day 56 pm	7	7.0		6.6	8.6	7.8	9.8	9.8	10.7	6.6	23.4	3.7	23.4	9.7	5.6
Day 57 am	22	40.6		19.4	36.0	17.1	24.9	27.7	28.0	14.9	35.1	19.4	36.0	24.7	7.6
Day 57 pm	31	38.0		26.3	73.7	32.1	29.0	38.8	34.0	20.8	51.8	31.0	73.7	37.5	16.1
Day 58 am	45	30.4		23.7	46.2	26.9	29.0	44.8	32.5	18.2	42.8	29.1	46.2	32.6	9.9
Day 58 pm	56	51.0		33.5	134.7	81.3	26.9	51.9	38.4	20.3	61.6	29.3	134.7	53.1	36.2
Day 59 am	70	104.3		27.9	43.9	44.8	27.8	48.1	33.8	25.1	54.5	30.6	54.5	37.4	10.6
Day 59 pm	79	92.6		32.4	71.0	67.3	33.8	55.7	37.0	17.9	61.5	34.7	71.0	45.7	18.5
Day 60 am	93	53.7		25.4	43.1	41.8	32.3	46.5	28.9	21.3	40.9	33.5	46.5	34.9	8.7
Day 60 pm	104	51.6		24.6	54.2	43.0	28.8	50.8	28.0	28.8	41.9	32.2	54.2	36.9	10.8
Day 61 am	117	44.1		24.1	35.8	34.5	27.0	44.0	26.7	29.0	38.4	30.6	44.0	32.2	6.4
Day 61 pm	128	49.5		24.6	39.4	46.4	22.3	44.6	25.5	25.0	31.2	30.0	46.4	32.1	9.1
Day 62 am	141	38.3		26.2	35.4	30.3	20.0	37.0	25.2	28.5	28.1	31.7	37.0	29.1	5.2
Day 62 pm	151	42.9		28.8	39.0	36.5	21.6	39.8	14.6	29.2	22.6	31.3	39.8	29.3	8.5
Day 63 am	167	33.1		23.2	32.3	28.2	25.9	32.7	25.0	23.3	19.0	33.5	33.5	27.0	5.0
Day 63 pm	175	37.2		29.7	36.5	38.2	18.7	36.2	71.7	30.4	20.3	39.5	71.7	35.7	15.4
Day 66 am	237	31.6		23.1	24.4	27.6	23.3	26.0	40.6	29.3	12.0	24.8	40.6	25.7	7.4

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 3c. Concentration of doramectin in milk (µg/kg) after injectable treatment with a dose of 0.23 mg/kg in northern Australia – Group 2

	Hours	Concentration of doramectin (µg/kg) / cow number										Max	Mean	Std dev
Day Time	Post-Dose	786	816	2546	2721	2774	7241	7244	8272	9329	9359			
Day 0 am	0													
Day 0 pm	7	10.4	12.9	6.7	11.1	26.0	5.4	8.2	14.5	9.7	5.6	26.0	11.0	6.1
Day 1 am	19	32.0	29.3	17.8	24.9	50.3	18.8	16.9	27.1	24.2	9.6	50.3	25.1	11.1
Day 1 pm	31	31.8	46.4	27.1	46.7	69.8	49.3	23.9	46.3	41.3	25.3	69.8	40.8	14.2
Day 2 am	43	14.5	43.1	34.2	46.2	63.9	47.6	32.5	53.6	49.6	23.4	63.9	40.9	14.8
Day 2 pm	55	45.4	41.6	41.2	46.4	80.0	37.8	24.4	41.5	51.6	26.7	80.0	43.7	15.3
Day 3 am	67	41.9	39.6	41.7	41.9	68.3	33.2	20.2	37.3	49.1	24.8	68.3	39.8	13.2
Day 3 pm	79	36.3	36.7	33.8	43.2	31.8	31.8	27.9	28.8	43.1	25.9	43.2	33.9	6.0
Day 4 am	91	38.8	34.1	53.7	43.1	51.8	27.9	30.4	37.0	45.1	31.4	53.7	39.3	8.9
Day 4 pm	103	32.5	30.9	36.8	44.5	29.9	27.8	22.3	40.1	38.1	22.8	44.5	32.6	7.3
Day 5 am	115	35.8	31.0	39.4	45.7	38.6	24.3	21.7	39.1	36.4	31.7	45.7	34.4	7.3
Day 5 pm	127	29.6	29.7	33.4	46.7	40.4	29.2	21.9	36.8	34.5	32.1	46.7	33.4	6.8
Day 6 am	139	26.7	24.7	28.1	42.8	29.7	32.1	22.5	29.0	31.2	32.1	42.8	29.9	5.5
Day 6 pm	151	19.5	21.4	33.4	24.6	23.6	27.3	23.3	29.9	28.6	29.8	33.4	26.1	4.4
Day 7 am	163	17.8	18.1	31.9	29.0	16.1	25.7	16.9	30.7	25.0	29.0	31.9	24.0	6.2
Day 7 pm	175	16.4	16.8	22.0	33.8	22.5	26.0	21.1	25.5	29.2	21.4	33.8	23.5	5.3
Day 10 am	235	9.7	8.6	16.2	24.4	12.1	19.7	13.4	15.1	12.1	21.7	24.4	15.3	5.2
Day 13 pm	319	4.1	4.3	6.0	18.2	6.8	11.4	10.1	14.6	4.5	15.6	18.2	9.5	5.2
Day 16 am	379	1.6	1.6	3.1	11.2	3.6	6.2	6.0	9.2	1.6	11.8	11.8	5.6	4.0
Day 19 pm	463	1.6	1.6	1.6	8.9	1.6	3.3	5.9	7.1	1.6	11.3	11.3	4.4	3.6
Day 22 am	523	1.6	1.6	1.6	5.2		1.6	1.6	1.6	1.6	8.7	8.7	2.8	2.5
Day 25 pm	607	1.6	1.6	1.6	3.5			1.6	1.6	1.6	5.3	5.3	2.3	1.4
Day 28 am	667	1.6	1.6	1.6	1.6				1.6	1.6	4.4	4.4	2.0	1.1
Day 32 pm	775		1.6	1.6	1.6				1.6	1.6	1.6		1.6	
Day 36 am	859			1.6						1.6	1.6		1.6	
Day 40 pm	967													
Day 49 am	1176													
Day 56 am	1340													

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 3d. Concentration of doramectin in milk (µg/kg) after a second treatment with a dose of 0.23 mg/kg in northern Australia – Group 2

	Hours	Retreatment: Concentration of doramectin (µg/kg) / cow number										Max	Mean	Stdev
Day Time	**Post-Dose**	**786**	**816**	**2546**	**2721**	**2774**	**7241**	**7244**	**8272**	**9239**	**9359**			
Day 56 am														
Day 56 pm	7	9.1	5.4	6.3	18.7	16.1	9.7	8.6	7.7	9.4	7.2	18.7	9.8	4.3
Day 57 am	22	27.7	19.1	13.2	47.1	57.9	24.3	13.1	17.1	23.3	17.8	57.9	26.0	14.9
Day 57 pm	31	35.0	24.5	27.2	51.3	82.8	28.7	22.6	26.0	32.3	26.4	82.8	35.7	18.5
Day 58 am	45	35.3	23.6	22.1	52.0	77.9	28.7	17.1	19.4	30.6	25.3	77.9	33.2	18.6
Day 58 pm	56	38.7	29.5	33.3	47.2	83.4	29.9	29.6	37.5	37.5	28.1	83.4	39.5	16.5
Day 59 am	70	42.8	26.7	28.5	50.7	79.9	30.5	17.4	33.2	33.6	23.0	79.9	36.6	17.9
Day 59 pm	79	35.1	43.6	33.1	52.4	78.3	32.9	30.1	37.6	37.7	26.9	78.3	40.8	15.0
Day 60 am	93	36.6	28.5	22.6	44.5	65.6	30.0	17.4	29.4	30.2	23.3	65.6	32.8	13.8
Day 60 pm	104	35.6	45.9	33.0	40.6	72.3	29.8	26.6	35.9	32.8	24.2	72.3	37.7	13.7
Day 61 am	117	32.9	24.4	23.0	41.5	57.3	27.2	24.7	28.8	29.4	20.6	57.3	31.0	11.0
Day 61 pm	128	28.8	37.3	33.5	34.5	48.1	26.5	23.4	30.6	28.8	21.5	48.1	31.3	7.7
Day 62 am	141	35.1	20.3	19.4	32.6	41.9	23.4	37.3	25.2	29.0	21.7	41.9	28.6	7.8
Day 62 pm	151	30.7	26.8	18.1	29.4	25.9	18.8	30.9	23.1	28.8	22.7	30.9	25.5	4.7
Day 63 am	167	29.5	17.7	15.5	27.0	38.2	23.8	22.0	25.5	26.0	20.7	38.2	24.6	6.4
Day 63 pm	175	28.3	21.9	19.1	28.5	57.3	27.3	26.4	37.3	29.0	23.4	57.3	29.8	10.8
Day 66 am	237	24.5	13.8	11.6	19.0	21.1	19.3	17.2	21.0	18.3	13.4	24.5	17.9	4.0

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Results

To facilitate sample handling, storage and analysis, cattle samples from trial were split into two groups by the Analytical Laboratory. The doramectin milk residue analysis was done using the same High Performance Liquid Chromatography – fluorescence detection method noted previously.

Doramectin concentrations in milk from animals treated with the injectable formulation increased gradually from non detectable concentrations at pre-treatment to a maximum mean value of 44.7 µg/kg at the morning milking on day 4 (91 hours post-treatment). Maximum individual milk doramectin concentrations occurred between the evening milking on day 1 and the morning milking on day 7, with the highest individual replicate value observed (80.0 µg/kg) at the evening milking on day 2 (55 hours post treatment). Between the evening milking of days 1 and 5 (31 to 127 hours post treatment) mean milk doramectin concentrations were fairly constant, ranging between 36.7 µg/kg and 44.7 µg/kg. Subsequently, milk doramectin residues gradually declined, with residues below the limit of quantitation in 9 of the 21 treated cows by the evening milking on day 19 (462 hours post treatment) and to below the LOQ in all animals by the evening milking on day 32 (774 hours post-treatment).Results are summarized in Table 3a and 3c.

Following retreatment on day 56, doramectin residues increased to a maximum mean value of 46.2 µg/kg by the evening milking on day 58 (56 hours post-retreatment). Mean doramectin milk residues were fairly constant between 31 and 104 hours post-retreatment, with values from 32.7µg/kg to 46.2 µg/kg. Residues then decreased to a mean value of 22.1µg/kg by 10 days (237 hours) after re-treatment. Individual maximum milk doramectin concentrations occurred between the evening milking on day 58 and the evening milking on day 63, with the highest individual replicate values in the two data sets (134.7 and 83.4 µg/kg) observed at 56 hours post re-treatment. The overall milk residue depletion profiles following both the initial and second treatment were similar. Results for the retreatment in the two groups of lactating cattle are summarized in Tables 3b and 3d.

Milk fat analysis were conducted using samples collected at the morning milking on day 1, day 4 and day 10 post treatment. Overall mean doramectin residues in milk fat at these time points were 557.0µg/kg, 1036µg/kg and 353.8 µg/kg, respectively. The concentration increases observed in the milk fat were consistent with the increases in doramectin residues in whole milk. .

The amount of doramectin residues resulting from the treatment with the injectable formulated product is distinctly different – approximately three times higher. While there are several possible reasons for this, the implication for recommendations on MRLs is noteworthy.

METHOD VALIDATION STUDIES

This study was conducted to validate an analytical methodology suitable for the recovery and quantification of doramectin in bovine milk, and to determine the partitioning of doramectin between the aqueous and fat components of whole milk, following milk fat separation by standard techniques. One animal was chosen for the second objective; the animal utilized had medium milk production (24 liters per day). The animal was weighed and treated with a 5 mg/ml doramectin pour-on formulation at a dose rate of 1.0 ml/8.6 kg (0.581µg/kg). Samples were collected on day 0, with replicates 4 to 6 fortified with 25 µg/kg doramectin, Replicates 7 to 9 were fortified with 50 µg/kg doramectin, Replicates 10 to 12 were fortified with 100 µg/kg doramectin with the remaining replicates 13 to 20 retained frozen.

The analytical method is described - "*A Determinative Procedure for the Detection and Quantitation of Doramectin in Cattle Plasma and Milk*". In the method, aliquots of milk were fortified with doramectin (UK-67,994) and the internal standard (UK-71,674) where appropriate and extracted prior to analysis by the high performance liquid chromatography (HPLC)-fluorescence method previously referred to.

The detection and quantitation of doramectin residues is based on the extraction procedure from plasma and milk and requires subsequent conversion to a fluorescent derivative (trifluoroacetic anhydride, triethylamine and acetonitrile). The conversion of doramectin is carried out in the presence of a fixed quantity of internal standard similar in structure to doramectin. The HPLC conditions are as follows:

Mobile phase:50% acetonitrile:	30% tetrahydrofuran: 20% Milli Q water. The mobile phase was filtered through a 0.45 µm Millipore filter.
The conditions for analysis:	
Flow rate	1.2 mL/min
Injection volume	10-15 µL
Detection (fluorescence) -	470 nm; Excitation (fluorescence) - 360 nm
Chromatographic run time	7-18 min.

The chromatographic system was highly satisfactory in terms of column efficiency, peak resolution, peak symmetry (tailing factors <1.03), system precision and linearity of response was satisfactory.

The limit of detection (LOD) for doramectin residues in milk was 0.061 µg/kg, determined from the analysis of blank samples and using the mean value plus three standard deviations, with a limit of quantification (LOQ) set at 3.12 µg/kg, determined from the fortified concentration in the method studies where the mean accuracy of quantification was 94.8% with the mean percentage imprecision of 6.1%.

The linearity of the assay was determined in milk fortified with doramectin at 50, 100, 200 µg/kg and had good linearity; the mean quantification at these concentrations being 50.7, 101.3 and 199.6 µg/kg for the 50, 100 and 200 µg/kg fortified samples. For the 50 µg/kg samples, the intra-assay accuracy is 97.7 - 104.2% with an intra-assay imprecision range of 0.4 – 4.4% while the mean inter-assay (inter day) accuracy and imprecision is 101.5% ± 3.5%, respectively. For the 100 µg/kg fortified samples, the intra-assay ranged from 97.0 – 103.8% with an intra-assay imprecision range of 0.6 – 4.3%. The mean inter –assay (inter day) accuracy and imprecision is 101.3% ± 4.0% respectively. For the 200 µg/kg samples, the intra-assay accuracy ranged from 95.0 – 104.4% with an intra-assay imprecision range from 0.8 – 3.9%. The mean inter-assay (inter day) accuracy and imprecision is 99.8% ± 4.5%, respectively.

The intra-day accuracy and recovery for doramectin at the LOQ varies from 87.8 to 115.4% with an intra-day imprecision of 3.9% - 6.6%. The inter-day accuracy and imprecision at the LOQ is 102.4%±12.4%.

The intra-day accuracy/recovery for doramectin at 50 µg/kg ranged from 85.3 to 106.4% with an overall intra-day accuracy/imprecision of 95.9%±14.3%. The intra-day accuracy and recovery for doramectin at 100 µg/kg are from 96.8 - 102.3% with an overall accuracy/imprecision of 99.0±7.8%. The inter-day accuracy and imprecision at 200 µg/kg is from 102.3 - 105.2% with an overall accuracy and imprecision of 101.4±8.5%.

Doramectin in milk was stable following a three-time freeze-thaw cycle. The concentration of doramectin in milk was within 7% of the freshly prepared samples. Doramectin fortified control milk samples (fortified with doramectin at 25, 50 and 100 µg/kg) were stable under frozen storage conditions (-20°C) for up to 6 months. Concentrations at the end of the storage period differed <10% from the initial values. Report analysis of milk (incurred residues) collected after 1 and 4 days from the pre-experimental doramectin pour on treated cow indicated that doramectin ranged from -7.6% to +5.2% of the original quantitation after 3 months of frozen storage and +7.8% and +20.4% following 6 months of frozen storage. At concentrations above the LOQ, incurred doramectin milk residues were stable under frozen conditions for a period of at least 6 months.

The partition of doramectin into milk fat was determined by analysis of doramectin in butterfat prepared from the cream of milk. A slightly modified procedure to a determinative procedure for the quantification of doramectin in cattle fat was used for quantitation of doramectin residues in butterfat

The recovery of doramectin from butterfat at 50 µg/kg with a fixed quantity of UK-71,647 internal standard was 101.4% for doramectin and 108.9% for the internal standard. At a doramectin concentration of 100µg/kg and the same internal standard concentration the recoveries are 110.2% and 116.1%, respectively. The accuracy of estimation of doramectin in the 50 and 100 µg/kg butterfat samples is 103.3% and 105.9%, respectively, with a imprecision (%CV) of 5.7% and 6.5%, respectively. Results of all method performance are summarized in Table 4.

Table 4. Summary of Method Validation Parameters

Parameter	Results
Intra-day accuracy (imprecision): LOQ	87.8 - 115.4% (3.9 – 6.6%)
Intra-day accuracy (imprecision): 50 µg/kg	97.7 - 104.2% (0.4 – 4.4%)
Intra-day accuracy (imprecision): 100 µg/kg	97.0 - 103.8% (0.6 - 4.3%)
Intra-day accuracy (imprecision): 200 µg/kg	95.0 - 104.4% (0.8 - 3.9%)
Inter-day accuracy and imprecision: LOQ	102.4% ± 12.4%
Inter-day accuracy and imprecision : 50 µg/kg	101.5% ± 3.5%
Inter-day accuracy and imprecision: 100 µg/kg	101.3% ± 4.1%
Inter-day accuracy and imprecision 200 µg/kg	99.8% ± 4.5%
Intra-day recovery (doramectin): 50 µg/kg	85.3% - 106.4%
Intra-day recovery (doramectin): 100 µg/kg	96.8% - 102.3%
Intra-day recovery (doramectin): 200 µg/kg	102.3% - 105.2%
Inter-day recovery and imprecision: 50 µg/kg	95.9% ± 14.3%
Inter-day recovery and imprecision: 100 µg/kg	99.0% ± 7.8%
Inter-day recovery and imprecision: 200 µg/kg	101.4% ± 8.5%
Inter-day recovery (imprecision):	. 96.4% (. 18%)
Accuracy(imprecision) for DOR in butterfat (50 µg/kg)	103.3% ± 5.7%
Accuracy(imprecision) for DOR in butterfat (100 µg/kg)	105.9% ± 6.5%
Limit of Quantification of DOR in milk	3.125 µg/kg
Limit of Detection of DOR in milk	0.061 µg/kg
Linearity of response	r-squared >0.999
Doramectin Stability in Milk (3X Freeze-Thaw cycles)	Stable
Doramectin Stability in Milk (Extended Frozen Storage)	Stable for at least 6 months

APPRAISAL

The 14th Session of the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) requested consideration of MRL for bovine milk. The sponsor submitted three new residue depletion studies for doramectin to extend its use to lactating cattle for the control of internal and external parasites. The recommended dosage for the pour-on formulation is 0.5 mg/kg bw and for the injectable formulation it is 0.2 mg/kg bw. At the present meeting two studies were reviewed using the pour-on formulation and one using the injectable formulation. In addition, performance data were provided for the analytical method to determine residues of doramectin in milk from lactating dairy cattle.

Milk Residue Studies.

The first study using a pour-on treatment was conducted with ten dairy Holstein cows. The treatment was at a dose of 0.58 mg/kg bw doramectin and re-treatment with the same dose 56 days later. Milk samples were collected for 49 days and 10 days, respectively, following the first and second treatments. The doramectin milk residue and milk/fat residue were determined using validated High Performance Liquid Chromatography and Fluorescence detector method. The doramectin residue concentrations in milk increased to a maximum mean value of 22µg/kg at 72 hours post-dose. Mean doramectin residues decreased to concentrations below the limit of quantitation (3µg/kg) at 384 hours (16 days). After re-treatment doramectin residues increased gradually to a maximum mean value of 12µg/kg at 48 hours post-dose; and decreased to less than 4µg/kg at 240 hr (10 days) post-dose. The milk/fat analyses were conducted at 1, 4, and 10 days post-dosing. Mean doramectin residues in the milk fat at these time points were 171µg/kg, 501µg/kg and 114µg/kg, respectively. The doramectin ratios in milk fat versus milk were estimated for each of the corresponding sampling times. Milk fat concentrations factors for doramectin residues were 29.6, 32.2 and 24.7, respectively.

In the second study, animals were treated with doramectin by a topical route (pour on) using a dose of 0.58 mg/kg and re-treatment with the same dose 56 days later. Milk samples were collected as the same study. Doramectin concentrations in milk increased to a maximum mean value of 9µg/kg at 45 hours post-dose and decreased to below the LOQ by 237 hours(10 days). Following re-treatment on day 56 residues increased to a mean maximum value of 8µg/kg at 93 hours and decreased to less than the LOQ at 237 hours (10 days) post re-treatment. Mean doramectin residues in the milk fat at 1, 4, and 10 days were 91µg/kg, 142µg/kg and 55µg/kg, respectively. Milk fat concentration factors for doramectin residues versus milk were 14.2, 20.9 and 14.1, respectively.

Differences in residue concentrations between the two studies were attributed to climatic and productions factors.

The third study determined the residue depletion profile of doramectin following the administration of subcutaneous 0.23 mg/kg doramectin injectable formulation in lactating cattle followed by re-treatment at the same dose 56 days later. Sampling followed the same protocol as the two previous studies. The doramectin milk residue analysis was conducted using the High Performance Liquid Chromatography-fluorescence detection method noted previously. Doramectin concentrations in milk increased gradually to a maximum mean value of 45µg/kg at 67 hours. Subsequently, doramectin residues gradually declined, with mean residues below LOQ at 523 hours (22 days). Following re-treatment, doramectin residues increased to a maximum mean value of 53µg/kg at 56 hours. Residues then decreased to a mean value of 25µg/kg at 237 hours(10 days) after re-treatment Residues resulting from the injection treatment were consistently higher at any given time point than from the pour-on formulation. Milk fat analysis were conducted using samples collected at the morning milking on day 1, day 4 and day 10 post treatment. Mean doramectin residues concentrations in milk fat at these time points were 557µg/kg, 1036µg/kg and 354µg/kg, respectively. Milk fat concentration factors were 24, 24.2 and 23.4 respectively.

Method Validation Studies

This study was conducted to validate analytical methodology for the recovery and quantitation of doramectin residues in bovine milk. In the method validation, aliquots of milk were fortified with doramectin and the internal standard and extracted prior to analysis by the high performance liquid chromatography (HPLC)-fluorescence method. The method is based on the extraction procedure used for tissue and requires on-column conversion to a fluorescence derivative. The limit of quantification (LOQ) was approximately 3µg/kg. The recovery estimated at the LOQ is 95%. Method performance data indicate it is suitable for use in residue depletion studies and for routine surveillance purposes.

MAXIMUM RESIDUE LIMITS

In considering MRLs for doramectin in milk, the Committee agreed to take into account the following factors:

- The acceptable daily intake for doramectin is 0-1µg/kg body weight, equivalent intake of up to 60 µg per day for a 60 kg person
- Based on MRLs for tissues in cattle and pigs, and the theoretical maximum daily intake of residues in tissue using 33µg/day, approximately 27 µg per day are available for milk.
- Based on its limited metabolism, the single component and the known large partitioning ratio for residues between milk fat and aqueous milk, the Committee considers that the ratio for marker residue to total residue for doramectin in milk would be equivalent to the ratio of doramectin residues in fat (0.80).
- The residue studies provided used a pour-on formulation at 0.58 mg/kg bw and the injectable formulation at 0.23 mg/kg, somewhat in excess of the recommended doses of 0.5 mg/kg bw and 0.2 mg/kg bw, respectively.

- The marker residue is doramectin.
- A suitable analytical method is available for determining residues in milk.

To accommodate the maximum daily intake of residues based in the ADI, The Committee recommends an MRL of 15µg/kg for doramectin residues in bovine milk with residues determined as parent drug.

Taking into account the doramectin marker residue to total residue ratio in milk (80%) and the MRL for residues for doramectin in milk, the theoretical daily intake from 1.5 kg of milk would be 28 µg per day. The estimated theoretical intake of doramectin residues from tissues and milk is 61µg.

The Committee draws attention to National regulatory authorities of the following comment:

The recommended MRL represents the highest value consistent with the residue limits permitted by the ADI. On the basis of the recommended 15 µg/kg MRL for doramectin in whole milk in cattle, it is important to note that this MRL may require milk discard times up to 240 hours for milking cattle based on one study using the pour-on treatment. Milk discard times would be approximately 480 hours following treatment using the injection formulated dose.

REFERENCES

Australian Pesticides & Veterinary Medicines Authority (2003). Evaluation Report for applicant proposal relevant to Doramectin pour-on endectocide. October 2003.

EMEA (1997). The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Summary report (2).

Pfizer Animal Health (2002a), "A field and laboratory study to determine the residue depletion profile of doramectin in milk following the administration of a 5 mg/ml doramectin pour on formulation in lactating dairy cattle" (site 1), Volume 3 ,October 2002. Study report 2539B-14-004 (PFD 1115).

Pfizer Animal Health (2002b) "A field and Laboratory study for the validation of the analytical procedures used in the quantitation of doramectin in milk from dairy cattle. Volume 2, October 2002. Study report 2939A-14-01-002 (PFD 1121).

Pfizer Animal Health (2003a), "Determination of the analytical depletion profile of doramectin in milk following the administration of a 5 mg/ml doramectin pour on formulation in lactating dairy cattle" (site 2), Volume 4, March 2002. Study report 2539B-14-01-004 (PFD 1253).

Pfizer Animal Health (2003b) "A field and Laboratory study to determine the residue depletion profile of doramectin in milk following administration of a 10 mg/ml doramectin injectable formulation in lactating dairy cattle. Volume 5, April 2003. Study report 2539A-14-01-003 (PFD 1205).

Pfizer Animal Health "monograph of doramectin MRL in bovine milk". Volume 1, August 2003.