MOXIDECTIN

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ADDENDUM
to the moxidectin residue monographs prepared by the 45th, 47th and 48th meetings of the Committee and published in FAO Food and Nutrition Paper 41/8, Rome 1996, FAO Food and Nutrition Paper 41/9, Rome 1997 and FAO Food and Nutrition Paper 41/10, Rome 1998, respectively

INTRODUCTION

At the 45th meeting of the Committee in Geneva, 1995, additional data on the marker residues in deer tissue was requested. Data presented for review at this 1995 meeting clearly demonstrated that moxidectin is the marker residue in cattle and sheep tissue. It was not possible to make moxidectin the Marker Residue for deer tissues because the metabolism of moxidectin in deer was not known and therefore the relationship between moxidectin and the total residues was also unknown. The sponsor initiated an *in vitro* program to address these questions.

IN VITRO LIVER MICROSOME ASSAY STUDY WITH ^{14}C-MOXIDECTIN

Summary of Study

An assay was employed to describe and compare the metabolic profiles obtained for moxidectin in liver preparations from various animal species. The original submission contained similar data with this technique, which confirmed the metabolic profile of moxidectin in cattle tissue. Table 1 demonstrates that moxidectin is the main component of the extract following incubation, representing 70.25, 65.06 and 69.18 % of the recovered radioactivity in the microsomal preparations for cattle, sheep and deer, respectively. This suggests that moxidectin should be the marker residue in deer, as is the case in cattle and sheep tissue evaluated previously. The profile of the other peaks in the chromatogram is similar for these species with only minor differences observed among cattle, sheep and deer. For deer preparations, no metabolite represents greater than 10% of the total radioactivity. Individual chromatograms for all preparations are shown in Figure 1.

Comparative metabolism of moxidectin by deer hepatic microsomes (Done to GLP).

Livers of the different animals (deer, cow, sheep and goat) were collected from 4 individuals for each animal species. The microsomes were prepared by differential centrifugation and stored at –80°C until used. The microsomal preparations were validated using a comprehensive range of oxidative enzyme assays. After microsomal preparation and before use for *in vitro* metabolism study, total cytochrome P450 was determined by the method of Omura and Sato (1964). A complementary interspecies characterization of P450 isoenzymes was carried out by western blotting analysis of each microsomal preparation. Antibodies directed against the major hepatic P450 subfamilies (1A, 2B, 2C, 2E, 3A) were used (Towbin et al, 1979).

The optimal conditions of incubation were determined using cow liver microsomes. A test incubation mixture consisted of microsomal proteins (1 mg), 1 ml buffer (pH 7.4) and ^{14}C- moxidectin (10 µg, 500 µCi, >95% purity) dissolved in acetonitrile. All reactions were started by the addition of a NADPH-generating system and carried out at 37-38°C for either 30, 60, 90 or 120 minutes. The incubates were stored at –20°C before analysis.

The incubate was extracted by acetonitrile and purified by solid phase extraction. The methanol eluate was evaporated to dryness and the residual radioactivity taken up in methanol. One aliquot was used to check the recovery by liquid scintillation counting and another aliquot was used for HPLC analysis with a radioactive detector. The HPLC profiles are shown in Figure 1. The identification of metabolites was made by comparison with chromatographic profiles obtained in similar experiments by Zulalian *et al.* (1994).

The "*in vitro*" metabolism study showed that:

1. The microsomal enzyme activity measurements corresponded to the normal values reported for microsomal preparations obtained for these animal species;
2. The metabolic profiles obtained for all the species investigated showed that the metabolism of ^{14}C moxidectin is low. This observation is in good agreement with previous studies in this field (Zulalian *et al.*, 1994); and
3. Qualitatively the same metabolites were observed in all species, however interspecies differences appeared in the repartition between the different metabolites. Deer, rat and goat liver microsomes can be considered as lower metabolizers by comparison to sheep and cows preparations. The results are shown in Table 1.

Further experiments are in progress to confirm these preliminary results.

Figure 1. HPLC radiochromatograms of microsomal incubates for different species

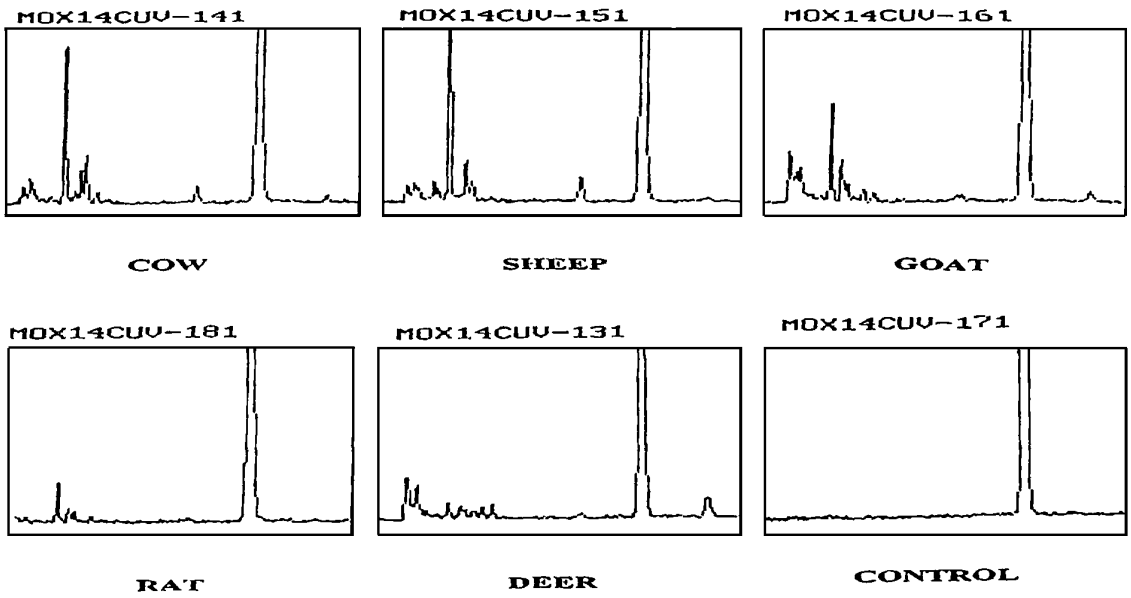

Table 1. Compared percentages after HPLC of microsomal incubates following the incubation of microsomes with [^{14}C]-moxidectin.

Peak Number	Retention Time (min)	Cow (%)	Sheep (%)	Goat (%)	Rat (%)	Deer (%)
1	3.52	1.84	1.69	6.11	1.12	9.34
2	4.73	3.16	2.65	2.29	0.73	4.83
3	5.85	0.24	0.10	0.21	0.19	0.38
4	6.18	0.39	1.10	0.07	0.24	0.09
5	7.41	0.10	0.72	0.31	0.12	0.60
6	8.61	13.12	21.25	5.15	3.07	1.77
7	9.92	0.61	0.21	2.70	1.19	1.61
8	10.72	2.11	2.84	1.01	0.80	0.85
9	11.51	3.72	1.61	--	0.06	1.08
10	12.70	1.09	0.22	0.85	0.51	1.53
11	14.00	0.48	0.21	0.74	0.18	2.12
12	24.62	2.01	2.78	0.90	0.94	1.24
13 (Moxidectin)	32.42	**70.25**	**65.06**	**78.63**	**90.37**	**69.18**
14	40.50	0.86	--	1.04	0.48	5.41

RESIDUE DEPLETION STUDY IN DEER.

Data for residues of unchanged moxidectin in red deer were presented at the 45th meeting of the Committee. Twenty red deer, 15-16 months old, were treated with moxidectin pour-on at a dose of 0.5 mg/kg BW. Five animals were sacrificed at 7, 14, 21 and 28 days after treatment. Edible tissues were collected and the moxidectin content assayed. All residues were below the LOQ in muscle (<24 μg/kg), liver (<6 μg/kg) and kidney (<11 μg/kg). The residues in fat are shown in Table 2.

Table 2. Residues (μg/kg) in fat of Red Deer after administration of a pour-on dose of 0.5 mg/kg BW

Withdrawal time (days)	Mean conc. in fat	Calculated 99% upper CL
7	126	266
14	155	226
21	57	185
28	31	144

APPRAISAL

Moxidectin is a macrocyclic lactone antiparasitic drug that is used to control a number of internal and external parasites in sheep, cattle and deer.

Data presented at the 45th meeting of the Committee clearly demonstrated that moxidectin is the marker residue in cattle and sheep tissue. However, it was not possible to recommend MRLs for moxidectin in deer because the metabolism of moxidectin in deer was not known and the relationship between moxidectin and the total residues was also unknown. Additional data on the marker residues in deer tissue was requested. The sponsor reported on *in vitro* studies to address these questions.

A liver microsome assay with ^{14}C-moxidectin was employed to describe and compare the metabolic profiles obtained for moxidectin in liver preparations from various animal species. Livers of deer, cow, sheep and goat were collected from four individuals for each species and microsomes were prepared. The microsomes were incubated with ^{14}C-moxidectin at 37-38°C. The incubates were extracted and analyzed by HPLC. Results of the liver microsome studies indicate that moxidectin is the main component of the extract following incubation, representing 70%, 65% and 69% of the recovered radioactivity in the microsomal preparations for cattle, sheep and deer, respectively. The chromatographic profile of the other metabolites is similar for each species with only minor differences observed among cattle, sheep and deer. For deer preparations, no moxidectin metabolite represents greater than 10% of the total radioactivity. Results indicate that moxidectin metabolism in deer is comparable to cattle and sheep and that moxidectin should be the marker residue in all three species.

Residue data for moxidectin in red deer were presented in a study at the 45th meeting of the Committee. Twenty deer, 15-16 months old, were treated with moxidectin pour-on at a dose of 0.5 mg/kg BW. Groups of five animals were sacrificed at 7, 14, 21 and 28 days after treatment. Edible tissues were collected and the moxidectin residues assayed. At all time points the residues were below the LOQ in muscle (<24 μg/kg), liver (<6 μg/kg) and kidney (<11 μg/kg) at each sampling time. The mean values (μg/kg) and the upper bound 99% CL (in parenthesis) in fat were at 7 days: 126 (266); 14 days, 155 (226); 21 days, 57 (185); and 28 days, 31 (144). These values are less than the proposed MRLs at all sampling times.

Maximum Residue Limits

The 45th meeting of the Committee established an ADI of 0-2 μg/kg, equivalent to 120 μg per day for a 60-kg person. The Committee recommended MRLs for cattle and sheep and provisional MRLs for deer of 500 μg/kg in fat, 100 μg/kg in liver, 20 μg/kg in muscle and 50 μg/kg for kidney expressed as parent drug based upon the following factors:

- Fat and liver are the target tissues;

- The marker compound is parent drug;
- 40% of the total residues in muscle, liver and kidney are unchanged drug;
- 75% of the total residues in fat are unchanged drug;
- Bound residues are 5-15% of the total residues and information is not available to discount them from the calculation of the MRL; and
- The LOQ of the analytical method is 10μg/kg.

The Committee recommends MRLs for deer as follows: 20 μg/kg in muscle; 100 μg/kg in liver; 50 μg/kg in kidney; and 500 μg/kg in fat expressed as parent drug.

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