IVERMECTIN

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ADDENDUM
to the Ivermectin residue monographs
prepared by the 36th and 40th meetings of the Committee and published in
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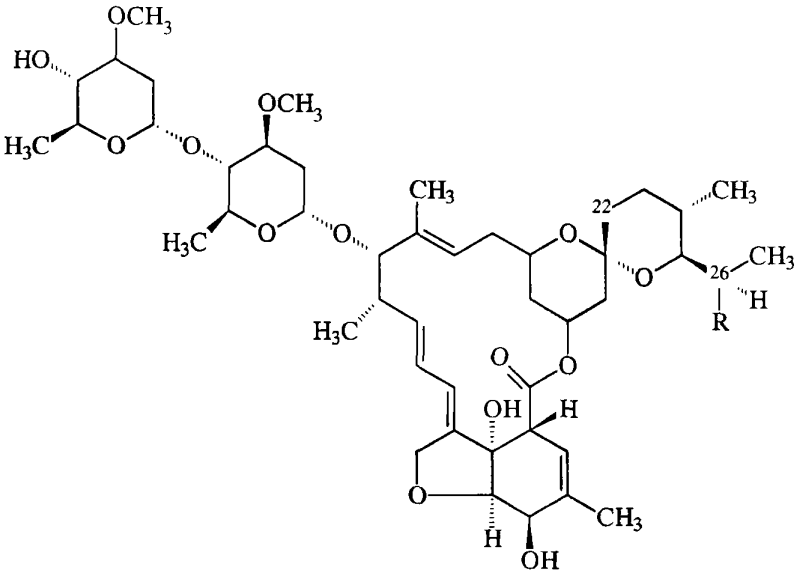
IDENTITY

Chemical name: 5-O-Demethyl-22,23-dihydroavermectin-A_{1a}
5-O-Demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl) avermectin-A_{1a}; 22,23 dihydroavermectin B_{1b} (Chemical Abstracts name)
CAS Number: 7-288-86-7

International Nonproprietary Name: Ivermectin

Structural formula:



Molecular formula: Component **B_{1a}**: $C_{48}H_{74}O_{14}$
Component **B_{1b}**: $C_{47}H_{72}O_{14}$

Molecular mass: Component **B_{1a}**: 874
Component **B_{1b}**: 860

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Off-white powder
Crystals from ethanol/water, m.p. 154.5 - 157°C

Optical rotation: $[\alpha]_D$: +71.5 ± 3° (c=0.755 in chloroform)

Ultraviolet-maxima: 238 nm (ε = 27100), 245 nm (ε = 30100) in methanol

Solubility: Solubility in water: 0.006-0.009 mg/L

INTRODUCTION

Ivermectin is widely used as a broad-spectrum antiparasitic drug against nematodes and arthropods in food-producing animals. In human medicine, it is mainly used for the treatment of *onchocerciasis*. Ivermectin was previously reviewed by the Committee at its thirty-sixth and fortieth meeting (Annex 1, reference 91, Annex 1, reference 104). At the 40th meeting the Committee recommended an ADI of 0-1 µg/kg of body weight per day and MRLs for ivermectin B_{1a} in cattle of 100 µg/kg in liver and 40 µg/kg in fat. At its present meeting, the Committee reviewed additional studies in which the drug was used topically in dairy cows. The recommended dose is 0.5 mg/kg of body weight. The drug is used as a solution (5g/L).

RESIDUES IN MILK AND THEIR EVALUATION

Six lactating Friesian dairy cows, were treated with a single dose of approximately 580 µg/kg of body weight (**O'Neill**, 1997). The characteristics of the animals, including treatment and performance are summarized in Table 1. The cows were grazing on irrigated pasture. During milking they were fed a specially formulated supplement. Milk samples were collected twice daily and were analyzed using a method with a postulated limit of detection (LOD) of 1µg/kg of milk for both Ivermectin B_{1a} and Ivermectin B_{1b}. Method recoveries were as given in Table 2. The results were corrected for recoveries.

Table 1. Characteristics of the Friesian cows and their performance and depletion of residues in milk

Friesian Cow Study						
Animal identification number	1	2	3	4	5	6
Number of lactations	2	3	6	5	5	8
Days in milk	134	125	128	127	99	125
Body weight [kg]	605	555	600	640	555	615
Dose [µg/kg of body weight]	579	586	583	578	586	585
Dose [mg/animal]	350	325	350	370	325	360
Milk yield [kg][a)]	247	311	289	264	279	298
Milkfat secreted [kg][a)]	10.2	11.7	10.4	11.0	10.6	9.9
Residues excreted [µg][a)]	784	579	756	927	832	990
% of dose excreted[a)] via milk	0.22	0.18	0.22	0.25	0.26	0.28

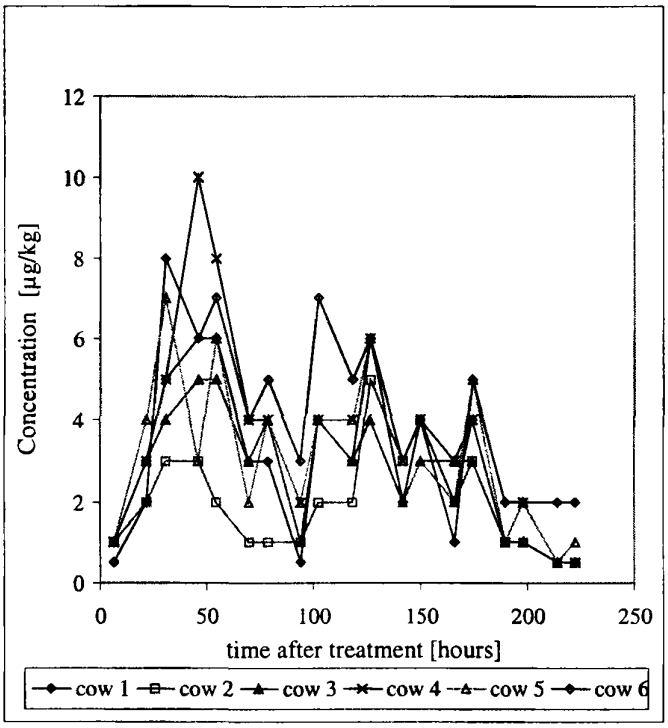
Note: [a)] summarized over the first 222.5 hours after treatment

Table 2. Recoveries of the analytical method used for the determination of Ivermectin B_{1a} in milk

Method recoveries for Ivermectin B_{1a} in milk				
Fortified level [µg/kg]	N	mean recovery [%]	standard deviation [%]	CV [%]
5	6	83	8.3	10
25	6	88	4.4	5
50	6	96	5.8	6

The concentrations of ivermectin B_{1a} in milk reached a maximum in samples obtained at the third or forth milking after treatment; subsequently one or more, usually broader, maxima, were reached. The later maxima were typically lower than the first maximum, except in milk obtained from one cow in which the highest concentration was reached at the tenth milking, about 130 h after treatment of the cow. The results are given in Figure 1

Figure 1. Milk excretion data on ivermectin H_2B_{1a} residues in Friesian cows treated with a single topical dose.



A similarly designed study was carried out using 3 - 6 year old lactating Jersey cows, 17 - 52 days in milk and treated with Coopers Paramax Pour-on for Cattle (**O'Neill** et al., 1998). This study was also carried out in a different geographical region. The animals were grazing on pasture. They were offered silage *ad libitum* in the paddock and received 5 kg of barley per day, split equally at each milking. The characteristics of the animals, including treatment and performance are summarized in Table 3. Milk samples were collected twice daily. Analyses were carried out using the same methodology as indicated for the study using Friesian cows.

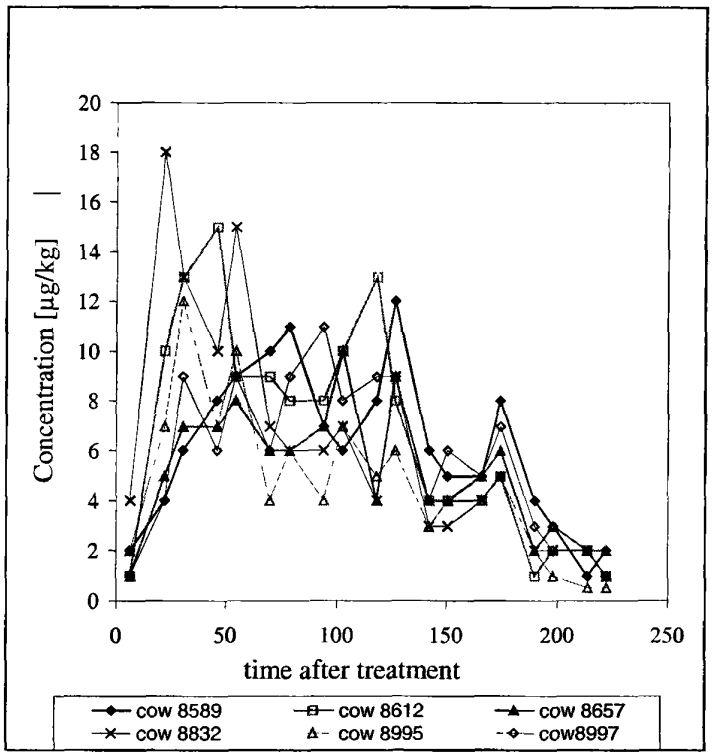
Table 3. Characteristics of the Jersey cows and their performance and depletion of residues in milk.

Jersey Cow Study						
Animal identification number	8612	8995	8832	8997	8657	8589
Number of lactations	4	3	4	3	4	4
Days in milk	26	36	45	36	17	52
Body weight [kg]	406	369	432	390	425	401
Dose [µg/kg of body weight]	579	583	579	577	576	586
Dose [mg/animal]	235	215	250	225	245	235
Milk yield [kg][a)]	202	149	191	161	187	142
Milkfat secreted [kg][a)]	12.9	7.39	11.8	9.21	10	9.13
Residues excreted [µg][a)]	1368	665	1203	923	908	868
% of dose excreted[a)] via milk	0.85	0.31	0.48	0.41	0.37	0.37

Note: [a)] during the first 222.5 hours post-dose

In this second study milk yield was lower than in the first one, fat excretion was only slightly lower and residue excretion was significantly higher. Thus, a significantly higher proportion of the administered dose was excreted via milk. The kinetics of depletion of residues was similar (see Figure 2), but the maxima in the milk of most animals, were higher. The highest concentrations of ivermectin B_{1a} in the milk of individual animals during the period of observation was 5-10 µg/ kg of milk in the study of Friesian cows. In the study of Jersey cows the range of the maxima was 10-18 µg/kg of milk. The contribution to the total residue of the concentrations of ivermectin B_{1b} was insignificant in both studies and typically below the reported limit of detection.

Figure 2. Milk excretion data on ivermectin H_2B_{1a} residues in Jersey cows treated with a single topical dose.



METHOD OF ANALYSIS

A method for the identification and quantification of ivermectin B_{1a} and ivermectin B_{1b} residues in milk was submitted (**Agal**, 1998). Ivermectin residues are extracted from milk by homogenisation with acetonitrile and evaporation of the acetonitrile under vacuum. The residue is dissolved in a mixture of hexane and dichloromethane; this solution is applied to a silica gel cartridge and ivermectin residues are eluted from the cartridge with ethyl acetate. The ethyl acetate is removed with a stream of nitrogen. The dry residue is treated with a mixture of 1-methylimidazole and acetic anhydride in dimethylformamide, the derivatisation mixture is cleaned up by application to a C_{18} cartridge and washing of the cartridge with 50% aqueous methanol. The fluorescent ivermectin derivatives are then eluted with methanol. Methanol is removed under vacuum and the residue re-dissolved in a known volume of methanol.

HPLC determination is performed on a C_{18} HPLC column, using an elution solvent of 95% aqueous methanol and fluorescence detection (360 nm excitation, 468 nm emission). Under these conditions, total analysis time is about 15 minutes and the derivatives formed from ivermectin B_{1a} and B_{1b}, respectively, are well separated

This method was not developed under GLP, and an incomplete set of the required validation data was made available by the sponsor. Neither the limits of detection (LOD) nor the limits of quantification (LOQ) for the two compounds was determined. The recovery of the method was estimated at concentrations of 5, 25, and 50 µg/kg of ivermectin B_{1a} and 2 and 4 µg/kg of ivermectin B_{1b} (see the above Table 2); however, many of the milk samples obtained in the studies of depletion had lower concentrations.

MAXIMUM RESIDUE LIMITS AND INTAKE ASSESSMENT

The ADI established at the 40th meeting of the Committee was 0-1 µg/kg body weight, equivalent to a maximum of 60 µg per person. Taking into account the TMDI of 39.4 µg/person, as estimated for daily consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, and 50g of fat and the MRLs recommended at the 40th Meeting, a TMDI of 21 µg/person represents the highest allowable intake from the consumption of 1.5 kg of milk which would not result in a total TMDI in excess of the ADI. A concentration of ivermectin B_{1a} of 10µg /kg of whole milk would result in a TMDI of 10 x 1.5 =15µg/person for a daily consumption of 1500 g of milk.

On this basis the Committee recommended a temporary MRL of 10 μg for ivermectin B_{1a}/kg of whole milk in cattle. It was aware that with the currently used formulation of the drug this MRL would require a milk discard time of up to 11 milkings. However, the recommended MRL could serve also as a basis to develop other formulations and/or other conditions of use.

REFERENCES

AGAL (1998). Determination of macrocyclic lactones (ivermectins) in milk using pre-column derivatisation and fluorescence detection. Australian Government Analytical Laboratories Method, Version Number 6, Date of issue, 1998

O'Neill, P. (1997) A research report dealing ivermectin residue levels in whole milk following treatment of lactating dairy cattle with a 5.0 g/L ivermectin pour-on formulation for cattle. Doc. No. 97/0633; Schering-Plough Animal Health Limited.

O'Neill, P., Baum, K., and Ho, K.B. (1998) A research report dealing ivermectin residue levels in whole milk following treatment of lactating Channel Island breed dairy cattle with 5.0 g/L ivermectin pour-on formulation for cattle. Doc.No. 98/0540; Schering-Plough Animal Health Limited.