PLASMA DISPOSITION, FAECAL EXCRETION, METABOLISM AND CHIRALITY OF ANTHELMINTIC DRUGS IN HORSES

A thesis submitted for the degree of Doctor of Philosophy

by

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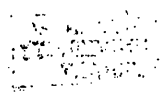
To my wife, Nurgul and my little daughter, Elif.



CONTENTS

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Declaration

The contents of this thesis are the work of the author. The thesis has not been submitted previously to any university for the award of a degree. The following publications are based in full or in part on the work contained in this thesis:

1. C. Gokbulut, A.M. Nolan & Q.A. McKellar (1999). Pharmacokinetics of ivermectin, doramectin and moxidectin after oral administration in horses. *17th International Conference of the World Association for the Advancement of Veterinary Parasitology*, g. 7. 06, Copenhagen, Denmark.

2. C. Gokbulut, & Q.A. McKellar (1999). Pharmacokinetics of oxfendazole, fenbendazole and oxibendazole after oral administration in horses. *17th International Conference of the World Association for the Advancement of Veterinary Parasitology*, g. 7. 07, Copenhagen, Denmark.

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Summary

The plasma disposition and faecal excretion of oxfendazole (FBZ.SO), fenbendazole (FBZ) and oxibendazole (OBZ), and enantiospecific disposition of FBZ.SO were investigated in horses following oral administration at a dose rate of 10 mg/kg. Fenbendazole and FBZ.SO were metabolised extensively to the sulphone metabolite. Significantly greater plasma concentrations were found for sulphone (SO_2) metabolites than for the parent molecules of FBZ and FBZ.SO. The sulphone metabolite is known to have relatively very low or no anthelmintic activity. Significantly higher mean maximum concentration (C_{max}) and larger area under the concentration time curve (AUC) of parent molecules were obtained for FBZ.SO (C_{max}: 0.35±0.07 μg/ml, AUC: 4.40±0.90 μg.h/ml) compared to FBZ (C_{max}: 0.04±0.01μg/ml, AUC: 0.61±0.01 μg.h/ml). At the same oral dose rate, it is possible that FBZ.SO produces greater activity in the systemic circulation than FBZ since the plasma concentration of active metabolite of FBZ.SO was larger than that of FBZ however the tubulin binding of FBZ.SO is much lower than FBZ and activity probably reflects both concentration present and inherent affinity for the known receptor conforming activity. High concentrations of FBZ in the gut following FBZ administration could confer good activity on the parent compound for gastrointestinal parasites. The estimated plasma concentrations of OBZ from samples were very low (C_{max}: 0.006 μg/ml). In faeces the highest faecal excretion was determined at 24 h for all molecules. A high FBZ concentration (~20% of parent molecule) was found in the faecal samples after FBZ.SO administration although faecal concentrations of administered moiety were always higher. Enantiospecific analysis of FBZ.SO showed that the first enantiomer (FBZ.SO-1) was predominant in six of the eight animals whereas in two animals the second enantiomer dominated in the plasma samples.

The effect of piperonyl butoxide, a cytochrome P450 inhibitor was determined on the pharmacokinetic and chiral disposition of FBZ.SO in ponies. Oxfendazole was given intravenously at a dose rate of 10 mg/kg bodyweight and piperonyl butoxide was administered by nasogastric intubation at a dose rate of 31 mg/kg bodyweight, 30 minutes prior to FBZ.SO administration. The plasma concentrations of FBZ.SO and its sulphide and sulphone metabolites were determined following administration of FBZ.SO alone or with PB. It was shown that piperonyl butoxide significantly inhibited the metabolism of FBZ.SO and increased its plasma concentration. It was apparent that one

of the FBZ.SO enantiomers (FBZ.SO-2) was metabolised much more rapidly than the other enantiomer (FBZ.SO-1). On the other hand, the plasma concentrations of FBZ.SO-2 were higher than those of FBZ.SO-1 when the animals were given FBZ.SO with piperonyl butoxide. Thus the metabolism or excretion of FBZ.SO-2 was inhibited by piperonyl butoxide more effectively than that of FBZ.SO-1.

Hepatic microsome samples were prepared from horse liver tissue to determine the *in vitro* metabolism of FBZ.SO, FBZ and OBZ with and without piperonyl butoxide. Only the sulphone metabolite was formed after FBZ.SO incubation and the sulphone, sulphoxide and hydroxy metabolite were formed after FBZ incubation. The sulphonation pathway of the metabolism of FBZ.SO and FBZ was inhibited significantly by piperonyl butoxide. The extent of metabolism for FBZ.SO and FBZ was significantly higher when the anthelmintics were incubated alone than in the presence of piperonyl butoxide. The enantiospecific metabolism was also determined following incubation of FBZ.SO as a racemate substrate and following incubation of FBZ as a prochiral drug with and without piperonyl butoxide. Microsomal metabolism was apparently enantiospecific since the enantiomers were metabolised differently from each other. There was a marked change in the enantiomer ratio when FBZ.SO was incubated with piperonyl butoxide. Fenbendazole (FBZ) metabolism to sulphoxide (FBZ.SO) was also shown to be enantiospecific since FBZ.SO-1 predominated in the reaction mixture. Piperonyl butoxide affected the enantiospecific character of the metabolism. Oxibendazole (OBZ) was metabolised extensively to its unidentified metabolites (M1, M2, M3 and M4) and piperonyl butoxide significantly inhibited the metabolism of OBZ. Three unidentified metabolites (M1, M2 and M4) were significantly decreased and one unidentified metabolite (M3) was significantly increased when OBZ was incubated with piperonyl butoxide.

The pharmacokinetic disposition and faecal excretion of IVM, MXD and DRM were reported in horses following oral administration at a dose rate of 0.2 mg/kg. Large interindividual variations of kinetic parameters were observed from animals in this study. A similar pattern of absorption and time till C_{max} (t_{max}) were found. The C_{max} and t_{max} were not significantly different for IVM, DRM and MXD. The AUC of MXD (86.81 ng.d/ml) was significantly larger than that of IVM (46.41 ng.d/ml) and the mean residence time (MRT) of MXD (16.31 day) was significantly longer than DRM (4.0 day)

and IVM (2.4 day). The faecal excretion patterns of IVM DRM and MXD were similar and no significant difference was observed for C_{max} and AUC values of all molecules in faeces. The highest faecal excretion was determined at 24 h for all molecules.

The plasma pharmacokinetic disposition and faecal excretion of pyrantel (PYR) was determined after oral administration. PYR was detected in plasma between 1 h and 72 h. The C_{max} was 0.09±0.02 µg/ml and was achieved at 7.50±1.41 h (t_{max}). The AUC and MRT of PYR were 1.06±0.24 µg.h/ml and 11.99±1.30 h, respectively. Pyrantel was detected in faeces between 12 h and 72 h. The highest faecal excretion (1.034 mg/g) was determined at 24 h.

Abbreviations

ABM	abamectin
ABZ	albendazole
ABZ.SO	albendazole sulphoxide
$ABZ.SO_2$	albendazole sulphone
AUC	area under the curve
AUC_{last}	area under the curve from time 0 to last measured concentration
$AUMC_{last}$	area under the first moment curve from time 0 to last measured concentration
BZ	benzimidazole
CBZ	cambendazole
CIBZ	ciclobendazole
C_{max}	peak plasma or faecal concentration
CNS	central neural system
d	day
DRM	doramectin
EPG	eggs per gram
EPM	eprinomectin
FBT	febantel
FBZ	fenbendazole
FBZ.SO	oxfendazole or fenbendazole sulphoxide
FBZ.SO-1	the first enantiomer of oxfendazole
FBZ.SO-2	the second enantiomer of oxfendazole
$FBZ.SO_2$	fenbendazole sulphone
FECs	faecal egg counts

FMO	flavine-containing monooxygenase
g	gram
GABA	gamma aminobutyric acid
h	hour
HPLC	high performance liquid chromatography
i.m.	intramuscular
i.r.	intraruminal
IVM	ivermectin
iv	intravenous
kg	kilogram
L	litre
LBZ	luxabendazole
L_3	third-stage larvae
L_4	fourth-stage larvae
L_5	fifth-stage larvae
kDa	kilodalton
LD_{50}	dose of drug which produces death in 50% of animals treated
Log	logarithm
M	molar
MBZ	mebendazole
MFO	mixed function oxidase
ml	millilitre
mmol	millimole
MRT	mean residence time
MXD	moxidectin

mol. wt.	molecular weight
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
nmol	nanomole
NS	no sample
NTB	netobimin
OBZ	oxibendazole
OH.FBZ	hydroxyfenbendazole
PB	piperonyl butoxide
PBZ	parbendazole
p.o.	per os
ppm	parts per million
S-oxidation	sulphoxidation
s.c.	subcutaneous
SD	standard deviation
SEM	standard error of the mean
susp.	suspension
t	topical
TBZ	thiabendazole
TCBZ	triclabendazole
TPT	thiophanate
t_{max}	time to peak concentration
ULQ	under the limit of quantification
uv	ultraviolet
µg	microgram

V_d	volume of distribution
μM	micromolar
°C	degrees centigrade

CHAPTER 1

General Introduction

1.1 INTERNAL PARASITIC INFECTIONS IN HORSES

In modern horse management, parasite infections are still a serious animal health problem. Horse parasites include large strongyles (*Strongylus vulgaris* and *Strongylus edentatus*), small strongyles (*Cyathostomum spp.*), ascarids (*Parascaris equorum*), bots (*Gastrophilus spp.*) and pinworms (*Oxyuris equi*). Other parasites of minor importance include strongyloides (*Strongyloides westeri*), stomach worms (*Trichostrongylus axei*), tapeworms (*Anoplocephala perfoliata*), eye worms and filaroides (Herd, 1992).

Nematode parasites, which cause serious pathological disorders in the gastrointestinal tract and its blood supply, are more important than other internal parasites with regard to parasite control programmes and anthelmintic chemotherapy. Nematodes are widespread in horses in all climatic regions of the world (Ogbourne, 1976; Reinemeyer *et al.,* 1984; Krecek *et al.*, 1989; Mfitilodze and Hutchinson, 1990). A variety of nematodes can be parasitic in the gastrointestinal tract of Equids. *Trichostrongylus axei*, *Drachia megastoma* and *Habronema spp.* are found in the stomach, mature forms of *S. westeri* and *P. equorum* inhabit the small intestine, and the mature and immature forms of the strongyles, *O. equi* and *Probstmayria vivipara* are found in the large intestine. In the host, these parasites can produce a number of pathological disorders including diarrhoea (Mair *et al.*, 1990; Love *et al.*, 1992), rapid progressive weight loss (Love *et al.*, 1991; Love, 1992a, b); functional disorders of the intestine (Ogbourne and Duncan, 1977); colic and pathological changes in the mesenteric arterial system (Wright, 1972; Duncan and Dargie, 1975; White, 1985; Drudge and Lyons, 1986).

The strongyles affect all ages of horses and represent the largest variety of parasitic nematodes in this animal species. They are classified in the family *Strongylidea* which is divided into subfamilies *Strongylinea* (large strongyles) and *Cyathostominea* (small strongyles). *Strongylinea* consist of the species of the *Oesophagodontus, Triodontophorus, Strongylus* and *Craterostomum* (Lichtenfels, 1975). After ingestion some strongyle larvae leave the gastrointestinal tract and migrate in the body of the host following a specific route. *Strongylus vulgaris*, the most harmful of the large strongyles migrates from the gastrointestinal tract and at the fourth-larval stages of development in the arterial system, causes arteritis and thrombosis, especially in the anterior mesenteric artery and its main branches. *Strongylus edentatus* reaches the liver via the portal system and arrives at the base of the caecum through the hepatorenal ligament (McCraw and

Slocombe, 1974). The parasites move easily by this route and may be found in the flanks, lungs and pancreas (McCraw and Slocombe, 1978).

Other members of the subfamily *Strongylinea* as well as all *Cyathostominea spp.* do not migrate from the mucosa or submucosa of the caecum and colon (Soulsby, 1965; Ogbourne, 1978). The main injury they cause is to the gut wall.

Strongyloides westeri and *P. equorum* are also important equine gastrointestinal nematodes. These parasites inhabit the small intestine and are frequently the cause of diarrhoea. After birth, the foals ingest the larvae of *S. westeri* in infected mare's milk (Lyons *et al.*, 1973; Enigk *et al.*, 1974). This parasite can cause diarrhoea in foals up to 6 months old (Lyons *et al.*, 1973). *Parascaris equorum* can also be a serious problem in young horses. In contrast with *S. westeri*, ascarid infections are not only restricted to foals, but also can be found in older horses (Mirck, 1985).

Oxyuris equi is the common pinworm in the colon. It causes anal pruritus induced by allergens in the gelatinous egg mass deposited by the female in the perianal region (Slocombe, 1985).

Microfilariae of *Onchocera cervicalis* are frequently found in the skin of the head, face, eyelid, neck and ventral thorax (Slocombe, 1985) and cause dermatitis (McMullan, 1972) and ocular lesions (Cello, 1971). Development of lesions may involve antigen release by microflariae, microfilarial death, and/or development of hypersensitivity to microfilariae in susceptible horses (McMullan, 1972).

Dictyocaulus arnfieldi is the only lungworm in equids and can cause chronic catharral bronchitis. Gross lesions in infected animals are similar with discrete areas of hyperinflated pulmonary parenchyma, mostly in the caudal lobe (Clayton and Duncan, 1981).

In horses, *Fasciola hepatica* infections are uncommon. Horses are considered to be more resistant to infection (Boray, 1969) than domestic ruminants. However, under environmental conditions favourable to the development of fasciola, horses and particularly donkeys may become patently infected with considerable burdens (Pankhurst, 1963; Hatch, 1966; Kearney, 1974).

1.2 ANTHELMINTICS IN HORSES

Modern equine anthelmintic drugs can be classified into seven distinct classes on the basis of their chemical structure and pharmacological behaviour. Namely benzimidazoles, pro-benzimidazoles, tetrahydropyrimidines, imidazothiazoles, simple heterocycles, organophosphates and macrocyclic lactones (Table 1-1). Each class of anthelmintics has a different spectrum of activity and pharmacokinetic behaviour. The agents which have a broad spectrum of anthelmintic activity can be classified into three groups: Benzimidazoles and pro-benzimidazoles, imidazothiazoles and tetrahydropyrimidines, and macrocyclic lactones. The other drugs including heterocyclics and most of the organophosphates have a narrow spectrum of activity (DiPietro and Todd, 1987).

1.2.1 BENZIMIDAZOLES IN HORSES

The benzimidazole anthelmintics are a large family of compounds that are related to thiabendazole (TBZ) (Brown *et al.*, 1961), which was first released for use in horses in 1961 (Drudge *et al.*, 1981). Benzimidazole anthelmintics licensed for use in the horse worldwide, include TBZ, mebendazole (MBZ) (Brugman *et al.*, 1971), oxibendazole (OBZ) (Theodorides *et al.*, 1973), fenbendazole (FBZ) (Baeder *et al.*, 1974), oxfendazole (FBZ.SO) (Averkin *et al.*, 1975), cambendazole (CBZ) (Hoff *et al.*, 1970) and the pro-benzimidazole, febantel (FBT) (Delatour and Euzeby, 1983). Cambendazole and FBT are not licensed for use in horses in the UK.

Benzimidazole and pro-benzimidazole anthelmintic drugs have common features such as broad-spectrum anthelmintic activity and relatively low mammalian toxicity.

1.2.1.1 Chemistry and Metabolism

The benzimidazole anthelmintics can be classified into four groups according to their chemical structure (Lanusse and Prichard, 1993). Namely, Group 1. Benzimidazole thiazolyl, including thiabendazole (TBZ) and cambendazole (CBZ); Group 2. Benzimidazole methylcarbamates, including parbendazole (PBZ), mebendazole (MBZ), flubendazole (FLBZ), ciclobendazole (CIBZ), oxibendazole (OBZ), luxabendazole (LBZ), albendazole (ABZ), albendazole sulphoxide (ABZSO), fenbendazole (FBZ) and oxfendazole (FBZ.SO); Group 3. Halogenated benzimidazole thiols, including

Table 1-1. Chemical classification of anthelmintics and related compounds used in horses.

Class	Route of administration	Dosage (mg/kg)	Formulation (not all licensed for horses)
Benzimidazoles			
Cambendazole*	p.o.	20	paste
Parbendazole*	p.o.	2.5	powder, susp.
Thiabendazole	p.o.	50	paste, susp.,powder
Mebendazole	p.o.	8.8	paste, granule
Fenbendazole	p.o.	7.5	paste,susp.,granule
Oxfendazole	p.o.	10	pellet, susp.
Oxibendazole	p.o.	10	paste, susp.
Albendazole*	p.o.	5	paste, susp.
Pro-benzimidazoles			
Netobimin+			
Febantel*	p.o.	6	paste, susp., granule
Thiophanate+	p.o.		powder
Tetrahydopyrimidines			
Pyrantel pamoate	p.o.	6.6	paste
Pyrantel tartrate	p.o.	6.6	powder
Morantel+			
Imidazothiazole			
Levamisole*	p.o.	10	solution
Simple heterocyclics			
Phenothiazine	p.o.	75	powder
Piperazine	p.o.	200	powder
Organophosphates			
Dichlorvos	p.o.	20	paste, pellet
Haloxon	p.o.	60	paste, powder
Trichlorfon	p.o.	40	paste, powder
Macrocyclic lactones			
Avermectins			
Ivermectin	p.o.	0.2	paste, solution
Abamectin+			
Doramectin+			
Milbemycins			
Moxidectin	p.o.	0.2	
Milbemycin D+			
Milbemycin oxime+			

p.o.: *per os*; susp.: suspension.
*Not licensed in UK
+Not licensed for horses

triclabendazole (TCBZ) and Group 4. Probenzimidazoles, including thiophanate (TPT), febantel (FBT) and netobimin (NTB). A 1,2 diaminobenzene ring forms the central ring structure of all the benzimidazole anthelmintics. The difference between each agent is associated with different substitutions at the 2 and 5 substituent positions of the central ring structure (Figure 1-1).

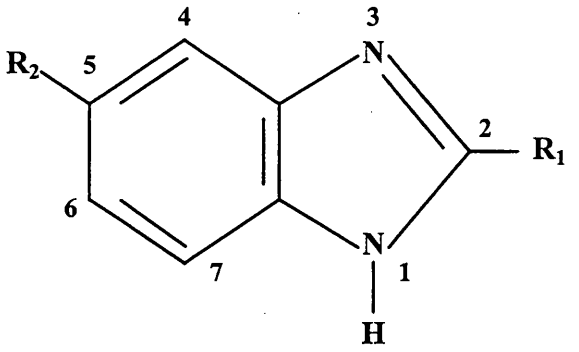


Figure 1-1. Primary substituent positions of the benzimidazole anthelmintics.

Although xenobiotic metabolism occurs mainly in the liver, extrahepatic tissues including plasma, kidney and gastrointestinal tract are also involved. Biotransformation of drugs generally results in metabolites of the parent drug which have reduced or no activity (Baggot, 1974). These reactions are usually biphasic. Phase I (non-synthetic) reactions include oxidation, reduction and hydrolysis and Phase II (synthetic, conjugation) reactions entail addition of sulphate groups, glucuronides, acetylation or methylation. A number of enzyme complexes determine the Phase I reactions. The cytocrome P450-mediated mixed function oxidase (MFO) system is the major one. The MFO comprises cytochrome P450, and NADPH-cytochrome P450 reductase and lipid. The flavin-containing monooxygenase (FMO) drug system is another important group of enzymes, which play a major role in oxidative drug metabolism. Functional groups such as hydroxy, carboxy, amino and sulphydryl groups are introduced into the organic substrates. The FMO enzyme system is found in many tissues but the highest concentrations occur in the microsomal fraction of the liver. It uses either NADH or NADPH as a source of reducing equivalents in the oxygenation of many nucleophilic organic nitrogen and sulphur compounds (Gibson and Skett, 1994), such as the

phenothiazines and sulphide benzimidazoles. Mixed function oxidase and FMO systems are involved in sulphoxidation and sulphonation. Both systems are equally involved in monogastric animals, whereas FMO is the predominant system in ruminants (Delatour *et al.*, 1994).

Benzimidazoles are extensively metabolised in all animal species. Generally the plasma elimination half-lives of the parent drugs are short and the metabolic moieties predominate in plasma and tissues and in excreta of the host as well as in parasites recovered from benzimidazole-treated animals (Delatour and Parish, 1986; Lanusse and Prichard, 1993). Albendazole, FBZ, TCBZ and the pro-benzimidazoles (FBT and NTB), which are commercially available thioether and sulphide benzimidazoles, commonly undergo microsomal sulphoxidation in liver. They are reversibly metabolised to their sulphoxide derivatives (Marriner and Bogan, 1980, 1981a; Gyurik *et al.,* 1981; Mohammed Ali *et al.*, 1987). Irreversible sulphonation follows sulphoxidation and is a slower oxidative step resulting in a sulphone metabolite (Averkin *et al.*, 1975)

Hydroxylation is another important metabolic pathway of the benzimidazoles. In sheep, it was demonstrated that hydroxy oxfendazole (OH-FBZ.SO) and hydroxy fenbendazole (OH-FBZ) were major hepatic metabolites of FBZ, 5 mg/kg, after intraruminal administration (Hennessy *et al.*, 1993). These metabolites are excreted directly into the bile as free or conjugated metabolites (Short *et al.*, 1987 a, b; 1988; Hennessy *et al.*, 1993). Lacey and co-workers (1987) reported that OH-FBZ was intrinsically more active than the parent FBZ *in vitro* in binding to parasite tubulin.

Mebendazole and FLBZ undergo carbonyl reduction to the corresponding alcohol (Van den Bossche *et al.*, 1982). The alcohol metabolite of MBZ was found at higher concentrations than the parent drug in sheep plasma (Behm *et al.*, 1983). However, MBZ metabolites are not thought to be biologically active (Meuldermans *et al.*, 1976).

Febantel, NTB and TPT are commercially available pro-benzimidazole drugs. Their anthelmintic activity is due to the fact that they are metabolised in the animal to form the biologically active benzimidazole carbamate nucleus. Febantel is a phenylguanidine that is hydrolysed and then cyclized to the active metabolite FBZ (Delatour *et al.*, 1982). Netobimin is a nitrophenylguinidine which undergoes both reduction and cyclization to form the active compound ABZ (McDougall *et al.*, 1985; Delatour *et al.*, 1986).

Thiophanate is cyclized to form the active compound, lobendazole and then loberdazole is oxidised to a phenol metabolite (Gardiner *et al.*, 1974).

1.2.1.2 Efficacy and Spectra of Activity

Benzimidazole and pro-benzimidazole drugs are used widely to treat gastrointestinal helminthiasis including migrating strongyle larvae and lungworm infections (Marriner and Bogan, 1985) (Table 1-2.). Most of the benzimidazoles (CBZ, FBZ.SO, FBZ, MBZ, OBZ, ABZ and PBZ) and the pro-benzimidazole (FBT) are highly effective (above 90%) against adult forms of the large strongyles, small strongyles, mature *O. equi*, the small pinworm (*Probstmayria vivipara*), and *T. axei* in horses (Courtney and Robertson, 1997).

There are some notable differences in the efficacy of benzimidazoles against particular parasite species. Thiabendazole needs to be given at a higher dosage for *P. equorum* and TBZ, FBZ.SO and FBZ are effective against *S. westeri* when given at a higher dose. At 7.5mg/kg daily for five days, FBZ is 94.6% effective against larval stages of small strongyles in mucosa, 80% effective against migrating larvae of *S. vulgaris* and fully effective against migrating stages of *S. edentatus* (Duncan *et al.*, 1980). Oxibendazole has little or no effect against migrating stages of *S. vulgaris, T. axei, H. muscae* and *D. megastoma* (Kates *et al.*, 1975; Nawaliski and Theodorides, 1976, 1977). Parbendazole, at 20 mg/kg body weight, is fully effective against migrating larvae of *S. edentatus* (Lyons *et al.*, 1980), but ineffective against 7-day-old larvae of *S. vulgaris* (Drudge and Lyons, 1970). Parbendazole has no activity against *T. axei*, Habronema *spp. D. megastoma* and *S. westeri* (Lyons *et al.*, 1980). Albendazole is effective against fourth-stage larvae of *S. vulgaris* with minor signs of toxicity at a dosage rate of 50 mg/kg twice a day for 2 days (Georgi *et al.*, 1980). The benzimidazoles have no activity against bots.

1.2.1.3 Mode of Action

Benzimidazoles and pro-benzimidazoles are considered to have a similar mode of action. Differences in their efficacy reflect differences in bioavailability. Benzimidazole anthelmintic compounds prevent polymerisation of the microtubules in eukaryotic cells by selectively binding to parasite β-tubulin (Martin, 1997). Borgers and co-workers (1975) reported that after exposure to mebendazole, the cytoplasmic microtubules disappeared and this caused disruption in the migration of subcellular organelles with a

Table 1-2. Activity of anthelmintics against gastrointestinal nematodes in equids (adapted from Mirck, 1985).

	Dose (mg/kg)	*Probstmayria vivipara*	*Oxyuris equi L4*	*Oxyuris equi L5*	Small *strongyles L4*	Small *strongyles L5*	*Strongylus equinus*	*Strongylus edentatus*	*Strongylus vulgaris*	*Parascaris equorum L4*	*Parascaris equorum L5*	*Strongyloides westeri*	*Habronema spp.*	*Draschia megastoma*	*Trichostrongylus axei*
Thiabendazole	50	+++	+	+++	+++	+++	+++	+++	+++	-	+	+++	-	-	+
Cambendazole	20	+++	+++	+++	+	+++	+++	+++	+++	-	+++	+++	-	o	+++
Parbendazole	2.5	-	-	+++	-	+++	-	+++	+++	-	-	o	o	o	o
Mebendazole	8.8	+++	+++	+++	+	+++	+++	++	+++	-	++	o	o	o	o
Fenbendazole	5	+++	+	+++	+	+++	+++	+++	+++	-	++	o	o	o	o
Oxfendazole	10	-	+++	+++	+++	+++	+++	+++	+++	-	+++	o	o	o	+++
Oxibendazole	10	+++	+++	+++	+	+++	+++	+++	+++	-	+++	+++	o	o	o
Albendazole	5	-	+++	+++	+	+++	+++	+++	+++	-	+++	-	-	-	-
Febantel	6	o	+++	+++	-	+++	-	+++	+++	+++	+++	o	o	-	-
Pyrantel	6.6	+++	+	-	+	+++	+++	+	+++	+++	+++	o	o	o	o
Levamisole	10	o	+	+++	-	+	-	+	+++	+++	+++	-	o	-	o
Phenothiazine	75	-	-	-	+	+++	+	+	+	-	+	-	-	-	-
Piperazine	200	-	o	++	-	+++	o	o	+	+++	+++	-	o	-	-
Trichlorfon	40	-	-	+++	-	-	-	-	-	+++	+++	-	-	-	-
Dichlorvos	40	+++	+++	+++	-	+++	-	o	+++	+++	+++	-	-	-	-
Haloxon	60	+++	+++	+++	-	++	+++	++	+++	-	+++	-	o	o	o
Ivermectin	0.2	-	+++	+++	+++	+++	+++	+++	+++	-	+++	+++	++	-	-

+++, 95-100% efficacy; ++, 80-100% efficacy; +, 0-100 efficacy; o, no efficacy; -, insufficient data.

failure of transport of secretory materials in the cells of the nematode parasites. Prolonged storage of the secretory granules caused lysis of the cell cytoplasm and disintegration of the cells. It was found that the benzimidazoles competed for the attachment site on β-tubulin with colchicine, a substance known to prevent cell division in the metaphase (Sangster *et al.*, 1985; Lacey and Gill, 1994).

The anthelmintic action of benzimidazoles is due to differences in the sensitivity of host and parasite cells to the tubulin binding effects of these drugs (Friedman and Platzer, 1980). Benzimidazoles bind reversibly to mammalian tubulin, whereas they bind irreversibly to nematode tubulin (Lacy and Gill, 1994) and thus prevent tubulin polymerisation to microtubules. In addition, the selective toxicity may be due to differences between the structure of host and nematode microtubules. Mammalian cells have 13 microtubule protofilaments whereas the nematode cells have 11, 12 and 14 protofilaments (Chalfie and Thompson, 1979; Davis and Gull, 1983).

The benzimidazole anthelmintics also inhibit the uptake of glucose (Van den Bossche, 1982; Van den Bossche and De Nollin, 1973) and inhibit the activity of various metabolic enzymes in nematode parasites such as malate dehydrogenase (Tejada *et al.*, 1987; Sharma *et al.*, 1989) and fumarate reductase (Prichard, 1970, 1973; Barrowman *et al.*, 1984). However, a much higher concentration of benzimidazole is required to inhibit fumarate reductase than to inhibit tubulin polymerisation in nematode parasites (Kohler and Backman, 1978; Dawson *et al.*, 1984).

1.2.1.4 Safety and Toxicity

The benzimidazole and pro-benzimidazole anthelmintics have a high therapeutic index and are extremely well tolerated by mammals. Benzimidazole compounds with low-solubility may be less toxic than the more water-soluble compounds because insufficient drug is absorbed to exert a systemic toxic effect (McKellar and Scott, 1990).

Sheep and cattle tolerated 5000 and 2000 mg/kg FBZ respectively and in rats the LD_{50} was greater than 10000 mg/kg (Baeder *et al.*, 1974; Duwel, 1977). For FBZ.SO the LD_{50} was greater than 6400 mg/kg in rats and 1600 mg/kg in dogs (Averkin *et al.*, 1975). In horses, including pregnant mares, FBZ at a dose of 100 mg/kg produced no adverse effects (Becker, 1975) and reproductive function of stallions was not affected (Squires *et al.*, 1978). In sheep FBZ is generally safe at high doses but when FBZ was given at

45mg/kg daily for 30 days, myocardial lesions and vacuolation of the liver parenchyma were observed (Booze and Oehme, 1982).

The main toxic effect of benzimidazoles is teratogenicity. This varies with the structure of the benzimidazole compound and the animal species (Delatour and Parish, 1986). The teratogenic activity of FBZ.SO and ABZ.SO has been shown when administered to rats in early pregnancy (Delatour *et al.*, 1984). Sheep and rats are much more sensitive than other species of animals to teratogenic activity of the benzimidazoles and extensive studies have been carried out in these species. Congenital malformations were reported during early gestation in ewes following administration of cambendazole (Delatour *et al.*, 1975), FBZ.SO (Delatour *et al.*, 1977) ABZ (Jhons and Philip, 1977) and FBT (Chement, 1982). The main congenital defects identified in the lambs were skeletal malformations, occurring mainly in the long bones, pelvis, joints and digits. The microtubular activity of benzimidazole compounds may lead to their teratogenic activity (McKellar and Scott, 1990). It is probably the unbound drug or metabolites, which are likely to exert toxic effects in mammals and the tightly protein-bound residues that persist in the tissues for longer periods of time, are thought to be of lower significance toxicologically (Delatour and Parish, 1986).

The teratogenic activity studies of the benzimidazoles in early pregnancy of rats have shown that this species has similar sensitivity to sheep (Delatour and Parish, 1986). Teratogenic and embryolethal effects have been reported for PBZ, MBZ, CBZ, ciclobendazole, carbendazim, FBZ.SO, ABZ, FBT and netobimin (Delatour and Parish, 1986). In male rats, testicular degeneration and abnormal spermatogenesis were produced by high doses of carbendazim (Styles and Garner, 1974) and mutagenic effects of carbendazim have also been shown in other species at high doses (Seiler, 1976).

1.2.1.5 Pharmacokinetics

The active benzimidazole anthelmintics are thought to have a similar mode of action (Coles, 1977). The different efficacies of the benzimidazoles *in vivo* have been attributed largely to different pharmacokinetics within the host (Bogan, 1983) and *in vitro*, to different solubility and therefore absorption of drug from culture media by the test parasites (Scott, 1988). The benzimidazole anthelmintics are only sparingly soluble in water. In ruminants, the absorption and pharmacokinetics of these drugs are affected by their aqueous solubility (Table 1-3.). The rapid dissolution and consequent absorption

and elimination of some benzimidazoles explain their relatively short residence in the body. Thus more soluble compounds have shorter residence in comparison to less soluble benzimidazoles, which are absorbed over prolonged periods. Thiabendazole and CBZ, the most water soluble of the therapeutic benzimidazoles, are extensively dissolved in

Table 1-3. The solubility (μg/ml) of benzimidazoles in phosphate buffer at different pH values (Ngumuo, 1983).

	pH 7.4	pH 6.0	pH 2.2
Thiabendazole	45.01	>24.67	>480.78
Albendazole	0.85	>0.48	>26.58
Fenbendazole	0.05	>0.07	>1.60
Oxfendazole	5.97	>3.01	>44.12
Parbendazole	0.2	>0.27	>27.07
Oxibendazole	0.44	>0.34	>18.51

rumen fluid and absorbed rapidly in ruminants (McKellar and Scott, 1990). The less soluble benzimidazole compounds are absorbed over prolonged periods and thus remain in the plasma for a long time and since an equilibrium exists between the plasma and the gastrointestinal tract, the duration of exposure of the gut parasites to effective concentration of drug is extended. Extremely insoluble anthelmintics may be less effective, since they may not be absorbed and are excreted unchanged in the faeces. This may explain the difference between the plasma concentrations of FBZ.SO achieved following oral administration of FBZ.SO, the parent drug and its interconvertible metabolite FBZ (Ngomuo, 1983). A large proportion of the less soluble FBZ is known to be excreted in the faeces (Duwel, 1977; Prichard, 1978).

There are significant differences in the pharmacokinetics of benzimidazole anthelmintics between monogastrics and ruminant species (Mariner and Bogan, 1981b; McKellar *et al.*, 1990). Anatomic features influence the passage of digesta, and the bioavailability and

pharmacokinetics of anthelmintics may be affected by different gut transit time in animal species (McKellar and Scott, 1990). The benzimidazole anthelmintic drugs have lower bioavailability in monogastric animals (e.g. dog and horse), which have relatively faster gut transit time than ruminants, since rapid passage of food in the gut causes a decrease in the bioavailability of the drugs. Dougherty (1992) reported gut transit time of 8.47 h in horses. In ruminants, this time may range from 30 to 80 h depending on the digestibility of food, since highly digestible food has a shorter retention time (Warner, 1981; McDonald *et al.*, 1995). The systemic bioavailability of most drugs administered orally is lower in herbivorous species (horse and ruminants) than in dogs and cats. The greater "first-pass effect" and higher drug metabolising capacity of the reticulo-rumen and liver may produce this effect in herbivorous species. In ruminants, the opposite is true for benzimidazole drugs. After oral administration to ruminants, greater bioavailability is observed than in monogastric species because in ruminants, the relatively slower gut transit time conferred by the fore stomach reservoir means that benzimidazoles are more extensively absorbed than in monogastric animals (Baggot and McKellar, 1994). In horses, after oral administration of FBZ.SO (10 mg/kg body weight), the area under the curve (AUC) for the parent drug and its metabolite FBZ were of 3.17 and 2.32 μg.h/ml respectively. In a similar study carried out with FBZ at the same dose rate, lower plasma concentrations of parent drug, FBZ, and its metabolite FBZ.SO were obtained, with FBZ.SO being below the limit of detection (<0.04μg/ml) and FBZ having an AUC of 1.77 μg.h/ml (Mariner and Bogan, 1985). In contrast to other species (sheep, cattle, man), horses generate relatively higher concentrations of sulphone metabolite than of sulphoxide. This may be due to relatively rapid conversion of sulphoxide to the sulphone by equine hepatocytes (Mariner and Bogan, 1985). Anthelmintic activity depends on the duration of parasite exposure to effective concentrations of the active compound (Baggot and McKellar, 1994), thus observations on the pharmacokinetics of benzimidazoles are extremely important.

Significant differences in the pharmacokinetics of benzimidazoles exist between ruminant species also. In sheep, after oral administration of FBZ.SO at a dose rate of 10 mg/kg body weight, the AUC of FBZ.SO was 49.6 ± 12.3 μg.h/ml whereas in goats the AUC was 19.9 ± 7.0 μg.h/ml (Bogan *et al.*, 1987). In addition the AUC for FBZ orally administered at 7.5 mg/kg to cattle was 11.4 ± 3.5μg.h/ml and for its metabolite, FBZ.SO, was 21.5 ± 7.7 μg.h/ml. However, in buffalo the AUC of FBZ and FBZ.SO

were 3.7±2.1μg.h/ml and 1.8 ± 0.5 μg.h/ml, respectively (Knox *et al.*, 1994). Different rates of hepatic metabolism and passage time of food in gut may contribute to cause these different pharmacokinetic profiles in ruminant species (McKellar and Benchaoui, 1994).

In animals, diet can substantially affect the bioavailability of drugs. Some non-steroidal anti-inflammatory drugs bind to food particles in horses which delay the absorption of the drugs significantly (Maitho *et al.,* 1986; Lees *et al.,* 1988; Welsh *et al.,* 1992; Landoni and Lees, 1995). Hennessy (1993) emphasised the role played by the adsorption of benzimidazoles on the ruminal particulate material in the extension of their bioavailability. The physicochemical nature of drug association with particulate material is likely to be due to physical adsorption rather than specific chemical binding (Lees *et al.*, 1988). In sheep, the relative bioavailability of benzimidazoles decreased when administered concomitantly with food (Taylor *et al.*, 1992; Ali and Hennessy, 1993). In cattle, it was demonstrated that fasting or restricted feed intake increased the relative bioavailability of ABZ metabolites (Sanchez *et al.*, 1996, 1997). However, in the dog (McKellar *et al.*, 1993a) and horse (McKellar, 1997a) feeding increased the bioavailability of benzimidazoles after oral administration.

The rumen affects the bioavailability of anthelmintics by acting as a metabolic compartment for foreign compounds. Mariner and Bogan (1981b) reported that the oral administration of FBZ resulted in higher and more sustained plasma concentrations of parent drug and its metabolites than when the anthelmintic had been given by the intra-abomasal route. In sheep, it was shown that partial or complete oesophageal groove closure occurred in 42% of animals treated with an oral FBZ.SO preparation and this caused a reduction in FBZ.SO bioavailability and efficacy (Prichard and Hennessy, 1981). Although similar results were obtained in goats (Sangster *et al.,* 1991), Ngomuo and co-workers (1984) did not find significant differences between the (AUC) of FBZ.SO and its metabolites in plasma following oral and intra-ruminal administration to cattle. In addition, the ruminal micro flora sometimes increase the bioavailability and efficacy of benzimidazoles. The conversion of the pro-benzimidazole netobimin into its active form ABZ is determined by the ruminal microflora (Delatour *et al.,* 1986).

The efficacy of benzimidazoles and pro-benzimidazoles can be improved by some potentiating agents. The hepatic cytochrome P450 metabolic pathway is considered to be responsible for sulphonation (Souhaili-el-Amri *et al.,* 1988) and the flavin-containing

mono-oxygenase for sulphoxidation (Galtier *et al.,* 1986). Metabolic inhibitors such as methimazole which inhibits flavin-containing mono-oxygenase and metyrapone which inhibits cytochrome P450 prevent the oxidative metabolism of netobimin and albendazole sulphoxide thus improving the efficacy of these anthelmintics and pharmacokinetic profile of the active metabolites (Lanusse and Prichard, 1991, 1992a, 1992b). The bioavailability of anthelmintic metabolites was also improved when FBZ and FBZ.SO were co-administered with methimazole (Lanusse *et al.*, 1995). Co-administration of PBZ with FBZ.SO has been shown to increase the anthelmintic activity of FBZ.SO and confer activity against benzimidazole-resistant nematodes. This has been attributed to a reduction in hepatic biotransformation and biliary secretion of FBZ.SO. Parbendazole promotes an increase in extra-biliary transfer of FBZ.SO into the intestinal lumen and thus exposure of the parasites to increased drug concentrations in the gastrointestinal lumen (Hennessy *et al.,* 1985, 1987, 1992). The methylenedioxiphenyl compound piperonyl butoxide is a potent inhibitor of the cytocrome P450 oxidation of benzimidazole sulphides to sulphoxides and it was shown to reduce the metabolism of FBZ in cultured rat hepatocytes by 50% in 24 h (Benchaoui and McKellar, 1996). In sheep, it was demonstrated that co-administration of piperonyl butoxide with FBZ significantly enhanced the pharmacokinetic profile and potentiated the antinematodal activity of the benzimidazoles. A dose-dependent inhibition of FBZ sulphoxidation by piperonyl butoxide improved the efficacy of the drug against nematodes of sheep, including benzimidazole-resistant *Ostertagia circumcincta* and *Haemonchus contortus* (Benchaoui, 1994; Benchaoui and McKellar, 1996). A similar study was carried out in horses and showed that piperonyl butoxide had a more dramatic pharmacokinetic effect in horses than in ruminants (McKellar, 1997a), probably because horses oxidise benzimidazole sulphides to sulphoxide more rapidly than ruminants. The AUC of parent FBZ was 0.32±0.11 μg.ml/h and 3.51±0.40 μg.ml/h after oral administration of FBZ at 10 μg/kg dose-level alone and in combination with piperonyl butoxide (63 mg/kg), respectively (Muzandu, 1997). The potentiating agents for the benzimidazoles and pro-benzimidazoles may be useful in practice since an increase in the ratio of sulphide + sulphoxide: sulphone metabolites occur, and the less oxidized metabolites (sulphide) of the benzimidazoles are considered to exhibit greater binding to nematode tubulin (Lacey *et al.*, 1987).

Parasitic infestation of the gastrointestinal tract has been demonstrated to affect the bioavailability of anthelmintics in animals. In sheep infected with *O. circumcincta* and treated with FBZ, plasma concentrations of both FBZ and FBZ.SO were reduced when compared with uninfected animals treated with the same dose of FBZ (Marriner *et al.*, 1984). Moreover, the plasma concentration of FBZ.SO was reduced 25% in goats infected with the same nematode compared to uninfected goats given the same dose of FBZ.SO (Bogan *et al.*, 1987). In contrast, after intraruminal administration of ABZ, the AUC of the ABZ.SO was higher in sheep naturally and artificially infected with *H. contortus* than in non-infected animals (Alverez *et al.*, 1997). It has been shown that the pH of abomasal fluid was decreased by the presence of nematode parasites (Mostofa and McKellar, 1989; McKellar *et al.*, 1990b). In addition helminthiasis produces a constant stimulus for gastrin secretion leading to hypergastrinaemia and pronounced hyperplastic changes in the abomasal mucosa (Anderson *et al.*, 1988). These changes probably affect absorption of benzimidazoles; hence the pharmacokinetic behaviour and expected efficacy may be reduced by the presence of the parasites. An elevation in gastric pH causes a reduction in solubility of the benzimidazoles and this would contribute to decreased bioavailability. It has been reported that there is a reduction in the bioavailability of the pro-benzimidazole, FBT in lambs infected with *Ostertagia* and in those infected with *T. colubriformis* (Debackere *et al.*, 1993). The *T. colubriformis* infection caused a reduction in gastrointestinal motility and mucosal villous atrophy, both of which could affect drug absorption (Debackere *et al.*, 1993). The parasitic infection may also alter drug metabolism, which could effect the disposition of the anthelmintics. It has been shown that the enzymatic activity of the hepatic microsomal cytochrome P450-dependent monooxygenase system is depressed in rats infected with *Fasciola hepatica* (Tekwani *et al.,* 1988). Similarly, in sheep, *F. hepatica* infection decreased sulphonation of ABZ and this was related to a decline in liver microsomal P450-dependent monooxygenase activity (Galtier *et al.*, 1986). In contrast to these observations, there were no significant differences in the bioavailability of anthelmintics (levamisole, ivermectin, NTB and ABZ) in sheep infected with *Nematodirus battus* and non-infected sheep (McKellar *et al.*, 1991, 1993b).

1.2.1.6 Chirality of Benzimidazoles

Chiral compounds are extensively used in veterinary practice. Pharmacodynamic and pharmacokinetic properties of enantiospecific pairs are commonly different and are of major importance for their effective and safe therapeutic use.

Sulphoxide benzimidazoles (FBZ.SO, ABZ.SO), which have a chiral centre about the sulphur atom, are formed as metabolites of sulphides and are metabolised into sulphones. The sulphones are anthelmintically inactive, whereas sulphides and sulphoxides are both active. The stereospecific behaviour of benzimidazole sulphoxides has been investigated in the plasma of various species following administration of the prochiral sulphide parent molecule (Delatour *et al.*, 1990a, b; 1991a, b). In sheep, the ratio of fenbendazole sulphoxide (FBZ.SO) enantiomers changed from 1.8 to 6.7 between 9 h and 120 h, and albendazole sulphoxide (ABZ.SO) enantiomer ratio changed from 3.3 to 22.4 between 3 h and 36 h. The enantiospecific ratio of AUC values of sulphoxide metabolites, following administration of sulphides, was 26:74 for fenbendazole sulphoxide and 14:86 for ABZ.SO enantiomers (Delatour *et al.*, 1990b). The stereospecific behaviour of ABZ.SO was shown to be different between monogastrics and ruminants (Delatour *et al.*, 1991a, b). The enantiospecific ratio (+/-) of plasma concentration of ABZ.SO changed over time in favour of (+) in all species examined, with the exception of rats, and the initial (+)/(-) plasma ratio was close to a racemate (50:50) in monogastrics, but was 75:25 in sheep. These results were confirmed by incubation of liver microsomes using ABZ as the substrate (Galtier *et al.*, 1986; Moroni *et al.*, 1995). It is thought that the flavine-containing monooxygenase (FMO) is mainly responsible for sulphoxidation, whereas cytochrome-dependent monooxygenase is responsible for sulphonation (Benoit *et al.*, 1992). The initial enantiospecific ratio was 30:70 when co incubated with methimazole (an inhibitor of FMO) and 65:35 in the presence of clotrimazole (an inhibitor of cytochrome P450). These data indicate that the FMO is product stereoselective and produces (+) ABZ.SO, whereas cytochrome specifically uses (-) ABZ.SO as substrate (Moroni *et al.*, 1995). Both systems act equally in rats and probably in other monogastrics (man, dog, horse), while the FMO system is predominant in ruminants. Differences of interspecies stereoselectivity for the benzimidazole sulphoxides exist and may be explained by different relative enzyme contributions.

The eudismic (potency) ratios of the enantiospecific pair of benzimidazole sulphoxides have not been determined yet, and their anthelmintic activity is still unclear (Landoni *et al.*, 1997).

1.2.2 AVERMECTINS AND MILBEMYCINS IN HORSES

Avermectins and milbemycins comprise a series of natural and semisynthetic molecules, including ivermectin (IVM), abamectin (ABM), moxidectin (MXD), doramectin (DRM), eprinomectin (EPM), selamectin (SLM) and milbemycine oxime, which share some structural and physicochemical properties, and have a similar antiparasitic activity at extremely low dosage rates based on a common mode of action (Lanusse and Prichard, 1993). They are highly effective against nematode and ectoparasitic arthropods in host species (Jones *et al.*, 1993; Kennedy and Philips, 1993; Logan, *et al.*, 1993; Miller *et al.*, 1994; Xiao *et al.*, 1994; Lyons *et al.*, 1996; DiPietro *et al.*, 1997; Dorchies *et al.*, 1998 and reviewed by Benz and Cox 1989; Benz *et al.*, 1989; Cambell *et al.*, 1989; Conder and Cambell, 1995; McKellar and Benchaoui, 1996). However, avermectins and milbemycins are not effective against trematodes or cestodes (Shoop *et al.*, 1995). It was the unique combination killing of endo- and ectoparasites by the avermectins that gave rise to the name "endectocide".

The milbemycins were discovered in 1973 as acaricidal and insecticidal compounds for crop protection (Takiguchi *et al.*, 1980). However, the full potential of these chemical groups was not realized until the acaricidal, insecticidal and nematocidal activity of the avermectins was documented in 1975 (Egerton *et al.*, 1979). This discovery started a new chapter in the treatment of endoparasitic and ectoparasitic infections in animal and human medicine.

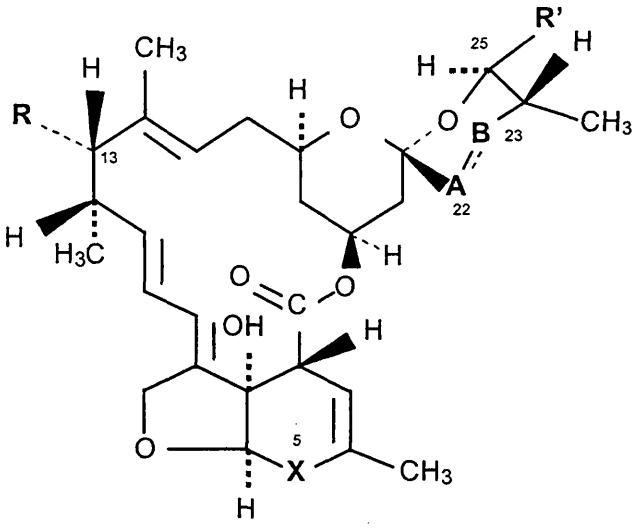
During anthelmintic development, the excellent microfilaricidal activity of IVM was recognized in *Dirofilaria immitis* infections in dogs (Blair and Campbell, 1979) and *Onchocerca cervicalis* infections in horses (Herd and Donham, 1983; Pulliam and and Preston 1989). Ivermectin was firstly introduced as an animal endectocide in 1981 in France (Shoop *et al.*, 1995) and within 5 years, it became the most popular anthelmintic worldwide (Bloomfield, 1988). It was shown that avermectins had good activity against microfilaria of *Onchocerca volvulus* in humans, and were well tolerated (Aziz *et al.*, 1982). Abamectin was introduced commercially as a veterinary parasiticide in 1985 in

Australia (Tahir *et al.*, 1986). Moxidectin, which is a milbemycin, was developed as an endectocide for livestock in the USA (Webb, *et al.*, 1991) and was marketed firstly in Argentina in 1990. It has been commercialized for use in cattle as an injectable and pour-on preparation, in sheep as an oral drench and in horses as an oral gel (at 200μg/kg therapeutic dose level). Milbemycin oxime has been licensed in the USA for use against adult *Ancylostoma caninum* as a therapeutic agent and against *Dirofilaria immitis* as a prophylactic agent since 1990 (Bowman, *et al.*, 1990; Stansfield and Hepler, 1991). Doramectin, the most recently introduced avermectin compound, was first marketed in 1993 in Brasil and South Africa (Vercruysse, 1993). Like other avermectins, it has prophylactic as well as therapeutic activity against gastrointestinal nematodes and arthropod parasites (Conder, 1995).

Avermectins and milbemycins are widely used in horses for the treatment of gastrointestinal and ectoparasitic infections. Ivermectin was first marketed for horses as a micellar formulation containing 20 mg of IVM per millilitre of sterile aqueous solution (2% w/v) for intramuscular injection. After parenteral administration, rare adverse reactions such as, Clostridial spp. infections and anaphylaxis were observed and these undesirable effects were responsible for the withdrawal of the parenteral preparation of IVM for use in the horse in 1984 (Randi, 1984; Campbell *et al.*, 1989). An oral paste formulation of IVM (1.87%) in titaniumdioxide and propylene glycol is now available in graduated delivery syringes of which each part is designed to administer sufficient IVM (at 200 μg/kg) for 100 kg of body weight. A liquid formulation of IVM is also marketed for administration by nasogastric intubation in some countries. Moxidectin 2% oral gel formulation is commercially available for antiparasitic treatment of horses in some markets. Doramectin has not yet been licensed for use in horses.

1.2.2.1 Chemistry and Metabolism

The chemical structures of avermectins and milbemycins are closely related. Avermectins are all 16-membered macrocyclic lactones, with a disaccharide substitution at C-13 (Fisher and Mrozik, 1989) (Figure 1-2). The major structural difference between the two groups is a bisoleandrosyloxy substituent found at the C-13 position of the macrolide ring of the avermectins whereas that position is unsubstituted in milbemycins. The sugar moiety at C-13 together with the hydroxy group at C-5 may determine



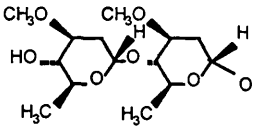
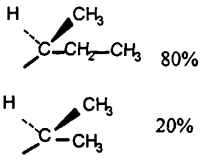
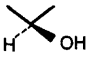
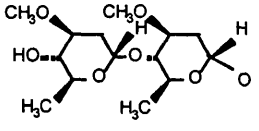
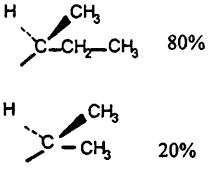
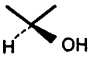
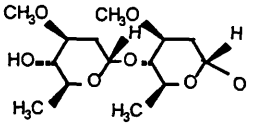
	R	R'	A	B	X
Ivermectin		 80% 20%	CH_2	CH_2	
Abamectin		 80% 20%	CH	CH	
Doramectin			CH	CH	
Milbemycin B-41D	H	$CH(CH_3)_2$	CH_2	CH_2	
Milbemycin A3/A4 5-oxime	H	CH_3/CH_2CH_3	CH_2	CH_2	
Moxidectin	H		CH_2	$CH{=}NOCH_3$	

Figure 1-2. Structures of macrocyclic lactones (adapted from Conder and Campbell, 1995).

anthelmintic and insecticidial activity (Jackson, 1989). Avermectins are derived from the mycelia of *Streptomyces avermitilis* and milbemycins are produced from fermentation of *Streptomyces hygroscopicus* and *Streptomyces cyanegriseus*. Fermentation of *S. avermitilis* produces eight different components and they are named avermectin A_{1a}, A_{1b}, A_{2a}, A_{2b}, B_{1a}, B_{1b}, B_{2a} and B_{2b}. The A-components have a methoxy group at the 5-position, whereas the B-components have a hydroxy group; the 1-components have a double bond between the 22- and 23-position, whereas the 2-components have a single bond with a hydroxy group at the 23-position; and a-components have a secondary butyl side chain at the 25-position, whereas the b-components have an isopropyl substituent at the 25-position. Abamectin (avermectin B_1) contains at least 80% of avermectin B_{1a} and not more than 20% avermectin B_{1b}. A semisynthetic derivative of the avermectin family, IVM, contains at lest 80% of 22-23 dihydroavermectin B_{1a} and less than 20% of the 22-23 dihydroavermectin B_{1b}.

Avermectins are highly lipophilic substances and dissolve in most organic solvents, such as chloroform, acetone, toluene and methylene chloride. Their solubility in water is very low (0.006-0.009 mg. l^{-1}) (Fisher and Mrozik, 1989). Moxidectin has a lower molecular weight (639.8 kDa) and higher water solubility (4.3 mg. l^{-1}) than ABM and IVM (mol. wt. ~870 kDa) (Lanusse and Prichard, 1993). These physicochemical differences may affect pharmacokinetic behaviour, development of drug resistance and parasite uptake mechanism (Lanusse and Prichard, 1993). Liver and fat are two main tissue sites of IVM biotransformation. Unchanged parent compound is the main liver residue in cattle, sheep and rats treated with IVM and the major liver metabolite is a 24-hydroxy-methyl-H2-B_{1a} and H2-B_{1b} (Chiu, *et al.*, 1987). The monosaccaride and aglycone forms of these metabolites were also identified in the liver. It was indicated that cytocrome P450 3A is the predominant isoform, which is primarily responsible for the metabolism of avermectins, by liver microsomes in rat (Zeng *et al.*, 1997) and humans (Zeng *et al.*, 1998). N-deacetylation of AAB1 has been shown to be the primary metabolic route of EPM in rats (Zeng *et al.*, 1996). The major excretion routes of avermectins are bile and faeces in all animal species (Chiu and Lu, 1989). Ivermectin has been shown to be excreted in high concentrations in the bile of ruminants (Bogan and McKellar, 1988) and primarily eliminated in faeces, with less than 2% of the total dose being excreted in urine (Chiu *et al.*, 1990). Considerable amounts of IVM are also excreted via the mammary gland. In lactating cows, plasma and milk concentration of IVM showed a parallel

disposition (Toutain, *et al.*, 1988). Ivermectin was detected in milk for 18 days and approximately 5% of the total given dose was recovered in milk in this period. In addition, in IVM treated ewes, their suckling lambs received about 4% of a therapeutic dose via the milk (Bogan and McKellar, 1988).

1.2.2.2 Efficacy and Spectra of Activity

Ivermectin and MXD which are licensed macrocyclic lactones for use in horses, are highly effective against bots, lungworms, thread worms, cutaneous onchocerciasis and ectoparasites (Christenson *et al.*, 1984; Herd, 1987; Lyons *et al.*, 1992; Klei *et al.*, 1993; Bello and Laningham, 1994; Miller *et al.*, 1994; Xiao *et al.*, 1994; Jacobs *et al.*, 1995; Monahan *et al.*, 1995; Lyons *et al.*, 1996; DiPietro *et al.*, 1997; Boersema *et al.*, 1998; Dorchies *et al.*, 1998). Ivermectin, 0.2 mg/kg body weight given orally, is highly active against adult and third-stage pulmonary larvae (L_3) of *Parascaris equorum* (French *et al.*, 1988) but has variable activity against fourth-stage (L_4) intestinal larvae (Campbell *et al.*, 1989) in horses. It is more than 90% effective against adult cyathostomes, but has very little or no activity against hypobiotic early third-stage or mucosal late third-or forth stage larvae (Eysker *et al.*, 1992; Klei *et al.*, 1993). An efficacy study of IVM for ascarids indicated that the oral formulation of IVM is 90 to 100% effective against pulmonary and small intestinal stages in foals and weanlings (Austin *et al.*, 1991). Ivermectin is highly effective against adult *Strongylus* spp., arterial stages of *Strongylus vulgaris*, migratory *Strongylus edentatus*, (Klei *et al.*, 1984; Slocombe and McCraw, 1984), *Strongyloides westeri* (Ryan and Best, 1985) and *Dictyocaulus arnfieldi* (French *et al.*, 1988). In ponies, IVM is fully effective against oral and gastric stages of *Gasterophilus* spp. after oral administration at 0.2 mg/kg body weight (Bello, 1989).

It has been shown that moxidectin has excellent activity against equine nematodes (Bella *et al.*, 1992; Lyons *et al.*, 1992; Xiao, 1994; DiPietro *et al.*, 1997; Dorchies *et al.*, 1998). It has higher activity than IVM against tissue strongyle larvae when given at an equal oral dose (DiPietro *et al.*, 1997). In MXD treated horses it was reported that strongyle mean egg per gram counts (EPG) remained low for longer than in IVM treated horses (Boersema *et al.*, 1998). The greater lipid solubility of MXD as compared to IVM may explain its increased activity against these parasites (Hayes, 1994; Zulalian *et al.*, 1994). The activity of MXD, given orally at 0.4 mg/kg, is 100% effective against *Trichostrongylus axei, Triodontophorus* spp., *O. equi* L_5 and Cyathostomes adult and L_5;

99% against *O. equi* L_4 and 92% against *S. edentatus* L_5 and *G. intestinalis* (Dorchies *et al.*, 1998). One hundred per cent reduction of strongyle Faecal Egg Counts (FECs) was reported after MXD treatment (Genchi *et al.*, 1995; Jacobs *et al.*, 1995; Demeulenaer *et al.*, 1997; Dorchies *et al.*, 1997, 1998). It was shown that IVM and MXD were equally effective in reducing *P. equorum* EPG (egg per gram counts) (DiPietro *et al.*, 1997). After oral administration, MXD and IVM were fully effective in the control of *Onchocerca cervicalis* microfilariae in horses (Mancebo *et al.*, 1997).

1.2.2.3 Mode of Action

Although the mechanism of action of avermectin and milbemycin anthelmintics is not fully understood, it is likely that they share the same mechanism of action. It appears that these compounds cause irreversible changes by increasing Cl^- permeability of the muscle membrane in nematodes (Martin and Pennington, 1989; Martin, 1997; McKellar and Benchaoui, 1996) but, the identity of the channel targeted by the avermectins has been controversial (Arena, 1994). In *Ascaris lumbricoides*, avermectins reduced inhibitory neuromuscular transmission by a GABA-mediated mechanism at a concentration of $5x10^{-6}$ M (Kass *et al.*, 1984). At this concentration, avermectins were thought to induce presynaptic GABA release or function as GABA agonists. At lower concentrations ($2x10^{-12}$ M), avermectins open GABA-independent chloride channels in *A. suum* muscle membranes and surprisingly, avermectins were shown to be antagonistic to GABA-gated chloride ion channels at concentrations $>10^{-8}$ M (Martin and Pennington, 1988). Thus, at high concentrations, avermectins open the GABA-gated ion channel directly and, at lower concentrations, they potentiate the effect of glutamate (Martin, 1997).

Early reports suggested that the chloride channels were associated with gamma aminobutric acid (GABA) receptors (Turner and Schaeffer, 1989), but more recent evidence indicates that there is no GABA-gated Cl^- channel association (Geary *et al.*, 1992; Martin, 1997). Expression cloning experiments using *Xenopus oocytes* have suggested an action of avermectins on avermectin-sensitive glutamate-gated Cl^- (GluCl) channels (Cully *et al.*, 1994) and avermectins and milbemycin mediate their nematocidal effects on *Caenorhabditis elegans* via interaction with a common receptor molecule at glutamate-gated channels (Arena *et al.*, 1995). Molecular studies showed that the Glu-Cl β-subunit of the glutamate channel was expressed in the pharyngeal muscle of *C. elegans* (Laughton *et al.*, 1995). It was demonstrated that avermectin-sensitive glutamate-gated

chloride channels were present on the pharyngeal muscle of *A. suum* by using a microelectrode current clamp technique (Martin, 1996). However, IVM has been shown to have excellent binding affinity for a P-glycoprotein which acts as a transmembrane efflux pump (Didier and Loor, 1996; Pouliot *et al.*, 1997) and that the high tolerance of mammals to IVM is abolished if the blood-brain barrier P-glycoprotein is deleted (Schinkel *et al.*, 1994). Thus, avermectins and milbemycin probably have more than one mechanism of action against nematodes (Martin *et al.*, 1997).

In ectoparasites (*Shistocerca gregaria*) it has been reported that IVM interacted with both a GABA receptor-Cl^- ion channel (10^{-10} M) in a reversible dose-dependent fashion and with GABA-insensitive neurones, where an irreversible increase in Cl^- conductance was noted at 10^{-10} M (Duce and Scott, 1985). In contrast, in the ectoparasite, *Periplaneta americans*, avermectin B_{1a} had no effect on GABA-independent neuronal conductance (Mellin *et al.*, 1983), although avermectin-sensitive GABA binding sites have been identified in nerve cord (Lummis and Satelle, 1995).

The selective therapeutic effects of avermectins and milbemycins may be explained by an action on a Glu Cl^- ion-channel, present in nematodes and ectoparasites, but not in mammals (Martin, 1997). In addition, the pharmacokinetic and pharmacodynamic properties of avermectins and milbemycins may determine their selective activity in nematodes (reviewed by McKellar and Benchaoui, 1996). Ivermectin distributes poorly into the brain of mammalian species (Chiu and Lu, 1989) and the affinity of IVM for specific binding sites in *C. elegans* is much higher (100-fold) than in rat brain (Turner and Schaeffer, 1989).

Transcuticular absorption of avermectins and milbemycins could be as effective as oral absorption for many nematodes (Court *et al.*, 1988). In blood sucking parasites such as *H. contortus* and arthropods, it is likely that the oral route contributes substantially to the uptake of these drugs (reviewed by McKellar and Benchaoui, 1996). This is supported by their greater activity against sucking lice, *Haematopinus eurysternus* and *Linognathus vituli*, than the biting lice, *Damalinia bovis*, and mites such as *Sarcoptes scabia var bovis* (Benz *et al.*, 1989).

The effects of avermectins are variable on different nematode species. While a slow rigid paralysis was observed in the free-living nematode, *C. elegans*, a flaccid paralysis was reported following injection into *A. suum* (Turner and Schaeffer, 1989). It was also

shown that avermectins reduced the fecundity (amount of eggs in uterus) of *Cooperia curticei* by 99% (McKellar *et al.*, 1988) and the reproductive potential of *Dermacentor alpictus* and *Amblyomma americanum* by >96% (Egerton *et al.*, 1980; Wilkins *et al.*, 1981).

1.2.2.4 Safety and Toxicity

Avermectins and milbemycins have a substantial margin of safety and high therapeutic indices when compared with other anthelmintic xenobiotics. Since GABA is a neurotransmitter limited to the central nervous system in mammals, neurotoxicity may occur when avermectins and milbemycins are administered at high doses (Lankas and Gordon, 1989). Neurotoxicity characterised by ataxia, tremors and coma was identified (Lankas and Gordon, 1989). Avermectins cause the release of endogenous GABA from cerebral cortex synaptosomes of the rat (Pong *et al.*, 1980). Avermectins bind to two different sites in the GABA-gated chloride channel, activating the channel on binding to the high-affinity site and blocking it on further binding to the low-affinity site in rat cerebellar granule neurones (Huang and Casida, 1997). The specific avermectin-binding sites may be blocked by GABA and GABA agonists (Draxler and Sieghart, 1984). Deficiency of P-glycoprotein, a transmembrane glycoprotein associated with multidrug resistance (Goldstein *et al.*, 1989), was shown in the intestinal epithelium and brain capillary endothelium in CF-1 mice which were 100-fold more sensitive to avermectin neurotoxicity than other species and normal mice (Lankas *et al.*, 1997).

The toxicity of macrocyclic lactones has been correlated with their disposition in susceptible species and breeds of animals (McKellar, 1997b). The distribution of IVM is restricted by the blood-brain barrier in the central nervous system of mammalian species (Chiu and Lu, 1989). The central nervous system of neonatal rats is more sensitive to toxicity than that of adult rats due to the immature blood-brain barrier (Lankas and Gordon, 1989). More recent studies have shown that IVM is pumped as an efflux by a P-glycoprotein in the blood-brain barrier of mice and this may prevent the accumulation of IVM in normally tolerant animals (Schinkel, *et al.*, 1994, 1996).

Although mammalian species have different sensitivity, similar signs of acute avermectin and milbemycin toxicity, such as ataxia, tremors and coma are observed in most species (Lankas and Gordon, 1989). The oral lethal doses for 50% of a population (LD_{50}) are 25, 50 and 80 mg/kg in mouse, rat and dog (beagle), respectively (Fisher and Mrozik, 1992).

Some Collie dogs are more sensitive to IVM than other dog species and this may be attributed to greater distribution of IVM into the CNS of sensitive dogs (Pulliam and Preston, 1989; Tranguilli *et al.*, 1991). Similar breed sensitivity was reported in Murray Grey cattle in Australia to abamectin (Seaman *et al.*, 1987).

In cattle, sheep, horses and swine, at more than double therapeutic dose administration, IVM was not embryotoxic and had no adverse effect on male reproductivity in target animal species (Hotson, 1983; Leaning *et al.*, 1983; Campbell and Benz, 1984; Schroder *et al.*, 1986; McKissick *et al.*, 1987). Ivermectin did not adversely affect the fertility of mares or the organogenesis of their developing foals following oral treatment at a 600μg/kg dose-rate (therapeutic dose x 3) (McKissick *et al.*, 1987).

Genotoxicity and carcinogenicity studies indicated that ABM and IVM had no genotoxic activity and no carcinogenic potential, respectively (Lancas and Gordon, 1989).

1.2.2.5 Pharmacokinetics

Pharmacokinetics and bioavailability studies of avermectins and milbemycins have been well documented in the last few years and are summarized in Table 1-4. The pharmacokinetic behaviour of IVM has been investigated more extensively than that of the other members of the macrocyclic lactones. The macrocyclic lactones possess large volumes of distribution and long persistence. The pharmacokinetic behaviour of this group of anthelmintics is significantly affected by route of administration, the formulation of the drug, and interspecies and interindividual variation (reviewed by McKellar and Benchaoui, 1996).

Avermectins and milbemycins are highly lipophilic substances that are extensively distributed from the bloodstream to different tissues and slowly eliminated from body compartments, especially liver and fat (Zulalian *et al.*, 1994), and for this reason larger volumes of distribution (V_d) may be obtained for these compounds than for other anthelmintics (Lanusse *et al.*, 1997). The volume of distribution of IVM has been reported to be 1.9 L/kg for cattle, 4.62 L/kg for sheep (Lanusse *et al.*, 1997) and 2.4 L/kg for dogs (Lo *et al.*, 1985b). The different chemical structure of MXD, in particular the lack of the avermectin glycosides, contributes extensive lipophilicity in subcutaneous fat (Hennessy, 1997). Hayes (1995) indicated that MXD is 100 times more lipophilic than

Table 1-4. Plasma pharmacokinetic parameters of macrocyclic lactones in different animal species.

Species	Drug	Dose (mg/kg)	n	C_{max} (ng/ml)	t_{max} (h)	AUC (ng.h/ml)	References
Cattle	IVM	0.2-s.c	4	42.8	96	11016	Lanusse *et al.* (1997)
		0.2-s.c.	5	54.6	35	10790	Toutain *et al.* (1988)
		0.5-pour-on	12	12.2	82	2760	Gayrard *et al.* (1997)
		0.5-pour-on	4	28.3	48	-	Herd *et al.* (1996)
		0.3-i.r.		29.0	24	3960	Chiu *et al.* (1990)
	MXD	0.2-s.c.	4	39.4	8	5208	Lanusse *et al.* (1997)
		0.2-s.c.	3	75.0	4-6	-	Miller *et al.* (1994)
	DRM	0.2-s.c.	20	27.8	72-144	11400	Nowakowski *et al.* (1995)
		0.2-s.c.	4	37.5	144	15048	Lanusse *et al.* (1997)
		0.5-pour-on	12	12.2	103	4032	Gayrard *et al.* (1997)
Sheep	IVM	0.2-s.c.	5	30.8	60	5718	Marriner *et al.* (1987)
		0.2-p.o.	5	22.0	16	2039	Marriner *et al.* (1987)
	MXD	0.2-s.c.	5	8.3	21	2688	Alvinerie *et al.* (1998)
		0.2-p.o.	5	28.07	5	2373	Alvinerie *et al.* (1998)
Goat	IVM	0.2-p.o.	6	16.0	<24	516	Scott *et al.* (1990)
		0.2-i.r.	5	10.54	29	831	Escudero *et al.* (1997)
		0.5-topical	6	4.0	48	317	Scott *et al.* (1990)
Horse	IVM	0.2-s.c.	3	60.7	80	13209	Marriner *et al.* (1987)
		0.2-p.o.	3	46.28	7	2646	Scott (1997)
		0.2-p.o.	5	43.99	9	3185	Perez *et al.* (1999)
		0.2-p.o.	3	82.3	3	4822	Marriner *et al.* (1987)
		0.2-p.o.	6	16.4	15	-	Asquith *et al.* (1987)
	MXD	0.4-p.o.	5	70.35	9	8726	Perez *et al.* (1999)
		0.4-p.o.		70.35	20	8712	Alvinerie & Galtier, (1997)
Donkey	IVM	0.3-p.o.	3	43.20	8	1811	Scott (1997)
Pig	IVM	0.3-s.c	5	28.4	27	1714	Scott & McKellar (1992)
Camel	IVM	0.2-s.c.		2.68	-	1591	Alvinerie & Galtier, (1997)
	MXD	0.2-s.c.	3	8.51	37	1761	Oukessou *et al.* (1997)
Dog	IVM	0.1-p.o.	16	44.3	4	1035	Daurio *et al.* (1992)
Deer	IVM	0.2-s.c.	10	15.3	28	-	Andrews *et al.* (1993)
		0.4-s.c.	10	28.3	28	-	Andrews *et al.* (1993)
Rabbit	IVM	0.4-s.c.	6	42.0	37	3543	McKellar *et al.* (1992)

IVM. In sheep, after oral administration of MXD, a significantly larger V_d (12.6 L/kg) was observed than that observed for IVM, 5.3 L/kg (Prichard *et al.*, 1985). Recently, in cattle, it was reported that there was no marked difference in the V_d obtained for IVM (3.35 L/kg) and DRM (2.92 L/kg), whereas a significantly larger V_d was obtained for MXD (13.6 L/kg) after their subcutaneous administration (Lanusse *et al.*, 1997). The concentrations of MXD in fat tissues 28 days after treatment in cattle have been shown to be ninety-fold higher than these detected in plasma following subcutaneous administration at 0.2 mg/kg body weight (Zulalian *et al.*, 1994). The half-life of MXD in fat was 14 days (Zulalian *et al.*, 1994) compared to that of IVM (7 days) (Chiu *et al.*, 1987) after subcutaneous administration to cattle. In addition, the fat /liver residue concentration ratio was 7:3 for MXD compared to 1:7 for IVM 7 days after treatment (Hayes, 1995).

The physicochemical properties of macrocyclic lactones may account for differences in formulation flexibility and in their kinetic behaviour (Lanusse *et al.*, 1997). In cattle, the pharmacokinetic profiles of IVM (Lo *et al.*, 1985), DRM (Wicks *et al.*, 1993) and MXD (Delay *et al.*, 1997) were significantly affected by the composition of the administered formulation. Ivermectin, administered in non-aqueous injectable form (60% propylene glycol / 40% glycerol formal), was absorbed more slowly from the site of subcutaneous injection than when the drug was administered as an aqueous solution in cattle, and the elimination half-life of the non-aqueous form of IVM was much longer than that obtained with an aqueous preparation (Lo *et al.*, 1985). The long persistence of the non-aqueous injectable formulation for cattle is reflected in the persistence of its clinical effect (Campbell and Benz, 1984). On the other hand, the elimination half-life of IVM given by the oral / intraruminal route to cattle has been shown to be similar to that obtained after the iv administration of the drug (2.8 days) (Fink and Porras, 1989).

After oral administration of the aqueous micelle and propylene glycol solutions, no significant difference was observed in the bioavailability of IVM in sheep. The maximum plasma concentrations of both formulations were achieved within 1 day, and elimination half-life ranged between 3 and 5 days (Fink and Porras, 1989). In horses, a paste formulation and an aqueous formulation of IVM were administered at the 200 μg/kg dose rate (Asquith *et al.*, 1987). Although the peak concentration was observed within 4 to 5 h for the liquid formulation, a delay of 15 h occurred before C_{max} was reached for the paste

form. In addition, after treatment of horses with a liquid form of IVM via nasogastric tube, 20% higher bioavailability was obtained than for the oral paste form. Nevertheless, the efficacy of the two formulations was similar in reducing faecal egg count (Asquith *et al.*, 1987). In dogs, after dosing with a beef-based chewable preparation better absorption was reported than after dosing with a tablet formulation (Daurio *et al.*, 1992). In man, 50% greater bioavailability was observed for aqueous IVM solution than for a capsule or tablet form (Fink and Porras, 1989). While, in cattle, a non-aqueous formulation of MXD provided a slower rate of absorption, lower peak plasma levels and longer residence time compared to the aqueous form of MXD (Delay *et al.*, 1997). Fat reservoirs of avermectins may determine their residence in plasma; but the slow distribution of avermectins from the bloodstream may also be related to slow absorption of their non-aqueous formulations (especially for DRM) (Lanusse *et al.*, 1997). This generates flip-flop kinetics by which the absorption constant is the rate-limiting step in the disposition of DRM (Nowakowski *et al.*, 1995).

The disposition kinetics of macrocyclic lactones is significantly affected by the route of administration (Table 1-4). Subcutaneous administration of IVM is more effective than oral administration against some endo- and ecto-parasites. After subcutaneous administration of IVM to sheep and horses, considerably greater plasma bioavailability and longer persistence were observed for both drug compared to oral administration at the same dose rate (Marriner *et al.*, 1987) (Table 1-4). In periparturient ewes, subcutaneously treated with IVM, more persistent plasma concentrations and extended efficacy as assessed by egg output were reported than when the drug was administrated as an oral drench form (McKellar and Marriner, 1987). Similarly, 100% persistent effect was found against reinfections with *H. contortus* and *C. curucei* for a period of at least 10 days when the injectable formulation was used, (Borgsteede, 1993).

It has been suggested that a more frequent treatment regimen is necessary when the oral formulation is used (Zajac *et al.*, 1992). In sheep, it was shown that systemic bioavailability of IVM was 25% following intraruminal administration compared with almost 100% bioavailability after intraabomasal injection. This marked difference was attributed to degradation or metabolism of the drug within the rumen (Prichard *et al.*, 1985). In cattle, similar biodegradation may explain the higher bioavailability of IVM obtained after subcutaneous administration compared to intraruminal treatment (Chiu *et*

al., 1990). But a recent study *in vitro* indicated that no metabolism of IVM occurred in rumen fluid (Andrew and Halley, 1996). An alternative explanation for the low bioavailability of orally or intrarumially administered IVM may be absorption or binding to the particulate phase of the digesta which has been shown to influence the pharmacokinetics of some drugs (Bogan *et al.*, 1984; Lees *et al.*, 1988). In swine, after subcutaneous injection of IVM, the absorption was much slower and the bioavailability was 41% higher than after oral administration (Fink and Porras, 1989). The precipitation of drug at the injection site also contributes to the slow absorption phase of these drugs (Lo *et al.*, 1985; Scott and McKellar, 1992). Different pharmacokinetic behaviour following subcutaneous injection and oral administration have been also shown for MXD. Following subcutaneous treatment of MXD, 200 μg/kg, achieved a maximum plasma concentration of 75 ng/ml, whereas following oral administration at the same dose rate, C_{max} was approximately 6.5 ng/ml, and a longer persistence was observed when administered by the subcutaneous route (Miller *et al.*, 1994). More recently, after oral administration of MXD to sheep, a faster absorption rate and shorter MRT were obtained compared to subcutaneous administration and these data suggest that gastrointestinal absorption is not rate limiting, in contrast with the slow absorption process following subcutaneous injection (Alvinerie *et al.*, 1998).

Topical (pour-on) formulations have been developed and marketed for IVM, MXD, DRM and EPM in cattle. Yazdanian and Chen (1995) modified the lipophilicity of the carrier to enhance IVM absorption using combinations of Miglyol (caprylic and capric acid triglyceride) with Transcutol (dietylene glycolmonoetyl ether: DGME). There is little information available, which describes pharmacokinetics of these drugs given transcutaneously. Hennessy (1997) claimed that this route of administration would be expected to enhance the bioavailability of IVM. Nevertheless, in goats, following transcutaneous administration of the IVM pour-on formulation at 500 μg/kg, more persistent but much lower plasma concentrations were observed than after oral administration at the 200 μg/kg (Scott *et al.*, 1990). The maximum plasma concentration (C_{max}) of IVM pour-on formulation was reported to be 28.3 ng/ml at 48 h following administration of 0.5 mg/kg in cattle (Herd *et al.*, 1996). In a recent study, topical administration of IVM pour-on formulation was compared with DRM pour-on formulation at 500 μg/kg in cattle (Gayrard *et al.*, 1997). These authors reported that C_{max}

and t_{max} were not significantly different, however, the mean AUC of DRM was 45% higher than following IVM administration.

Interspecies differences also significantly affect the pharmacokinetic behavior of avermectins and milbemycins. The higher V_d for IVM in sheep (5.3 L/kg) (Prichard *et al.*, 1985) compared with cattle (2.41L/kg) (Wilkinson *et al.*, 1985), correlates with the lower plasma AUC found in sheep than in cattle after intravenous administration (Fink and Porros, 1989). Thus the plasma concentration of IVM is markedly lower and the clearance rate more rapid in sheep because of its large distribution volume (Stell, 1993). A marked difference was reported between goats and sheep in IVM disposition, with goats clearing the drug more rapidly (Scott *et al.*, 1990). After oral administration at 200 µg/kg, IVM was absorbed from the gastrointestinal tract and reached peak plasma concentrations more slowly in donkeys than in ponies and the persistence of drug in plasma was greater in ponies (Scott, 1997). In camels, lower AUC (1760 ng.h/ml) and C_{max} (8.51 ng/ml) were reported following subcutaneous injection of MXD at 0.2 mg/kg (Oukessou *et al.*, 1997) compared to cattle (AUC=5208 ng.h/ml, C_{max}=39.4 ng/ml) at same dose rate (Lanusse *et al.*, 1997). In general, herbivore species (horses and ruminants) metabolize lipid soluble drugs more rapidly than do carnivores (dogs and cats) (Baggot and McKellar, 1994). However, there are notable exceptions to this trend that defy explanation, such as IVM which has a $t_{1/2}$ of 1.8 days in dogs compared with 2.8 days and 2.7 days in cattle and sheep, respectively (Lo *et al.*, 1985). In addition, swine eliminate IVM much faster than ruminants with a half-life of 0.5 day following oral administration with feed (Lo *et al.*, 1985). In guinea-pigs, after subcutaneous, oral and topical treatments, the bioavailability of IVM at 500 µg/kg was much lower than in other species given equivalent and lower doses (McKellar *et al.*, 1992). Similarly, lower AUC of IVM was observed in rabbits after subcutaneous administration at a 400 µg/kg dose level compared with other species (McKellar *et al.*, 1992).

Interindividual variations have been reported to affect the pharmacokinetic disposition of IVM (Marriner *et al.*, 1987; Scott and McKellar, 1992; Andrews *et al.*, 1993; Nowakowski *et al.*, 1995; Scott, 1997). The reason for such differences could not be explained. Shoop and co-workers (1997) have shown that there was a correlation (r^2 = 0.922) between body weight and the zero detectable time of MXD in plasma i.e. the lightest lamb (20.9 kg) reached zero detectable plasma concentration of MXD at day 24,

while the heaviest lamb (38.6 kg) still possessed detectable plasma concentrations at day 60. It was reported that in sheep, IVM was associated with particulate matter in the rumen (Hennessy *et al.*, 1994) and kinetic disposition was increased with reduced feed intake (Ali and Hennessy, 1995, 1996). In grazing lambs, a lower AUC of IVM was reported than in housed lambs and this was attributed to shorter gastrointestinal transit time in the animals eating grass (Taylor *et al.*, 1992). The bioavailability of IVM was not affected by intestinal parasitism (*Nematodirus battus*) in lambs after parenteral or oral administration (McKellar *et al.*, 1991).

1.2.3 PYRANTEL IN HORSES

1.2.3.1 Chemistry and Metabolism

Pyrantel (PYR) is an imidazothiazole derivative (Figure 1-3) which belongs to the tetrahydropyrimidine class of anthelmintics (Roberson, 1988). It is available as a pamoate (syn. embonate) salt, which is almost insoluble in water, and as a tartrate, which is soluble in water (180 mg/ml). Pyrantel pamoate was developed as an anthelmintic, which is poorly absorbed from the gastrointestinal tract since 50%-70% of an ingested dose is excreted in the faeces (Arundel, 1983), and blood levels do not exceed 1 μg/ml (Davis, 1973). Reduced systemic absorption of PYR potentially increases availability in the lumen of the intestine (Bjorn *et al.*, 1996). Hydroxylation of the thiophene ring seems

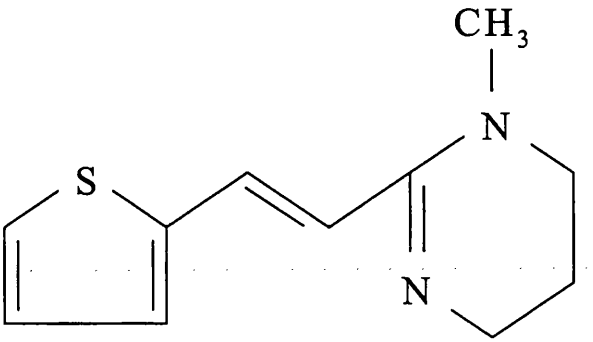


Figure 1-3. Chemical structure of pyrantel.

to be the main route of metabolism of PYR since the *n*-methyl-1,3-propanediamidine skeleton of the tetrhydropyrimidine ring is relatively resistant to metabolic processes. It is

assumed that polar metabolites recovered in urine and bile are anthelmintically inactive (Lanusse and Prichard, 1993).

Pyrantel citrate is absorbed rapidly and extensively from the intestine of monogastric animals and the maximum concentration (C_{max}) of PYR in the blood stream of pigs was observed within 3 h of administration (Faulkner *et al.*, 1972).

1.2.3.2 Efficacy and Spectra of Activity

Pyrantel is highly effective (95%-97%) against small strongyles, *P. equorum* and *S. vulgaris*, and has moderate active against *S. edentatus* (70%) and *O. equi* (65%) (Mirck, 1985). Pyrantel is not effective against *Gasterophilus* spp., but it has activity against the Anoplocephalan tapeworms if used at 13.2 mg/kg (Theodorides, 1985). Continuous low-level daily administration of pyrantel tartrate to horses was highly effective against common gastrointestinal parasitic infections of horses, including large strongyles (*S. vulgaris*, *S. edentatus* and *Triodontophorus* spp.), adult small strongyles (*Cyathostomum* spp., *Cylicocyclus* spp., and *Cylicostephanus* spp.), and adult and fourth-stage *P. equorum* (Valdez *et al.*, 1995).

1.2.3.3 Mode of Action

Pyrantel acts selectively as an agonist at synaptic and extrasynaptic nicotinic acetylcholine receptors on muscle cells of nematodes. It produces contraction and spastic paralysis, which serves to eliminate them from the host (Martin, 1997). Simultaneous application of acetylcholine and PYR showed that both agonists acted on the same nicotinic receptors (Harrow and Gration, 1985) and PYR is up to 100 times more potent than acetylcholine in inducing muscular contraction (Aubry *et al.*, 1970). It has been shown that PYR increases the membrane conductance and depolarizes the membrane by opening non-selective ion channels that are permeable to both Na^+ and K^+ (Harrow and Gration, 1985).

1.2.3.4 Safety and Toxicity

The oral LD_{50} of PYR was 175 mg/kg in mice and 170 mg/kg in rats (Van Den Bossche, 1985). First signs of toxicosis associated with pyrantel tartrate were observed at a dose rate of 55 mg/kg and consisted of sweating, dyspnoea and even death (Cornwell and Jones, 1968). In horses, no adverse effects occurred at doses up to 20 times greater than the therapeutic dose of PYR pamoate (Slocombe and Smart, 1975). The maximum

tolerated oral dose of pyrantel tartrate in sheep was 173 mg/kg (Austin *et al.*, 1966) although the toxicity of pyrantel tartrate in water in adult sheep varies according to the method of administration (Cornwell, 1966).

The reproductive performance of stallions and pregnant mares was not affected by pyrantel (Bentley *et al.*, 1978).

1.2.3.5 Pharmacokinetics

Pyrantel citrate was absorbed rapidly from the intestine in mono gastric animals and achieved C_{max} within 3 h in pigs (Faulkner *et al.*, 1972). Due to the poor solubility of pyrantel pamoate, little is absorbed from the gastrointestinal tract and most passes unchanged in the faeces (Arundel, 1983). The pharmacokinetic disposition of PYR was determined following intravenous and oral administration in pigs (Bjorn *et al.*, 1996). Pyrantel citrate was extensively distributed (2.74 L/kg) and cleared rapidly (1.09 L/kg.h) following intravenous administration. After oral administration t_{max} (3.26 h) was significantly longer, C_{max} (0.23 μg/ml) lower and AUC (3.18 μg.h/kg) smaller for pyrantel pamoate than pyrantel citrate (t_{max}: 1.51 h, C_{max}: 1.92 μg/ml, AUC: 8.42 μg.h/kg). Although a significantly greater quantity of pyrantel citrate was absorbed (mean bioavailability of 41%) than pyrantel pamoate (mean bioavailability of 16%), the rapid clearance of the citrate resulted in a lower MRT (4.92 h) compared to the pamoate form (11.74 h). The more insoluble pamoate salt is less well absorbed and therefore provides a safer dosage formulation with greater passage into the large intestine. This may contribute significantly to the increased efficacy of the pamoate against the parasites at this site (Bjorn *et al.*, 1996).

The influence of the digesta flow rate and pyrantel solubility on pharmacokinetics was recently investigated in pigs (Prasliska *et al.*, 1997). The authors reported that lower fibre diets provided lower digesta flow rate and allowed more time for absorption of the drug than diets with high fibre content.

1.3 ANTHELMINTIC RESISTANCE IN HORSES

In recent years, anthelmintic resistance to chemotherapeutic agents has become an increasing problem in human and domestic animals throughout the world. Anthelmintic drug resistance is a heritable reduction in the sensitivity of the nematode population to the action of a drug at a recommended therapeutic dose level (Conder and Campbell, 1995). Irreversible resistance develops in helminths, usually within 5 years of introduction of the drug (Roos, 1997). It has been reported that resistance occurres in nematode parasites to all major groups of the anthelmintics including benzimidazoles, the levamisole/morantel group, salicylanilides and avermectins (McKellar and Scott, 1990). Resistance to one drug of the benzimidazole group in a nematode population generally confers resistance to other drugs in the same group (Donald, 1983). This is also the case imidazothiazoles and avermectins (Sangster, 1999). This phenomenon is designated side-resistance and has been defined as resistance to a compound, which is the result of selection by another compound with a similar mode of action (Prichard *et al.*, 1980). Furthermore there is evidence for cross-resistance (resistance among different anthelmintic classes) between the imidazothiazoles and organophosphates (Sangster, 1996).

The key issue in the development of resistance is the contribution that helminths surviving therapy make to the next generation (Geerts *et al.*, 1997). These survivors are the most resistant component of the population and carry resistance genes, which they pass to their offspring (Sangster, 1996). After several generations, the number of survivors of therapy increases as the number of genes conferring resistance accumulate (Sangster, 1996). There are three phases in the selection process (Prichard, 1990). Firstly, an initial anthelmintic susceptibility phase occurs where the frequency of resistant individuals within the population is low. Secondly, development of a phase in which the frequency of heterozygous resistant individuals increases within the population and finally, sustained selection pressure results in a resistant phase where homozygous resistant individuals predominant within the population.

In horses, anthelmintic resistance was first described to phenothiazine by Drudge and Elam (1961). A few years later, resistance to thiabendazole was also observed (Drudge and Lyon, 1965). In both cases only small strongyles were involved. Since 1974, the existence of side-resistance has been reported among benzimidazoles (Drudge *et al.*,

1974, Hope and Camp, 1980). However, oxibendazole was highly effective against strongyles resistant to other benzimidazoles for unknown reasons (Drudge *et al.*, 1979). Unfortunately, recent studies have reported that further use of oxibendazole has produced resistance in horses (Lyon *et al.*, 1996; Rolfe, 1998). Anthelmintic resistance has been recognised throughout the world in small strongyles to various anthelmintics including piperazine (Drudge *et al.*, 1988), benzimidazoles (McKellar and Scott, 1990) and pyrantel (Conder and Campbell, 1995). Avermectins/milbemycins resistance to small strongyles have not been reported in horses (Sangster, 1999). The mechanisms of anthelmintic resistance among the small strongyles are unknown and knowledge is available only by extrapolation from studies in sheep nematodes and the free-living *Caenorhabditis elegans* (Lloyd and Soulsby, 1998). Resistance in large strongyles has not been clearly demonstrated.

To delay anthelmintic resistance and to prevent multiple resistance, a single class of anthelmintic is used annually and rotation of classes may limit passage of resistant genes to next the generations (Conder and Campbell, 1995). Benzimidazole-resistant small strongyles may be treated by using the non-benzimidazole drugs including piperazine, avermectins, dichlorvos or pyrantel. Mixtures of two broad-spectrum drugs from different classes could be used against anthelmintic-resistant parasites in preventive strategies (Martin *et al.*, 1990; Anderson *et al.*, 1990) which have been shown to be beneficial in mathematical models. The co-administration of oxfendazole with parbendazole (Hennessy *et al.,* 1985) and fenbendazole with piperonyl butoxide (Benchaoui and McKellar, 1996) in sheep increased systemic availability of the oxfendazole and fenbendazole, respectively and thus efficacy even against resistant parasites, and these strategies may be used to extend the useful lifespan of the benzimidazole drugs. In recent years, mathematical models have been used to evaluate factors contributing to resistance and/or strategies to limit its development (Echevarria *et al.*, 1993; Jackson, 1993).

1.4 STUDY OBJECTIVES

The aim of the studies described in this thesis were:

1. To determine the pharmacokinetics and faecal excretion of benzimidazoles (FBZ.SO, FBZ, OBZ), macrocyclic lactones (IVM, DRM, MXD) and PYR in horses.

2. To determine whether the efficacy of benzimidazole anthelmintics can be improved by the co-administration of piperonyl butoxide, a metabolic inhibitor of hepatic drug oxidative metabolism (cytochrome P450).

3. To study *in vitro* metabolism of FBZ.SO, FBZ and OBZ using horse liver microsomes and to investigate the effects of piperonyl butoxide on the metabolism of these benzimidazole anthelmintics.

4. To determine the *in vivo* and *in vitro* enantioselective nature of the disposition and metabolic inhibition of sulphoxide benzimidazoles.

CHAPTER 2

Pharmacokinetics, metabolism and chirality of benzimidazoles in horses

Section 1

Pharmacokinetics and faecal excretion of oxfendazole, fenbendazole and oxibendazole in horses

2.1.1 Introduction

Benzimidazoles and pro-benzimidazoles are highly efficacious anthelmintics widely used in the horse for treatment of gastrointestinal helminthiases including migrating strongyle larvae and lungworm infections (Marriner and Bogan, 1985). Helminth parasites cause serious pathological changes in the horse including diarrhoea (Mair *et al.*, 1990), rapid progressive weight loss (Love, 1992), functional disorders of the intestine (Ogbourne and Duncan, 1977), colic and pathological changes in the mesenteric arteries (Duncan and Dargie, 1975). The selection of anthelmintic resistant populations has increased since phenothiazine resistance was first recognized in horses in 1961 (Drudge and Elam, 1961). Anthelmintic resistance has developed rapidly in the horse (Love *et al*, 1989) probably because the epidemiology of equine parasites is less seasonal than that of ruminant parasites. Horses are consequently treated frequently throughout the year even in temperate climates, thus exerting great selection pressure on the nematodes.

Benzimidazole anthelmintics have limited water solubility and differences in solubility may influence their absorption and clinical efficacy. Oxfendazole and FBZ are interconvertable and irreversibly metabolised to their sulphone metabolite (Figure 2-1-1). The pharmacokinetics of oxfendazole (FBZ.SO) and fenbendazole (FBZ) in horses has been investigated by Marriner and Bogan (1985) who observed that the maximum plasma concentration (C_{max}), mean residence time (MRT) and area under the curve (AUC) were lower for the parent molecules and the active metabolites of these anthelmintics in the horse than in ruminant species which had been investigated previously (Marriner and Bogan, 1981; Ngomuo *et al*, 1984). Furthermore, the sulphoxidation and sulphonation of the benzimidazoles appeared to be extremely rapid in horses (Marriner and Bogan, 1985). Sulphide and sulphoxide benzimidazoles are known to bind nematode tubulin (Lacey *et al*, 1987) and therefore have activity against nematodes. In most animal species examined, the sulphoxide moiety predominates in plasma and is though to confer activity against gut dwelling nematodes following

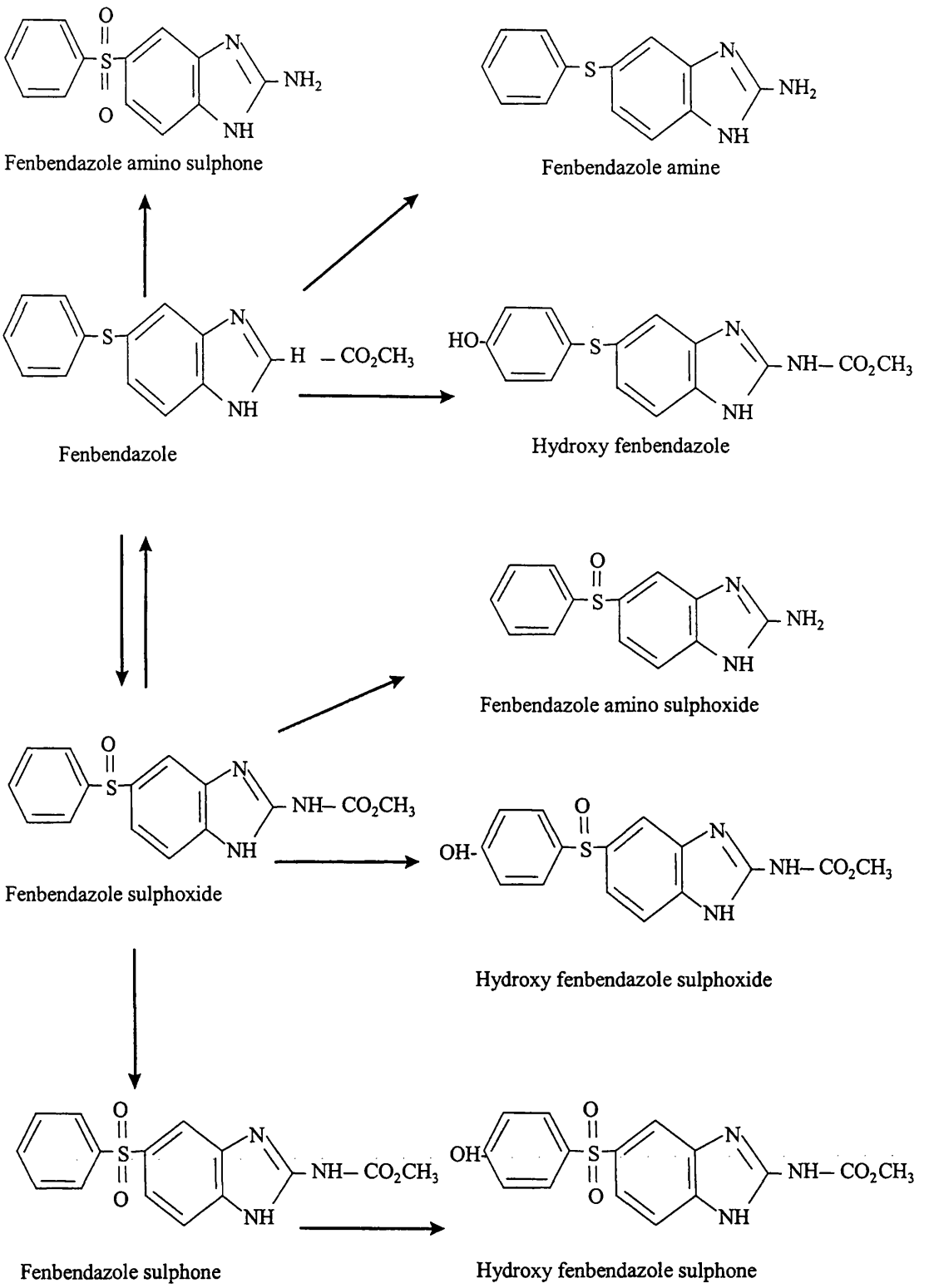


Figure 2-1-1. Major metabolic pathways of fenbendazole.

secretion across the gastrointestinal wall into the gut lumen. There is a paucity of data available in the literature on the pharmacokinetics of OBZ in animals including horses.

The excretion of anthelmintics in the faeces of livestock has given rise to concern since it was observed that the avermectins have adverse effect in the dipteran flies and coleopteran beetles which inhabit and feed in dung (Wall and Strong, 1987). While it is recognized that benzimidazoles are unlikely to affect dung dwelling arthropods (McKellar, 1997b), their excretion in the faeces of horses has not been characterized and consequently the associated environmental impact is not known.

The aim of this study was to determine and compare the plasma disposition kinetics and faecal excretion of FBZ.SO, FBZ (and their metabolites) and OBZ in horses after oral administration. Stereospecific disposition of FBZ.SO was also examined in this study.

2.1.2 Materials and methods

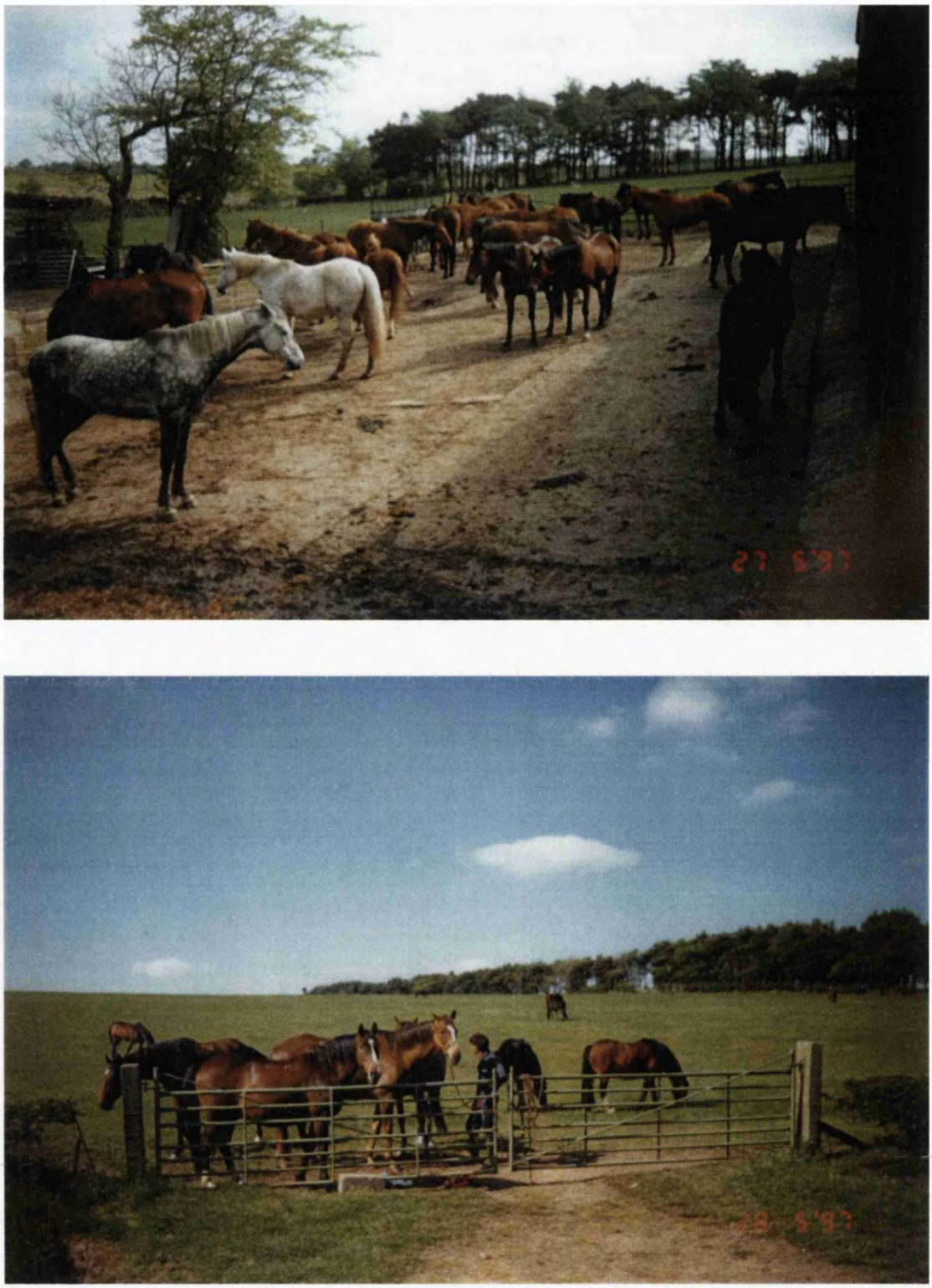
2.1.2.1 Animals

Thirty-two horses weighting 390-720 kg were used in this study. Animals were kept at pasture but were put into a corral for the period of drug administration and for 4 hours thereafter. Water was available *ad libitum* during the experimental period (Picture 2-1-1). No invasive procedures were involved beyond blood and faecal sampling procedures. Horses were allocated into four groups of eight such that the mean weight of animals in each group was similar and the horses were identified by unique freeze brand or natural markings.

The animals used in the study were treated with Panacur (10%) biannually however they were not treated prior to the study.

2.1.2.2 Drug administration and sampling procedure

Commercially available formulations of FBZ.SO (Systamex 906®, 9.06% w/v, Mallinckrodt), FBZ (Panacur®, 18.75% w/w, Hoechst) and OBZ (Equidin Paste®, 30% w/w, Vetoquinol) were administered orally as a single bolus dose on the back of the



Picture 2-1-1. The animals used in this study were kept in the corral for the period of drug administration (A) and then kept at the pasture during the experimental period (B).

tongue at a dose rate of 10 mg/kg bodyweight. Heparinized blood samples were collected by jugular venipuncture prior to drug administration and 1, 2, 4, 8, 12, 20, 24, 32, 48, 72, 96 and 120 h thereafter. Faecal samples (>10 g) were collected *per rectum* throughout the blood-sampling period, before drug administration and then at 4, 8, 12, 20, 24, 32, 48, 72, 96 and 120 h in order to determine faecal excretion of the benzimidazoles under study. Blood samples were centrifuged at 1825 g for 30 min and plasma was transferred to plastic tubes. All the plasma and faecal samples were stored at -20°C until estimation of drug concentration.

2.1.2.3 Drug analysis

Plasma and wet-faecal concentration of FBZ.SO, its sulphide (FBZ) and sulphone ($FBZ.SO_2$) metabolites for the FBZ.SO study, FBZ and its sulphoxide (FBZSO), sulphone ($FBZSO_2$) and hydroxy (OH.FBZ) metabolites for the FBZ study and OBZ were estimated by high performance liquid chromatography (HPLC) with a liquid phase extraction procedure adapted from that described by Marriner and Bogan (1980).

2.1.2.3.1 Standard preparation

Stock solutions (100 µg/ml and 1 mg/ml) of pure standards of FBZ.SO, FBZ, $FBZ.SO_2$ and OH.FBZ (Hoechst, Frankfurt, Germany), and OBZ (Vetoquinol Ltd., UK) were prepared using acetonitrile (Rathburn Chemical Ltd., UK) as the solvent. These were diluted to give 0.1, 0.5, 1, 5, 10 and 10, 50, 500 µg/ml standard solutions for plasma and faecal samples, respectively for calibration as standard curves and to add to drug-free plasma and faecal samples to determine the recovery.

2.1.2.3.2 Extraction from plasma

Drug-free plasma samples (1 ml) were spiked with standards of FBZ, and its metabolites (FBZ SO, FBZ SO_2 and OH.FBZ) for the FBZ study, FBZ.SO and its metabolites (FBZ, $FBZ.SO_2$ and OH.FBZ) for the FBZ.SO study and OBZ to reach the following final concentrations: 0.01, 0.05, 0.1, 0.5, and 1 µg/ml. Ammonium hydroxide (200µl, 0.1N, pH 10) was added to 10 ml-ground glass tubes containing 1 ml spiked or experimental plasma samples. After vortexing for 15 seconds, 6 ml chloroform (Rathburn Chemical ltd., UK) was added. The tubes were shaken on a slow rotary mixer for 10 min. After

centrifugation at 1825 g for 15 min, the supernatant was removed with a pasteur pipette. The organic phase (4 ml) was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 43°C in a sample concentrator (model SC210A, Svant Instrument Inc., Holbrook, NY, USA). The dry residue was resuspended with 50 µl dimethyl sulphoxide (DMSO) and 200 µl of 25% acetonitrile. Then the tubes were placed in ultrasonic bath and finally, 50 µl of this solution was injected into the chromatographic system.

2.1.2.3.3 Extraction from faeces

Wet-faecal samples were mixed finely with a spatula to obtain homogeneous concentrations. Drug-free wet faeces samples (0.5 g) were spiked with benzimidazole standards to reach the following final concentrations: 1, 5, 50, 100, 200 µg/g. Sodium hydroxide buffer (200 µl, 0.4M, pH 10) and 2 ml acetonitrile were added to 10 ml-ground glass tubes containing 0.5 g spiked or experimental wet-faecal samples. After vortexing for 15 seconds, 8 ml chloroform was added. The tubes were shaken on a slow rotary mixer for 15 min. After centrifugation at 1825 g for 15 min, the supernatant was removed with a pasteur pipette. The organic phase (5 ml) was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 43°C in the sample concentrator. The dry residue was resuspended with 50 µl dimethyl sulphoxide (DMSO) and diluted appropriately with 35% acetonitrile. After ultrasonication, the samples were filtered with GF/C glass microfibre filter (Whatman International Ltd., Maidstone, England). Finally, 50µl of this solution was injected into the chromatographic system.

2.1.2.3.4 HPLC system

The mobile phase was a mixture of acetonitrile-water to which glacial acetic acid was added (0.5%, v/v). For FBZ.SO, FBZ and their metabolites it was pumped through the column (Genesis nukleosil C_{18} 4 µm, 150mm x 4.6mm, Crawford Scientific, Strathaven, UK) in a linear gradient fashion changing from 35:65 (acetonitrile-water) to 60:40 for 9 min, 60:40 to 35:65 for 1 min and the last ratio was maintained for 2 min. The flow rate was 1 ml/min. Samples were processed on a computerized HPLC system (PC 1000, Spectra Physics Analytical Inc., UK) comprising a gradient pump (model SP 4000), a UV-detector (SP Focus) set at 292 nm, an autosampler (model AS 3000) and a controller (model SN 4000). The retention times were 5.51 min (FBZ.SO), 6.82 min (OH.FBZ),

7.42 min ($FBZSO_2$) and 10.23 min (FBZ) for plasma samples (Figure 2-1-2). For OBZ, acetonitrile:water (35:65) with trifluoro acetic acid 0.5% (w/w) pumped through the column (Nemesis nukleosil C18, 4μ, 150 mm x 4.6mm, Phenomenex, Cheshire, UK) as a mobile phase for 9 min at 1ml/min flow rate. The retention time was 5.20 min for OBZ.

2.1.2.3.5 Recovery and precision

Recovery of the three parent molecules and the metabolites under study was measured by comparison of the peak areas from spiked plasma samples with the areas resulting from direct injections of standards. The inter-assay precision of the extraction and chromatography procedures was evaluated by processing replicate aliquots of drug-free horse plasma and faecal samples containing known amounts of the drugs on different days. Recoveries and coefficients of interassay variations for plasma and faecal extractions are reported in Appendices A-1 and A-8 for the FBZ.SO and FBZ study, respectively and A-17 and A-19 for the OBZ study. The limit of detections of the plasma and faecal assays were 0.005 μg/ml and 0.2 μg/g, respectively for FBZ.SO and FBZ studies, and 0.001 μg/ml and 0.2 μg/g, respectively for the OBZ study.

The concentrations of parent molecules and their metabolites in unknown samples were calculated by reference to plasma samples to which known amounts of drug (and metabolites) had been added and taken through the analytical procedure.

To determine the dry proportion of wet faecal samples, 1.0 g of wet faeces from each sample was weighed exactly into an evaporating bowl and heated in an oven at 70°C for 10 h. The weight of each was determined and the percentage of each dry sample was calculated.

2.1.2.3.6 Pharmacokinetic and statistic analysis of data

The plasma concentration versus time curves obtained after each treatment in individual animals, were fitted with the WinNonlin software program (Scientific Consulting Inc., North Carolina, USA). The value of zero (0.00) shown in the time-plasma concentration tables in Appendices indicates the drug measurement was under the limit of detection. Pharmacokinetic parameters for each animal were analysed using non-compartmental

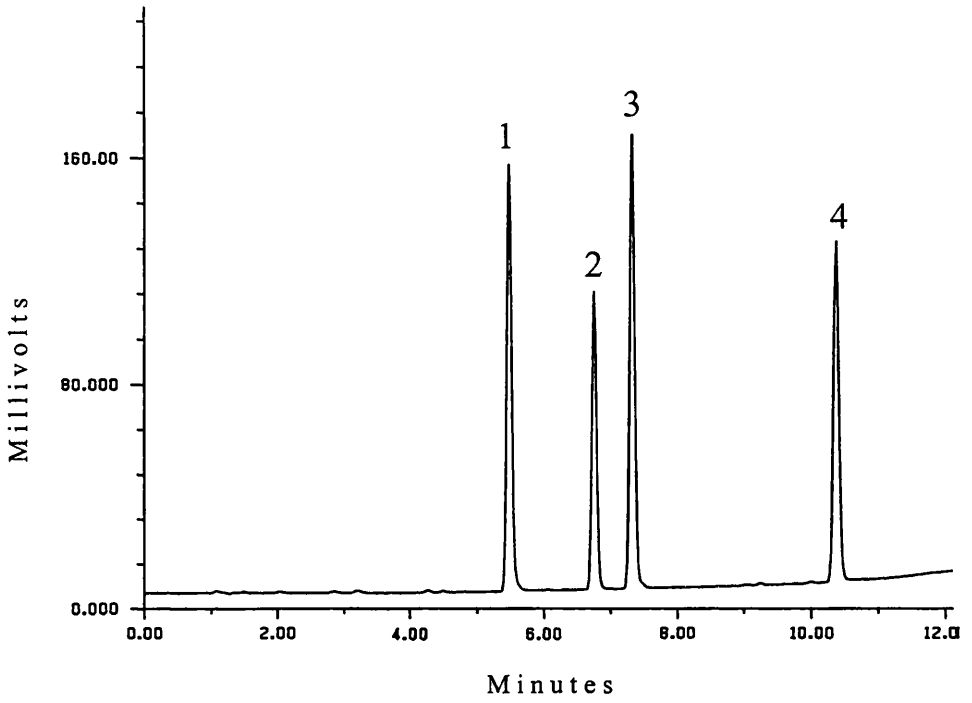


Figure 2-1-2. Typical chromatogram for standard mixtures of oxfendazole (FBZ.SO) (1), hydroxy fenbendazole (OH.FBZ) (2), fenbendazole sulphone ($FBZ.SO_2$) (3) and fenbendazole (FBZ) (4).

model analysis with extravascular input. The C_{max} and time to reach C_{max} (t_{max}) were obtained from the plotted concentration-time curve of each drug in each animal. The linear trapezoidal rule was used to calculate the area under the plasma concentration time curve (AUC):

$$AUC_{0\text{-last}} = \sum_{i=1}^{n} \frac{C_i + C_{i-1}}{2} \times (t_i - t_{i-1})$$

Where C represents the plasma concentration, i-1 and i are adjacent data point times. The area under the first moment curve (AUMC) was calculated using the equation:

$$AUMC_{0\text{-last}} = \sum_{i=2}^{n} \frac{C_i t_i + C_{i-1} t_{i-1}}{2} \times (t_i - t_{i-1})$$

and, the mean residence time (MRT) was calculated as:

$$MRT_{0\text{-last}} = AUMC_{0\text{-last}} / AUC_{0\text{-last}}$$

The pharmacokinetic parameters are reported as mean ± SEM. Individual pharmacokinetic parameters for FBZ.SO, FBZ, their metabolites and OBZ obtained following oral administration to horses were statistically compared by the Mann-Whitney U test. Mean values were considered significantly different at $p < 0.05$.

2.1.2.4 Chiral analysis

Plasma samples of FBZ.SO obtained from this study were extracted in a similar fashion to the procedure outlined previously. The residue was re-suspended with 50 µl DMSO and 150 µl H_2O. Finally, 50 µl of this solution was injected into the chromatographic system.

The enantiomers of FBZ.SO were estimated by using chiral chromatography adapted from that described by Delatour and colleagues (1990b). A mobile phase of acetonitrile:water (7:93) was pumped at a flow rate of 0.9 ml/min through a Chiral-AGP column (5µ, 100x40 mm) (BAS Technical, Cheshire, UK) with ultraviolet detection at 296 nm. Retention times were 7.87 min for the first enantiomer (FBZ.SO-1) and 10.43 min for the second enantiomer (FBZ.SO-2) (Figure 2-1-3).

Recovery of the enantiomers was measured by comparison of the peak areas from spiked plasma with the areas resulting from direct injections of racemate (50:50) standard

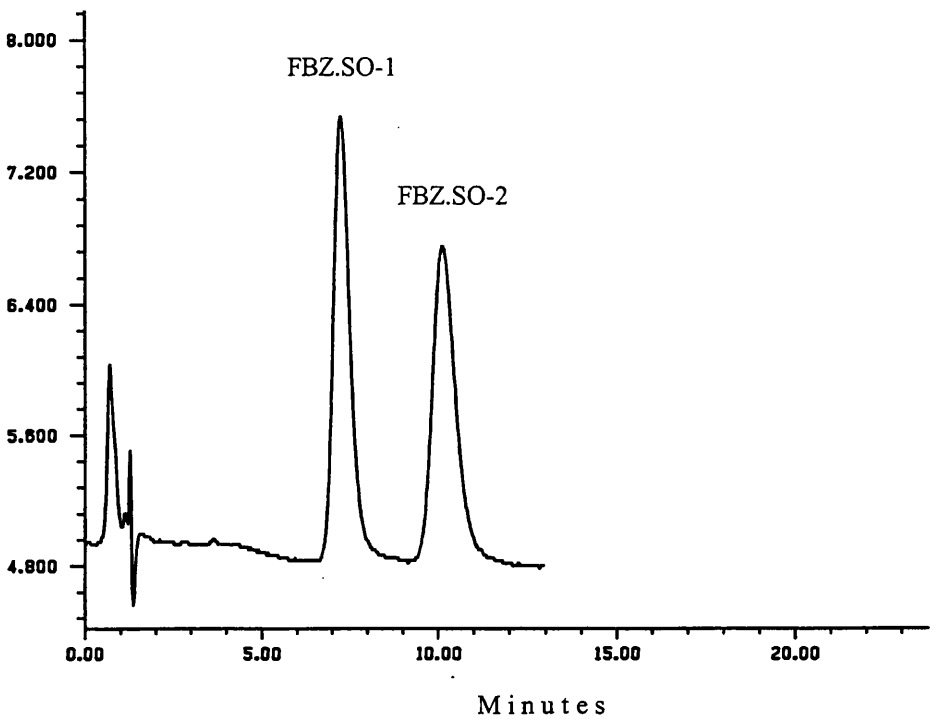


Figure 2-1-3. Typical chromatogram for enantiomers (FBZ.SO-1 and FBZ.SO-2) of oxfendazole (FBZ.SO) as racemate.

solution of FBZ.SO. The limit of detection of the assay was 0.01 μg/ml for both enantiomers. Mean recoveries were 88.35% (inter assay CV=8.0%) for FBZ.SO-1 and 88.60% (inter assay CV=10.3%) for FBZ.SO-2 (Appendix A-21).

2.1.3 Results

Mean plasma concentrations of FBZ.SO and its metabolites, FBZ and FBZ.SO_2 following administration of FBZ.SO (Table 2-1-1, Appendices A-2, A-4, A-6 for individual values, respectively) are plotted in Figure 2-1-4 and their mean plasma pharmacokinetic parameters are shown in Table 2-1-2 (Appendices A-3, A-5, A-7 for individual values). Oxfendazole was detected in plasma between 1 h and 72 h after administration. An unidentified absorbance peak, which achieved larger chromatographic peak than the parent molecule, and sulphide and sulphone metabolites, was detected at 2.77 min, between 1 h and 48 h in the plasma. Double peaks were observed for FBZ.SO at 4 h and 20 h. Maximum plasma concentrations (C_{max}) (0.35 μg/ml- FBZ.SO, 0.09 μg/ml-FBZ, 0.76 μg/ml-FBZ.SO_2) were obtained at (t_{max}) 8.88 h (FBZ.SO), 13.50 h (FBZ) and 12.00 h (FBZ.SO_2). The areas under the curves (AUC) were 4.36 μg.h/ml (FBZ.SO), 1.40 μg.h/ml (FBZ) and 13.00 μg.h/ml (FBZ.SO_2). The AUC ratios of sulphoxide:sulphide:sulphone were approximately 3:1:9 following FBZ.SO administration. Mean dry-faecal concentrations of FBZ.SO and its metabolites (Table 2-1-3, Appendix A-9 for individual values) are plotted in Figure 2-1-5. The highest dry faecal concentrations (1.02 mg/g- FBZ.SO, 0.21 mg/g-FBZ, 0.014 mg/g-FBZ.SO_2) were detected at 24 h for all molecules. In contrast to plasma, the parent molecule predominated and higher concentrations of the sulphide metabolite were detected than that of the sulphone metabolite following FBZ.SO administration in faeces. Mean plasma concentrations of FBZ and its metabolites, FBZ.SO and FBZ.SO_2 (Table 2-1-4, Appendices A-10, A-12, A-14 for individual values, respectively) are plotted in Figure 2-1-6 and their mean plasma pharmacokinetic parameters are shown in Table 2-1-5 (Appendices A-11, A-13, A-15 for individual values). Fenbendazole was detected in plasma between 1 h and 48 h after administration. The hydroxy metabolite was not detected in plasma at any time. Maximum plasma concentration (C_{max}) (0.04 μg/ml-FBZ, 0.01 μg/ml-FBZ.SO, 0.06 μg/ml-FBZ.SO_2) was obtained at (t_{max}) 8.00 h (FBZ), 9.50 h (FBZ.SO) and 10.50 h (FBZ.SO_2). The areas under the curves (AUC) were 0.61 μg.h/ml

Table 2-1-1. Mean (± SEM) plasma concentrations (μg/ml) of oxfendazole (FBZ.SO) and its metabolites, fenbendazole (FBZ) and fenbendazole sulphone ($FBZ.SO_2$) following oral administration of oxfendazole at 10 mg/kg bodyweight in horses.

	Mean ± SEM (n = 8)		
Time (h)	FBZ.SO	FBZ	$FBZ.SO_2$
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	0.10 ± 0.03	0.00 ± 0.00	0.15 ± 0.02
2	0.12 ± 0.02	0.01 ± 0.00	0.29 ± 0.03
4	0.23 ± 0.05	0.02 ± 0.01	0.54 ± 0.09
8	0.20 ± 0.08	0.05 ± 0.01	0.63 ± 0.14
12	0.08 ± 0.02	0.07 ± 0.01	0.41 ± 0.10
20	0.11 ± 0.05	0.06 ± 0.02	0.29 ± 0.05
24	0.10 ± 0.06	0.05 ± 0.02	0.27 ± 0.07
32	0.03 ± 0.02	0.02 ± 0.02	0.14 ± 0.08
48	0.01 ± 0.00	0.00 ± 0.00	0.02 ± 0.01
72	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
96	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
120	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

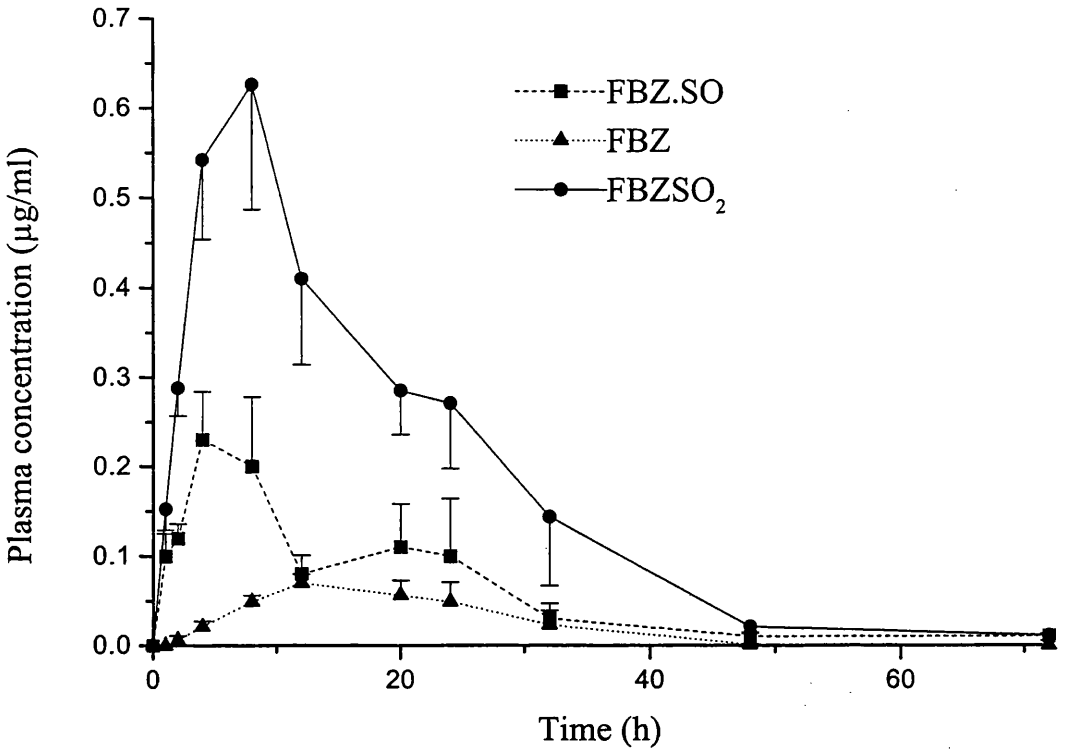


Figure 2-1-4. Mean (± SEM) plasma concentrations (µg/ml) of oxfendazole (FBZ.SO) and its metabolites, fenbendazole (FBZ) and fenbendazole sulphone ($FBZ.SO_2$) following oral administration of oxfendazole at 10 mg/kg bodyweight in horses.

Table 2-1-2. Mean (± SEM) pharmacokinetic parameters of oxfendazole (FBZ.SO) and its metabolites fenbendazole (FBZ) and fenbendazole sulphone ($FBZSO_2$) following oral administration of oxfendazole (10 mg/kg) to horses.

	Mean ± SEM		
Pharmacokinetic parameters	FBZ.SO	FBZ	$FBZ.SO_2$
C_{max} (μg/ml)	0.35 ± 0.07	0.09 ± 0.02	0.76 ± 0.09
t_{max} (h)	8.88 ± 3.02	13.50 ± 1.99	12.00 ± 3.63
AUC_{last} (μg.h/ml)	4.36 ± 0.89	1.40 ± 0.32	13.00 ± 1.55
$AUMC_{last}$ (μg.h^2/ml)	69.07 ± 21.85	23.45 ± 6.98	210.24 ± 45.84
MRT_{last} (h)	14.77 ± 2.32	15.58 ± 1.01	15.45 ± 2.10

C_{max}: peak plasma concentration; t_{max}: time to reach peak plasma concentration; AUC_{last}: area under the (zero moment) curve from time 0 to the last detectable concentration; $AUMC_{last}$: area under the moment curve from time 0 to the last detectable concentration t; MRT_{last}: mean residence time.

Table 2-1-3. Mean (± SEM) dry faecal concentrations (mg/g) of oxfendazole (FBZ.SO) and its metabolites, fenbendazole (FBZ) and fenbendazole sulphone ($FBZ.SO_2$) following oral administration of oxfendazole at 10 mg/kg bodyweight in horses.

	Mean ± SEM (n = 8)		
Time (h)	FBZ.SO	FBZ	$FBZ.SO_2$
0	0.00 ± 0.00	0.00 ± 0.00	0.000 ± 0.000
4	0.00 ± 0.00	0.00 ± 0.00	0.000 ± 0.000
12	0.00 ± 0.00	0.00 ± 0.00	0.000 ± 0.000
20	0.76 ± 0.11	0.14 ± 0.00	0.010 ± 0.001
24	1.02 ± 0.22	0.21 ± 0.05	0.014 ± 0.002
32	0.53 ± 0.07	0.12 ± 0.01	0.009 ± 0.001
48	0.06 ± 0.01	0.03 ± 0.00	0.003 ± 0.001
72	0.00 ± 0.00	0.00 ± 0.00	0.000 ± 0.000
96	0.00 ± 0.00	0.00 ± 0.00	0.000 ± 0.000
120	0.00 ± 0.00	0.00 ± 0.00	0.000 ± 0.000

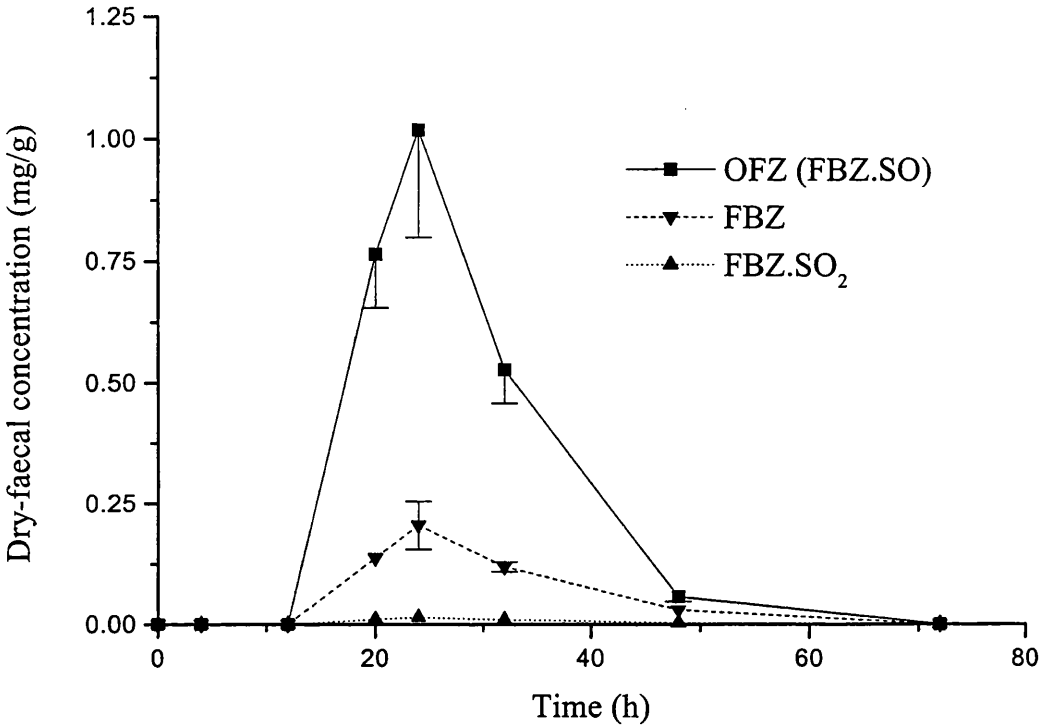


Figure 2-1-5. Mean (± SEM) dry faecal concentrations (mg/g) of oxfendazole (FBZ.SO) and its metabolites, fenbendazole (FBZ) and fenbendazole sulphone ($FBZ.SO_2$) following oral administration of oxfendazole at 10 mg/kg bodyweight in horses.

Table 2-1-4. Mean (± SEM) plasma concentrations (μg/ml) of fenbendazole and its metabolites, fenbendazole sulphoxide (FBZ.SO) and fenbendazole sulphone ($FBZ.SO_2$) following oral administration of fenbendazole at 10 mg/kg bodyweight in horses.

	Mean ± SEM (n = 8)		
Time (h)	FBZ	FBZ.SO	$FBZ.SO_2$
0	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
1	0.009 ± 0.002	0.002 ± 0.001	0.004 ± 0.002
2	0.022 ± 0.006	0.006 ± 0.002	0.021 ± 0.006
4	0.031 ± 0.007	0.008 ± 0.001	0.049 ± 0.007
8	0.023 ± 0.005	0.007 ± 0.001	0.054 ± 0.005
12	0.021 ± 0.005	0.010 ± 0.002	0.037 ± 0.005
20	0.016 ± 0.005	0.004 ± 0.001	0.030 ± 0.005
24	0.013 ± 0.004	0.003 ± 0.001	0.026 ± 0.004
32	0.007 ± 0.003	0.002 ± 0.000	0.015 ± 0.003
48	0.002 ± 0.001	0.000 ± 0.000	0.002 ± 0.001
72	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000
96	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
120	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000

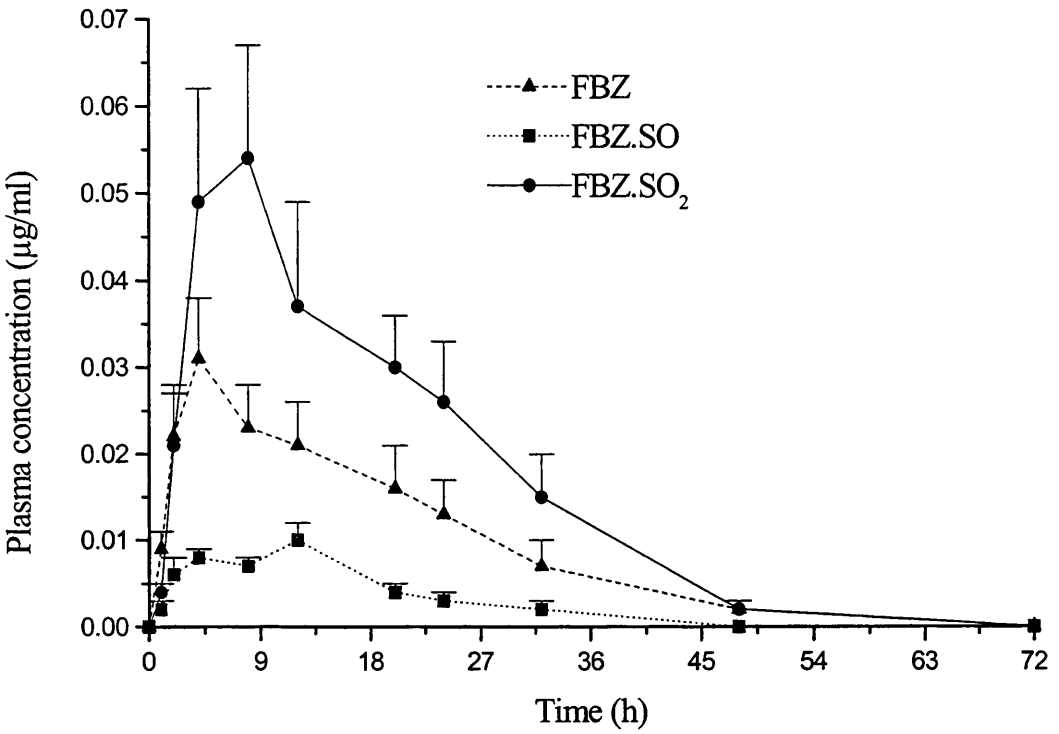


Figure 2-1-6. Mean (± SEM) plasma concentrations (µg/ml) of fenbendazole and its metabolites, fenbendazole sulphoxide (FBZ.SO) and fenbendazole sulphone ($FBZ.SO_2$) following oral administration of fenbendazole at 10 mg/kg bodyweight in horses.

Table 2-1-5. Mean (± SEM) pharmacokinetic parameters of fenbendazole (FBZ) and its metabolites fenbendazole sulphoxide (FBZ.SO) and fenbendazole sulphone ($FBZSO_2$) following oral administration of fenbendazole (10 mg/kg) to 8 horses.

	Mean ± SEM		
Pharmacokinetic parameters	FBZ	FBZ.SO	$FBZ.SO_2$
C_{max} (μg/ml)	0.04 ± 0.01	0.01 ± 0.00	0.06 ± 0.01
t_{max} (h)	8.00 ± 2.70	9.50 ± 3.52	10.50 ± 3.20
AUC_{last} (μg.h/ml)	0.61 ± 0.11	0.17 ± 0.02	1.12 ± 0.19
$AUMC_{last}$ (μg.h^2/ml)	9.33 ± 2.89	2.26 ± 0.46	17.54 ± 2.43
MRT_{last} (h)	14.21 ± 1.74	12.90 ± 1.33	16.50 ± 1.00

C_{max}: peak plasma concentration; t_{max}: time to reach peak plasma concentration; AUC_{last}: area under the (zero moment) curve from time 0 to the last detectable concentration; $AUMC_{last}$: area under the moment curve from time 0 to the last detectable concentration t; MRT_{last}: mean residence time.

(FBZ), 0.17 μg.h/ml (FBZ.SO) and 1.12 μg.h/ml ($FBZ.SO_2$). The AUC ratios of sulphoxide:sulphide:sulphone were approximately 1:4:7 following FBZ administration. Mean dry-faecal concentrations of FBZ and its sulphoxide metabolites (Table 2-1-6, Appendix A-18 for individual values) are plotted on Figure 2-1-7. Fenbendazole was detected between 20 h and 72 h and the sulphoxide metabolite was detected between 20 h and 48 h in faeces after FBZ administration. The highest dry faecal concentration (0.97 mg/g-FBZ, 0.017 mg/g-FBZ.SO) was detected at 24 h for both molecules. No sulphone or hydroxy metabolites were detectable in faeces at any time.

Mean plasma concentrations of OBZ (Table 2-1-7, Appendix A-18 for individual values) are plotted in Figure 2-1-8. An unidentified absorbance peak, which generated larger chromatographic peak than the parent molecule, was detected at 1.87 min, between 1 h and 72 h in the plasma. Oxibendazole was detected only at 0.5, 1 and 2 h in plasma after administration. Maximum plasma concentration (C_{max}) of OBZ (0.008 μg/ml) was obtained at (t_{max}) 0.81 h. Mean dry-faecal concentrations of OBZ (Table 2-1-8, Appendix A-22 for individual values, respectively) are plotted in Figure 2-1-9. Oxibendazole was detected in faeces between 12 h and 72 h after administration. The highest dry faecal concentration (0.53 mg/g) was detected at 24h.

Mean plasma concentrations of enantiomers (FBZ.SO-1 and FBZ.SO-2) of FBZ.SO (Table 2-1-9, Appendices A-24 and A-25 for individual values) are plotted in Figure 2-1-10 and the mean percentages of each enantiomer are shown in Figure 2-1-11. The first enantiomer (FBZ.SO-1) was predominant in six of the eight animals whereas in two animals (animal 2 and 3), the second enantiomer dominated in plasma.

Table 2-1-6. Mean (± SEM) dry faecal concentrations (mg/g) of fenbendazole (FBZ) and its metabolites, fenbendazole sulphoxide (FBZ.SO) and fenbendazole sulphone ($FBZ.SO_2$) following oral administration of fenbendazole at 10 mg/kg bodyweight in horses.

	Mean ± SEM (n = 8)		
Time (h)	FBZ	FBZ.SO	$FBZ.SO_2$
0	0.00 ± 0.00	0.000 ± 0.000	0.00 ± 0.00
4	0.00 ± 0.00	0.000 ± 0.000	0.00 ± 0.00
12	0.00 ± 0.00	0.000 ± 0.000	0.00 ± 0.00
20	0.59 ± 0.21	0.004 ± 0.000	0.00 ± 0.00
24	0.97 ± 0.09	0.017 ± 0.005	0.00 ± 0.00
32	0.89 ± 0.20	0.014 ± 0.002	0.00 ± 0.00
48	0.20 ± 0.06	0.004 ± 0.002	0.00 ± 0.00
72	0.01 ± 0.00	0.000 ± 0.000	0.00 ± 0.00
96	0.00 ± 0.00	0.000 ± 0.000	0.00 ± 0.00
120	0.00 ± 0.00	0.000 ± 0.000	0.00 ± 0.00

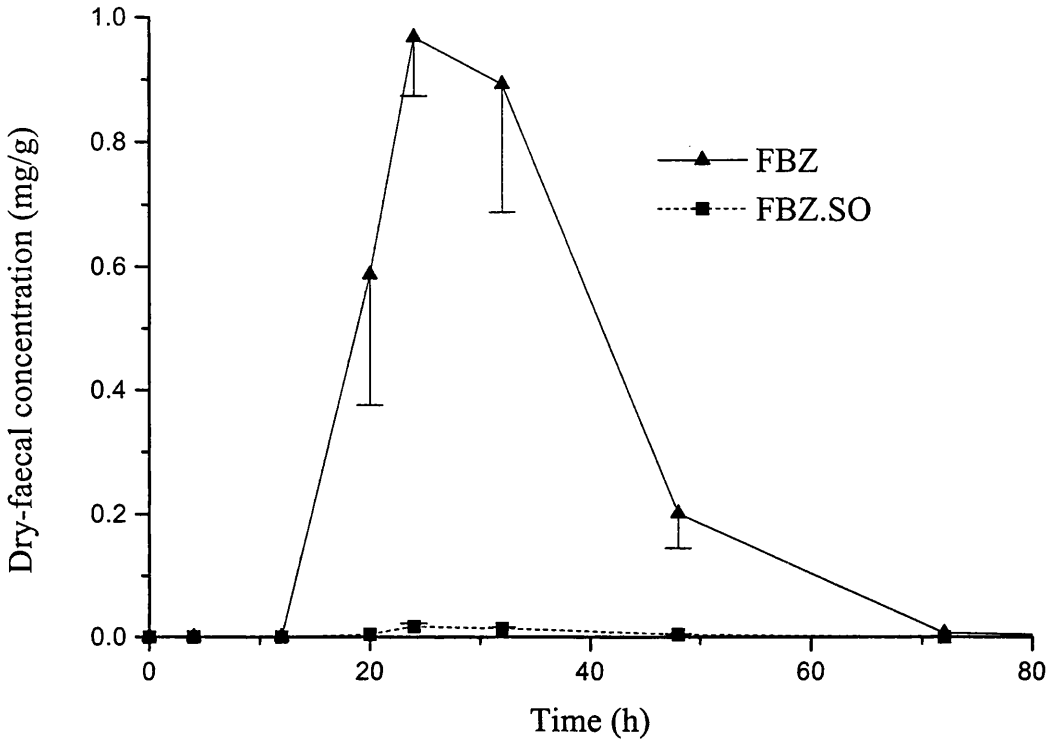


Figure 2-1-7. Mean (± SEM) dry faecal concentrations (mg/g) of fenbendazole (FBZ) and its metabolites, fenbendazole sulphoxide (FBZ.SO) and fenbendazole sulphone ($FBZ.SO_2$) following oral administration of fenbendazole at 10 mg/kg in horses (n=8).

Table 2-1-7. Mean (± SEM) plasma concentrations (μg/ml) of oxibendazole (OBZ) following oral administration to horses at 10 mg/kg bodyweight (n=8).

	Mean ± SEM
Time (h)	OBZ
0	0.000 ± 0.000
0.5	0.005 ± 0.000
1	0.006 ± 0.003
2	0.000 ± 0.000
4	0.000 ± 0.000
6	0.000 ± 0.000
8	0.000 ± 0.000
12	0.000 ± 0.000
16	0.000 ± 0.000
24	0.000 ± 0.000
32	0.000 ± 0.000
48	0.000 ± 0.000
72	0.000 ± 0.000
96	0.000 ± 0.000
120	0.000 ± 0.000

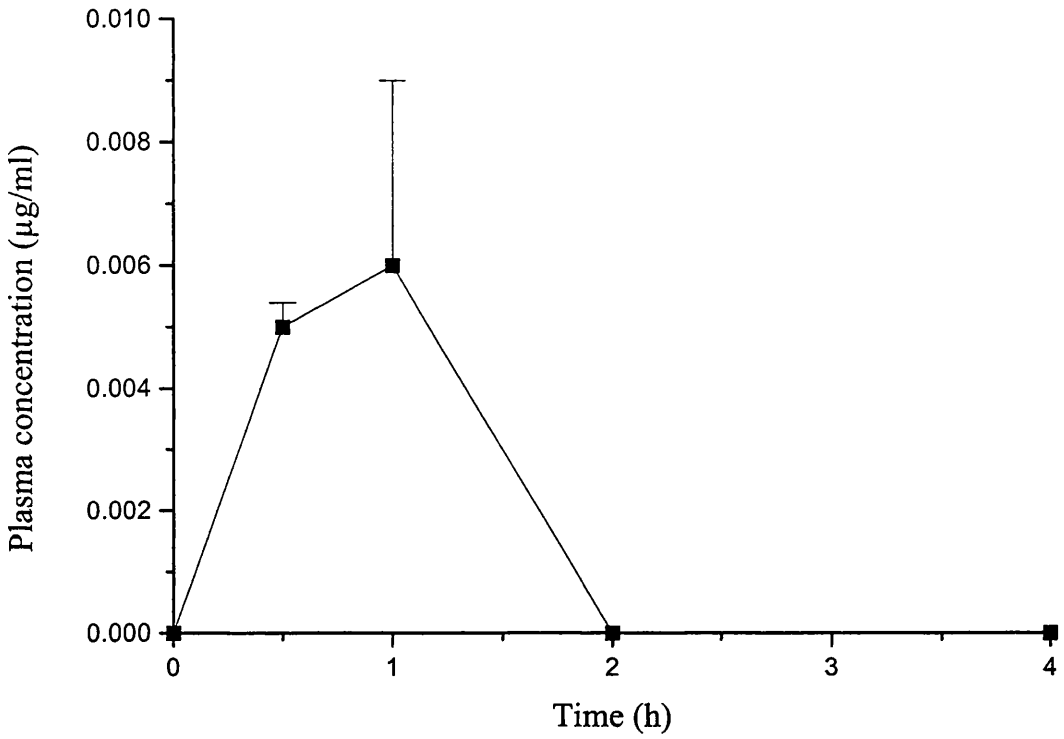


Figure 2-1-8. Mean (± SEM) plasma concentrations (µg/ml) of oxibendazole (OBZ) following oral administration of oxibendazole at 10 mg/kg bodyweight in horses (n=8).

Table 2-1-8. Mean (± SEM) dry faecal concentrations (mg/g) of oxibendazole (OBZ) following oral administration of oxibendazole at 10 mg/kg bodyweight in horses (n=8).

	Mean ± SEM
Time (h)	OBZ
0	0.00 ± 0.00
4	0.00 ± 0.00
6	0.00 ± 0.00
8	0.00 ± 0.00
12	0.02 ± 0.00
16	0.20 ± 0.06
24	0.53 ± 0.15
32	0.34 ± 0.10
48	0.03 ± 0.01
72	0.01 ± 0.01
96	0.00 ± 0.00
120	0.00 ± 0.00

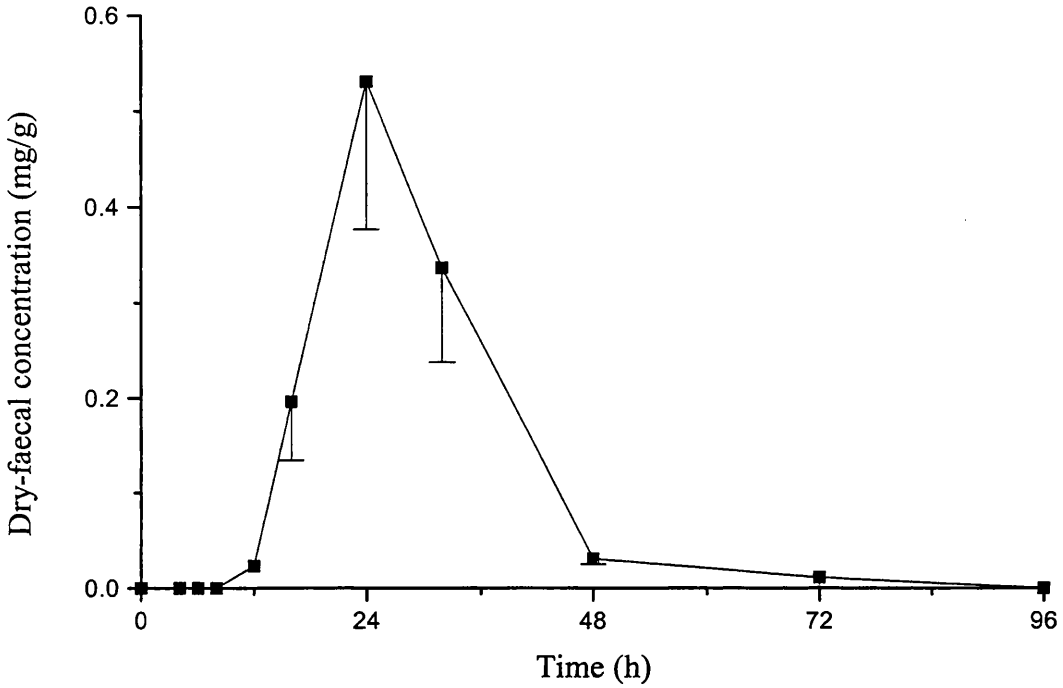


Figure 2-1-9. Mean (± SEM) dry-faecal concentrations (mg/g) of oxibendazole (OBZ) following oral administration of oxibendazole at 10 mg/kg bodyweight in horses (n=8).

Table 2-1-9. Mean (± SEM) plasma concentration (μg/ml) of enantiomers (FBZ.SO-1 and FBZ.SO-2) of oxfendazole (FBZ.SO) following oral administration in horses.

	Mean ± SEM (n=8)	
Time (h)	FBZ.SO-1	FBZ.SO-2
0	0.000 ± 0.000	0.000 ± 0.000
1	0.032 ± 0.011	0.036 ± 0.009
2	0.054 ± 0.015	0.052 ± 0.009
4	0.068 ± 0.012	0.073 ± 0.018
8	0.085 ± 0.031	0.057 ± 0.021
12	0.040 ± 0.009	0.028 ± 0.014
20	0.041 ± 0.019	0.043 ± 0.027
24	0.043 ± 0.030	0.029 ± 0.017
32	0.007 ± 0.007	0.002 ± 0.002
48	0.000 ± 0.000	0.000 ± 0.000
72	0.000 ± 0.000	0.000 ± 0.000
96	0.000 ± 0.000	0.000 ± 0.000
120	0.000 ± 0.000	0.000 ± 0.000

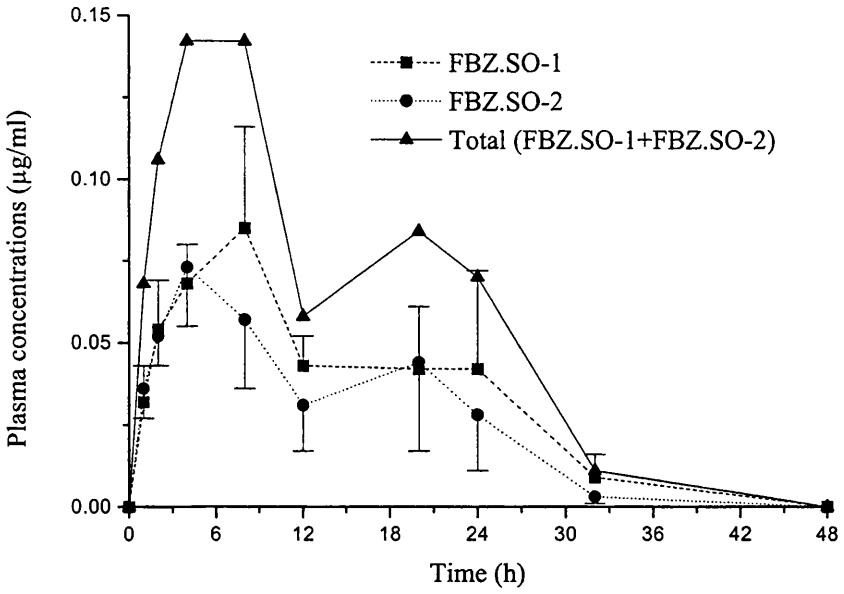


Figure 2-1-10. Mean (± SEM) plasma concentrations (μg/ml) of enantiomers (FBZ.SO-1 and FBZ.SO-2) of oxfendazole (FBZ.SO) in horses following oral administration of oxfendazole (10 mg/kg).

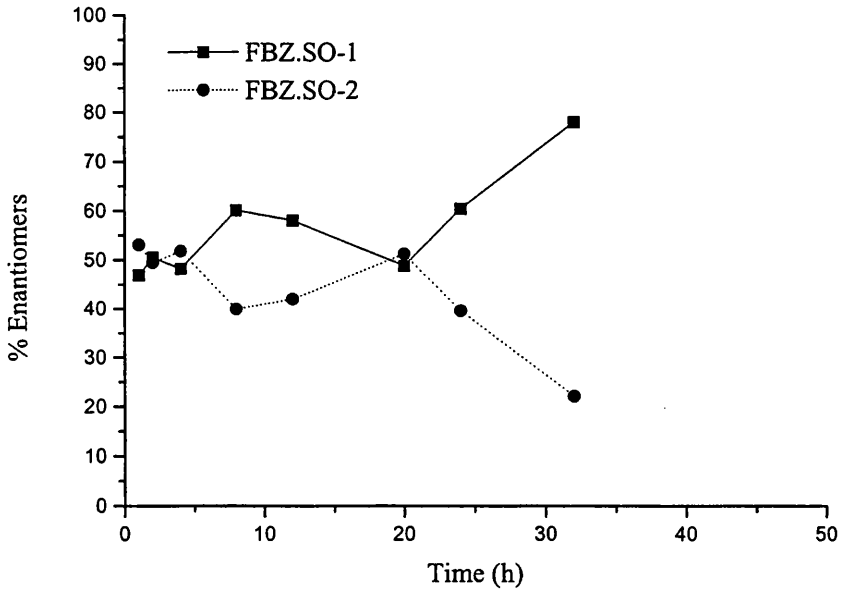


Figure 2-1-11. Ratio of the percentage of enantiomers (FBZ.SO-1 and FBZ.SO-2) (μg/ml) in horses following oral administration of oxfendazole (10 mg/kg).

2.1.4. Discussion

Oxfendazole (FBZ.SO) and FBZ are both known to be anthelmintically active and metabolically interconvertible. The results reported here indicate that the plasma concentrations of FBZ.SO and FBZ were relatively low and that they are extensively metabolised to their sulphone metabolite at the same oral dose rate (10 mg/kg). These results support the work of Marriner and Bogan (1985) who reported that in horses, the bioavailability and residence time of FBZ.SO and FBZ were lower and shorter, respectively than in ruminants (Marriner and Bogan, 1981a, b). The AUCs for FBZ.SO following its oral administration at dose rate of 10 mg/kg in sheep and goats were 49.6 and 19.9 μg.h/ml, respectively (Bogan *et al.*, 1987), whereas in the present study, the AUC of FBZ.SO at the same oral dose rate was 4.36 μg.h/ml in horse. It is also apparent that the sulphone metabolite ($FBZ.SO_2$) was predominant in plasma following administration of either FBZ.SO or FBZ administration whereas in ruminants FBZ.SO was predominant after either FBZ.SO (Ngomuo *et al.*, 1984) or FBZ administration (Benchaoui and McKellar, 1996). It is probable that horses metabolise sulphide and sulphoxide benzimidazoles to their sulphone metabolites more quickly than ruminants since the large AUC of the sulphone in the horse is not associated with a substantially increased MRT. In goats, the MRT of $FBZ.SO_2$ was 34.4 h after FBZ administration at 7.5 mg/kg (Benchaoui and McKellar, 1996) whereas in this study, MRT was 16.50 h for the sulphone metabolite following FBZ administration at 10 mg/kg in horse. Relatively different enzyme contributions could explain the faster sulphonation in horses. The FMO system, which is responsible for sulphoxidation, predominates in ruminants in comparison to monogastrics in which the FMO and MFO systems act equally (Delatour *et al*, 1994). The sulphone metabolite has relatively little anthelmintic activity and this may contribute to its relatively poor efficacy.

The low plasma concentrations of active moieties (FBZ, FBZ.SO) following FBZ administration probably accounts for the higher and repeated dosage required for the treatment of migrating larval and tissue stages of strongyles and lung worms (Marriner and Bogan, 1981). In the present study, the AUC of FBZ.SO (4.36 μg.h/ml) was significantly larger than that of FBZ (0.61μg.h/ml) following their oral administration at the same dose rate. This reflects the better absorption and greater systemic availability of FBZ.SO compared to FBZ since each compound produces the same metabolites and

these would be expected to have similar metabolic and excretory rates. It is of interest that the AUC of the sulphide moiety was 2.3 times greater in horses administered FBZ.SO than in horses given FBZ as the parent molecule. Much higher solubility of FBZ.SO (3.01 mg/l) than that of FBZ (0.07 mg/l) (Marriner and Bogan, 1985) may explain its greater absorption at a rate exceeding its oxidative clearance and thus provide sufficient substrate for reductive metabolism to the sulphide in horses. The limited adsorption rate of FBZ may be matched by rapid oxidative metabolism with consequent low concentrations achieved in plasma following its oral administration in the present study.

It is probable that faster gut transit time in horses (between 25.9 h and 37.9 h - Wolter *et al.*, 1974) than in ruminants (between 30 and 80 h - Warner, 1981; McDonald *et al.*, 1995) contributes to the shorter absorption time and lower plasma concentration of parent molecules after oral administration. In the present study, the 12 h delay in appearance of benzimidazoles in faeces and the time for the maximum faecal concentrations (24 h) reflect the gut transit time following oral administration. The parent drugs were predominant in faecal samples although substantial concentrations (>0.1 mg/g) of the sulphide (FBZ) were detected following oral administration of FBZ.SO. In sheep and cattle, it was reported that the reduction of FBZ.SO to sulphide (FBZ) occurred in ruminal fluid (Beretta *et al*, 1987). The reductive environment of the gastrointestinal tract of the horse could be responsible for metabolic reduction and this could be the source of sulphide metabolites in plasma since no sulphide metabolite was observed after FBZ.SO incubation in the *in vitro* studies with horse liver microsomes reported in section 3. Very low concentrations of sulphone were detected in faeces following FBZ.SO administration, possibly as a consequence of redistribution from the plasma compartment into the gut.

The unidentified absorbance peak detected between 1 h and 48 h after FBZ.SO treatment could be the hydroxy metabolite of FBZ.SO. It was reported that in sheep, hydroxy oxfendazole was the major biliary metabolite after intraruminal administration of FBZ (5 mg/kg) but, this metabolite was not observed in plasma samples (Hennessy *et al*, 1993). The double peaks were observed in the plasma concentration-time profiles following FBZ.SO administration (Figure 2-1-4) and this was also confirmed by chiral analysis (Figure 2-1-11). Some of the administered drug may have been absorbed directly from

the small intestines, but the bound drug may have been absorbed at the later stage in the colon and caecum as it was being released by fermentative digestion.

Plasma levels of the parent drug were very low following oral administration of OBZ in horses. Gottschall and Wang (1996) reported that in swine, OBZ was quickly metabolised and liver was the only tissue which contained significant residues following oral administration at 15 mg/kg. The unidentified absorbance peak detected between 1 h and 72 h in the plasma could be the hydroxy- metabolite of OBZ since 5-hydroxy- and 6-hydroxy-oxibendazole were identified in swine urine and tissues following oral administration (Gottschall and Wang, 1996) and their anthelmintic activities are unclear. It is likely that the high first-pass metabolism decreased OBZ bioavailability in horses.

The chiral analysis indicated that FBZ.SO displayed inter-individual variation in the enantiospecific disposition after its oral administration. In six of the eight animals, the first enantiomer was predominant in the plasma and these findings support the results described in the *in vivo* study in section 2 and in the *in vitro* study in section 3. However in two animals (animal 2 and 3 in Appendices A-22 and A-23) the second enantiomer was predominant. The reasons for this are unclear, but some pathological changes in the liver could affect the activities of the enzyme systems that are responsible for the different enantiospecific disposition of FBZ.SO. However, all horses were clinically healthy and had normal haematological profiles.

In conclusion, this study showed that the bioavailability of FBZ.SO was significantly higher than that of FBZ and that the plasma concentrations of OBZ were very low following their oral administration at the same dose rate (10 mg/kg) in horses. High intestinal concentrations could be effective against gastrointestinal nematodes that inhabit the gut lumen, but very low plasma concentrations of FBZ and OBZ may not be effective against the for migrating fourth-larval stages of large strongyles, lungworms. Repeated dosage regimes of FBZ and OBZ or co-administration with metabolic inhibitors could be utilised to migrating larval, tissue stages of strongyles and lungworms.

Section 2

Effect of piperonyl butoxide on the pharmacokinetics and chirality of oxfendazole in ponies: *in vivo* studies

2.2.1. Introduction

Oxfendazole, a member of the benzimidazole group of anthelmintics, is used worldwide for the treatment of gastrointestinal parasites in horses.

Piperonyl butoxide, which belongs to a group of chemicals known as methylenedioxyphenyl compounds or benzodioxole compounds, has been extremely widely used in medicine and animal and crop production for many decades with extremely low toxicity (Breathnach, 1998). Piperonyl butoxide was well-adsorbed following oral administration and 65% and 73% of the dose was excreted in the urine of mice and rats, respectively (Casida *et al.*, 1966; Kamienski and Casida, 1970). It was shown that piperonyl butoxide inhibited the microsomal oxidation of xenobiotics in a number of mammalian species whether the piperonyl butoxide was administered *in vivo* prior to the preparation of the microsomes or if both substrate and inhibitor were administered together *in vitro* (Hodgson and Philpot, 1974). Early studies demonstrated that the inhibition of microsomal oxidations associated with piperonyl butoxide was due to a direct effect on the P450 enzyme system (Perry and Bucknor, 1970) and that the apparent loss of P450 activity was due to the formation of a metabolite-inhibitory complex, which blocked CO binding to the cytochrome (Philpot and Hodgson, 1971). More recently, it was demonstrated clearly that the major route of metabolism of piperonyl butoxide involved the opening of the methylenedioxy ring followed by loss of the methylene group into the endogenous metabolic pool (Cockburn and Needham, 1998). This is also believed to be the basis of the initial inhibition of the cytochrome P450 enzyme system, which is essential for the compound's efficacy as a synergist. Current evidence suggests that it is the carbene intermediate formed with the methylene group that complex with the Fe^{++} ion of cytochrome P450 (Wilkinson *et al.*, 1984; Ortiz de Montellano and Reich, 1986) that induces its effect. Isozyme specificity of piperonyl butoxide has been shown in rats using different probe drugs for hepatic drug-metabolising activity (Bachmann, 1989). While the clearance of antipyrine, used as a probe for the cytochrome P450 IIB1 and P450 IIB2 isoforms, was decreased by 50%

following pre-treatment with piperonyl butoxide, the disposition of quanidine, expressing the activity of the cytochrome P450 IIIA subfamily, was unaffected by this inhibitor.

Piperonyl butoxide also induces of xenobiotic P450 metabolising enzymes. Recent studies concerned primarily with the isozyme specificity of the induction process have reported that piperonyl butoxide not only induced P450 2B10, but also induced P450 1A2 in mice by an Ah-receptor-independent mechanism and at high doses, P450 1A1 by an Ah-receptor-dependent mechanism (Adams *et al.*, 1993a, b). Several studies using fish have shown an increase in P450 activities after treatment with piperonyl butoxide (Vodicnik *et al.*, 1981; Erickson *et al.*, 1988). However, the identity of the most part of the P450 species induced by piperonyl butoxide has not been defined clearly (Hodgson and Levi, 1998).

The present study was carried out to investigate the effect of piperonyl butoxide as a metabolic inhibitor on the pharmacokinetics and chiral disposition of oxfendazole (FBZ.SO) in horses.

2.2.2. Materials and methods

2.2.2.1 Animals

Six ponies weighing 164-250 kg were used for this study. They were kept indoors (University of Glasgow, Veterinary School, Cochno Research Farm) and hay and water were provided *ad libitum* throughout the experimental period. They were photographed and the photographs used to distinguish them from each other.

2.2.2.2 Experimental design

The ponies were randomly allocated into two groups and each group consisted of three animals. Oxfendazole and piperonyl butoxide were administered according to a two-phase crossover design protocol. In phase I, group 1 received FBZ.SO alone while group 2 received FBZ.SO and piperonyl butoxide. In phase II, group 1 received FBZ.SO and piperonyl butoxide and group 2 received FBZ.SO alone. A four-week washout period was allowed between the two phases.

2.2.2.3 Drug administration and sampling procedure

Oxfendazole (Syntex, 99.9%) (FBZ.SO) was prepared in dimethylsulphoxide (DMSO) (500 mg/ml) for intravenous injection. This solution was given at a dose rate of 10 mg/kg bodyweight by right jugular venipuncture. Piperonyl butoxide (Aldrich Chemicals, 90%) was administered by nasogastric intubations at a dose rate of 31 mg/kg bodyweight, 30 minutes prior to FBZ.SO administration. Heparinized blood samples were collected by jugular venipuncture prior to drug administration and 10, 20, 30, 45, 60, 75, 90, 105 min and 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24, 30, 36, 48, 54, 72, 96 h thereafter. Blood samples were centrifuged at 1825 g for 30 min and plasma was transferred to plastic tubes. The samples were stored at -20°C until estimation of drug concentration.

2.2.2.4 Drug analysis

Oxfendazole and its sulphide and sulphone metabolites were analysed according to the methods described in section 1. The plasma concentrations of piperonyl butoxide were not measured in this study.

2.2.2.5 Chiral analysis

Plasma samples obtained from this study were extracted and analysed in a similar fashion to the procedure outlined in section 2.1.2.4.

2.2.3. Results

The plasma concentrations of FBZ.SO (Table 2-2-1 for mean values and Appendices B-2 and B-3 for individual values), FBZ (Table 2-2-2 for mean values and Appendices B-4 and B-5 for individual values) and $FBZ.SO_2$ (Table 2-2-3 for mean values and Appendices B-6 and B-7 for individual values) are plotted in Figures 2-2-1, 2-2-2 and 2-2-3 following administration of FBZ.SO alone or in combination with piperonyl butoxide. The mean pharmacokinetic parameters (t_{max}, C_{max} and AUC) are presented in Table 2-2-4.

The plasma profile of FBZ.SO alone or in combination with piperonyl butoxide was atypical for iv administration, with an early increase in plasma drug concentration such that zero time did not represent maximum concentration. After an initial short decline phase (a similar initial short decline phase was observed for its sulphide metabolite

Table 2-2-1. Mean (± SEM) plasma concentrations (μg/ml) of oxfendazole (FBZ.SO) in ponies following iv administration of oxfendazole (10 mg/kg) either alone or in combination with piperonyl butoxide (PB) (31 mg/kg).

	Mean ± SEM (n=6)	
Time	FBZ.SO alone	FBZ.SO with PB
10 min	1.31 ± 0.14	1.51 ± 0.11
20 min	1.19 ± 0.07	1.33 ± 0.08
30 min	1.14 ± 0.08	1.20 ± 0.02
45 min	0.98 ± 0.07	1.43 ± 0.12
60 min	1.04 ± 0.04	1.53 ± 0.10
75 min	1.01 ± 0.10	1.75 ± 0.10
90 min	0.95 ± 0.10	1.77 ± 0.16
105 min	1.03 ± 0.11	1.82 ± 0.16
120 min	1.08 ± 0.10	2.01 ± 0.14
2.5 h	1.04 ± 0.08	2.36 ± 0.18
3 h	1.12 ± 0.08	2.67 ± 0.13
3.5 h	1.15 ± 0.07	2.88 ± 0.26
4 h	1.22 ± 0.08	3.07 ± 0.29
5 h	1.00 ± 0.13	3.19 ± 0.41
6 h	1.07 ± 0.11	3.33 ± 0.38
7 h	1.03 ± 0.10	3.69 ± 0.50
8 h	1.50 ± 0.31	2.98 ± 0.44
10 h	1.08 ± 0.08	2.72 ± 0.32
12 h	1.18 ± 0.21	1.81 ± 0.39
16 h	0.86 ± 0.10	1.58 ± 0.29
20 h	0.65 ± 0.08	0.87 ± 0.25
24 h	0.44 ± 0.07	0.52 ± 0.12
30 h	0.32 ± 0.10	0.26 ± 0.04
34 h	0.20 ± 0.06	0.14 ± 0.02
48 h	0.17 ± 0.04	0.09 ± 0.01
54 h	0.14 ± 0.03	0.07 ± 0.01
72 h	0.11 ± 0.04	0.04 ± 0.01
96 h	0.06 ± 0.03	0.03 ± 0.00

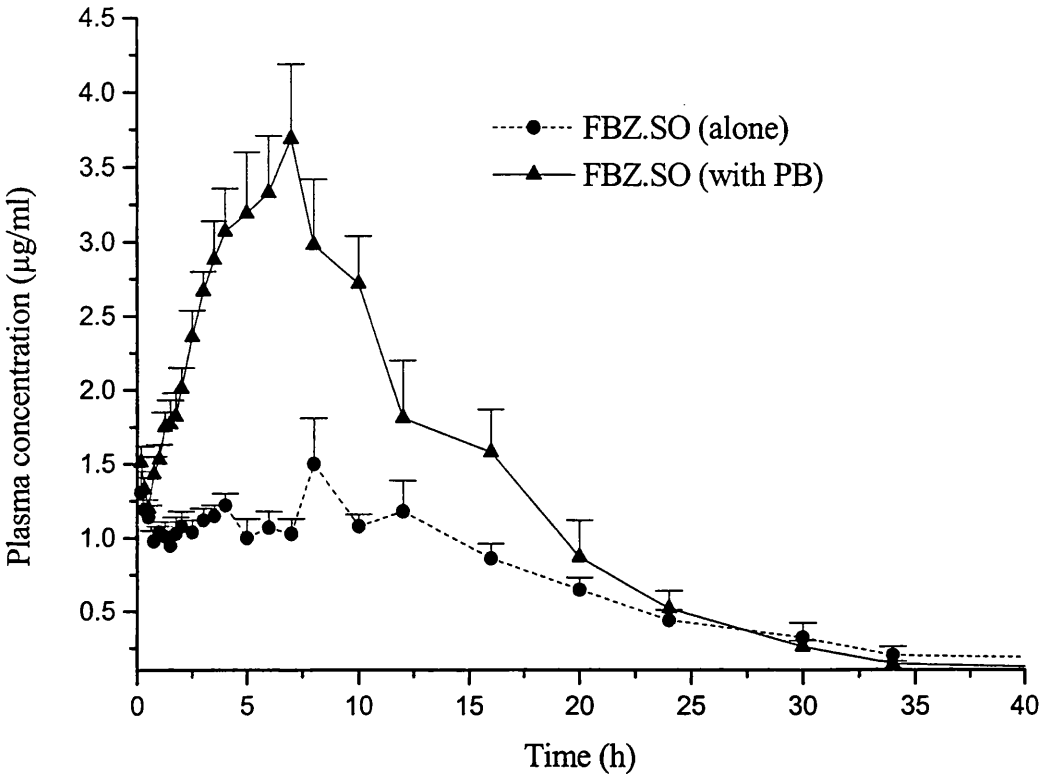


Figure 2-2-1. Mean (± SEM) plasma concentrations (µg/ml) of oxfendazole (FBZ.SO) following administration of oxfendazole alone or with piperonyl butoxide (PB) in ponies (n=6).

Table 2-2-2. Mean (± SEM) plasma concentrations (μg/ml) of fenbendazole (FBZ) in ponies following iv administration of oxfendazole (FBZ.SO) (10 mg/kg) either alone or in combination with piperonyl butoxide (PB) (31 mg/kg).

	Mean ± SEM (n=6)	
Time	FBZ.SO alone	FBZ.SO with PB
10 min	0.012 ± 0.004	0.027 ± 0.008
20 min	0.011 ± 0.006	0.022 ± 0.008
30 min	0.017 ± 0.004	0.020 ± 0.004
45 min	0.010 ± 0.004	0.020 ± 0.005
60 min	0.009 ± 0.003	0.013 ± 0.004
75 min	0.007 ± 0.004	0.009 ± 0.003
90 min	0.003 ± 0.002	0.017 ± 0.007
105 min	0.000 ± 0.000	0.012 ± 0.003
120 min	0.005 ± 0.005	0.017 ± 0.004
2.5 h	0.002 ± 0.002	0.015 ± 0.003
3 h	0.006 ± 0.006	0.018 ± 0.004
3.5 h	0.017 ± 0.011	0.020 ± 0.003
4 h	0.007 ± 0.003	0.025 ± 0.005
5 h	0.010 ± 0.007	0.032 ± 0.004
6 h	0.020 ± 0.006	0.044 ± 0.005
7 h	0.026 ± 0.012	0.054 ± 0.006
8 h	0.030 ± 0.007	0.056 ± 0.009
10 h	0.033 ± 0.004	0.076 ± 0.008
12 h	0.032 ± 0.008	0.074 ± 0.009
16 h	0.038 ± 0.009	0.100 ± 0.010
20 h	0.030 ± 0.007	0.079 ± 0.010
24 h	0.034 ± 0.013	0.064 ± 0.007
30 h	0.027 ± 0.010	0.066 ± 0.013
34 h	0.020 ± 0.013	0.038 ± 0.011
48 h	0.004 ± 0.004	0.018 ± 0.012
54 h	0.000 ± 0.000	0.005 ± 0.002
72 h	0.000 ± 0.000	0.000 ± 0.000
96 h	0.000 ± 0.000	0.000 ± 0.000

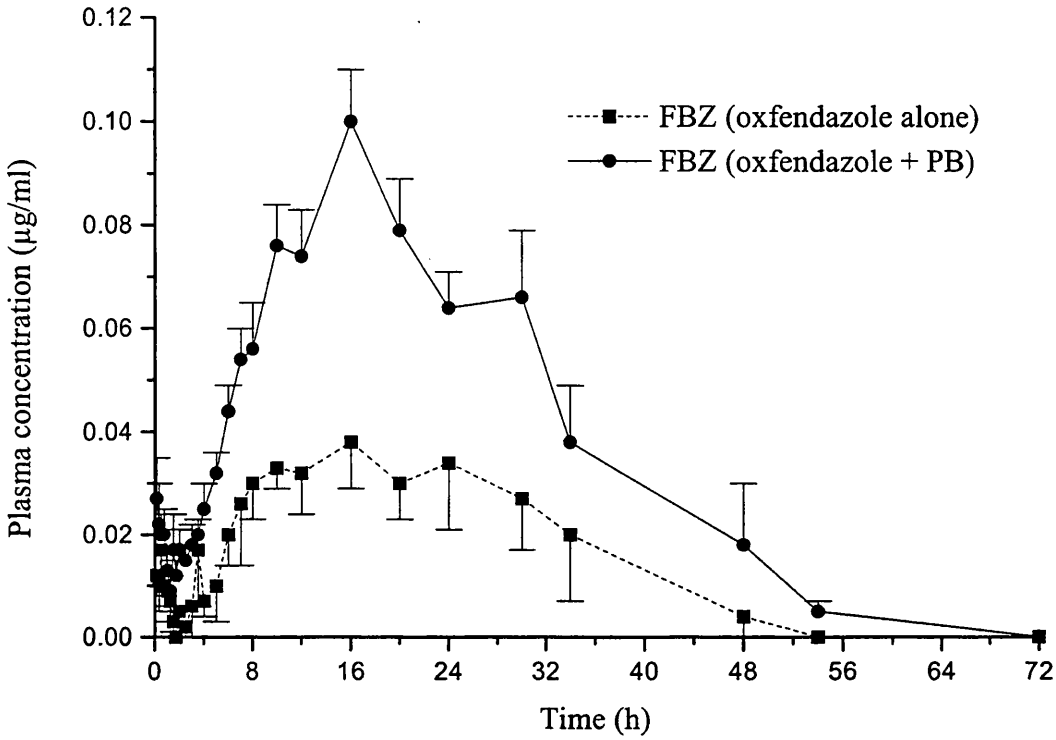


Figure 2-2-2. Mean (± SEM) plasma concentrations (µg/ml) of fenbendazole (FBZ) (mean ± SEM) following administration of oxfendazole (FBZ.SO) alone or with piperonyl butoxide (PB) in ponies (n=6).

Table 2-2-3. Mean (± SEM) plasma concentrations (μg/ml) of fenbendazole sulphone ($FBZ.SO_2$) in ponies following iv administration of oxfendazole (10 mg/kg) either alone or in combination with piperonyl butoxide (PB) (31 mg/kg).

	Mean ± SEM (n=6)	
Time	FBZ.SO alone	FBZ.SO with PB
10 min	0.25 ± 0.03	0.23 ± 0.02
20 min	0.33 ± 0.01	0.29 ± 0.04
30 min	0.41 ± 0.04	0.30 ± 0.03
45 min	0.44 ± 0.03	0.39 ± 0.04
60 min	0.55 ± 0.03	0.42 ± 0.05
75 min	0.59 ± 0.05	0.47 ± 0.06
90 min	0.62 ± 0.05	0.45 ± 0.06
105 min	0.59 ± 0.14	0.48 ± 0.09
120 min	0.68 ± 0.15	0.46 ± 0.06
2.5 h	0.84 ± 0.07	0.52 ± 0.06
3 h	1.01 ± 0.05	0.57 ± 0.06
3.5 h	1.05 ± 0.07	0.62 ± 0.12
4 h	1.18 ± 0.07	0.65 ± 0.10
5 h	1.09 ± 0.14	0.64 ± 0.11
6 h	1.28 ± 0.14	0.75 ± 0.11
7 h	1.28 ± 0.14	0.82 ± 0.11
8 h	1.47 ± 0.19	0.92 ± 0.14
10 h	1.49 ± 0.12	0.84 ± 0.09
12 h	1.36 ± 0.12	0.90 ± 0.15
16 h	1.42 ± 0.14	1.40 ± 0.13
20 h	1.23 ± 0.15	1.35 ± 0.23
24 h	0.84 ± 0.18	1.34 ± 0.18
30 h	0.67 ± 0.18	1.14 ± 0.17
34 h	0.55 ± 0.12	0.73 ± 0.13
48 h	0.28 ± 0.06	0.27 ± 0.05
54 h	0.21 ± 0.03	0.17 ± 0.03
72 h	0.08 ± 0.01	0.07 ± 0.01
96 h	0.04 ± 0.01	0.04 ± 0.01

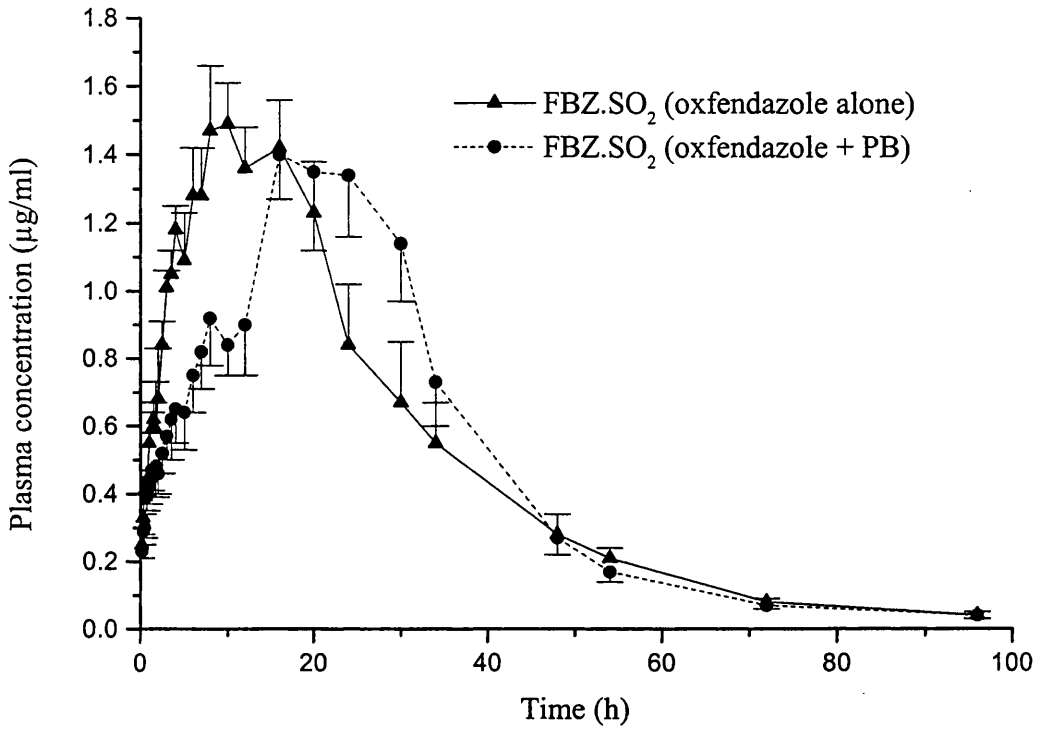


Figure 2-2-3. Mean (± SEM) plasma concentrations (µg/ml) of fenbendazole sulphone ($FBZ.SO_2$) following administration of oxfendazole (FBZ.SO) alone or with piperonyl butoxide (PB) in ponies (n=6).

Table 2-2-4. Mean (±SEM) pharmacokinetic parameters of oxfendazole (FBZ.SO) and its metabolites fenbendazole (FBZ) and fenbendazole sulphone ($FBZ.SO_2$) following iv administration of oxfendazole (10 mg/kg) alone or in combination with piperonyl butoxide (PB) (31 mg/kg) to ponies (n=6).

	Mean (± SEM)					
	FBZ.SO		FBZ		$FBZ.SO_2$	
Parameters	- PB	+ PB	- PB	+ PB	- PB	+ PB
t_{max} (h)	2.35±1.28*	5.92±0.88	14.56±3.85	21.33±2.82	11.33±1.53*	20.00±1.47
C_{max} (µg/ml)	1.72±0.26*	3.92±0.40	0.06±0.01*	0.11±0.01	1.65±0.13	1.59±0.14
AUC_{last} (µg.h/ml)	33.97±3.29*	53.17±6.51	1.03±0.28*	2.50±0.30	47.00±4.56	46.94±6.02

* $P < 0.05$; -PB significantly different from +PB.

t_{max}: time to reach peak plasma concentration; AUC_{last}: area under the (zero moment) curve from time 0 to the last detectable concentration.

[FBZ]) lasting approximately 45 minutes, the concentration of FBZ.SO plateaued at between 0.94 and 1.5 μg/ml until approximately 12 h from which time concentration declined to the limit of detection by 96 h following FBZ.SO administration alone. When FBZ.SO was administered with piperonyl butoxide, the concentration of FBZ.SO declined after administration for 30 minutes then increased until 7 h by which time the plasma concentration was 3.69 ± 0.50 μg/ml. Subsequently, plasma concentrations decreased until 96 h, by which time they were lower than concentrations following administration of FBZ.SO alone. The C_{max} and AUC of FBZ.SO (1.72 ± 0.26 μg/ml and 33.97 ± 3.39 μg.h/ml, respectively) were significantly smaller following administration of FBZ.SO alone than after administration of FBZ.SO in combination with piperonyl butoxide (3.92 ± 0.40 μg/ml and 53.17 ± 6.51 μg.h/ml, respectively).

The concentrations of FBZ following FBZ.SO alone or in combination with piperonyl butoxide displayed a similar pattern to those of the parent molecule except that maximum concentrations were achieved earlier (mean, 15 h) compared to those of FBZ.SO (21 h). The C_{max} and AUC of FBZ (0.06 ± 0.01 μg/ml and 1.03 ± 0.28 μg.h/ml, respectively) were significantly lower following administration of FBZ.SO alone than those of FBZ following administration of FBZ.SO in combination with piperonyl butoxide (0.11 ± 0.0.1 μg/ml and 2.50 ± 0.30 μg.h/ml).

The plasma concentrations of $FBZ.SO_2$ reached a maximum (C_{max}: 1.65 ± 0.13 μg/ml) 11 h after administration of FBZ.SO alone. When FBZ.SO was given in combination with piperonyl butoxide, a similar maximum concentration was achieved (1.59 ± 0.14 μg/ml) but not until 20 h. The AUC ratios for sulphide:sulphoxide:sulphone were 1:33:46 following FBZ.SO alone and 1:21:19 following FBZ.SO in combination with piperonyl butoxide.

The plasma concentrations of the enantiomers (FBZ.SO-1, FBZ.SO-2) of FBZ.SO following administration of FBZ.SO (as a racemate) either alone or in combination with piperonyl butoxide are presented in Table 2-2-5 (mean values) and Appendices B-8, B-9, B-10 and B-11 (individual values), respectively. The plasma concentration versus time curves and the ratios (as percentages) of each enantiomer are shown in Figures 2-2-4 and 2-2-5, respectively. The FBZ.SO-1 enantiomer was predominant in plasma following administration of racemate FBZ.SO alone. The ratio (FBZ.SO-1:FBZ.SO-2) was

Table 2-2-5. Mean (± SEM) plasma concentration (μg/ml) of enantiomers (FBZ.SO-1 and FBZ.SO-2) of oxfendazole (FBZ.SO) in ponies following iv administration of oxfendazole (10 mg/kg) either alone or in combination with piperonyl butoxide (PB) (31 mg/kg).

	Mean ± SEM (n=6)			
	FBZ.SO alone		FBZ.SO with PB	
Time	FBZ.SO-1	FBZ.SO-2	FBZ.SO-1	FBZ.SO-2
10 min	0.66 ± 0.10	0.77 ± 0.12	0.81 ± 0.13	0.88 ± 0.17
20 min	0.65 ± 0.08	0.73 ± 0.09	0.75 ± 0.09	0.76 ± 0.11
30 min	0.56 ± 0.07	0.59 ± 0.10	0.69 ± 0.07	0.70 ± 0.08
45 min	0.56 ± 0.07	0.56 ± 0.09	0.79 ± 0.06	0.81 ± 0.06
60 min	0.56 ± 0.08	0.52 ± 0.09	0.80 ± 0.07	0.85 ± 0.09
75 min	0.59 ± 0.08	0.51 ± 0.08	0.87 ± 0.07	0.92 ± 0.07
90 min	0.58 ± 0.07	0.47 ± 0.07	0.92 ± 0.07	0.97 ± 0.07
105 min	0.67 ± 0.05	0.52 ± 0.05	0.99 ± 0.10	1.07 ± 0.12
120 min	0.65 ± 0.05	0.47 ± 0.05	1.02 ± 0.10	1.14 ± 0.11
2.5 h	0.69 ± 0.06	0.51 ± 0.06	1.17 ± 0.11	1.39 ± 0.15
3 h	0.72 ± 0.07	0.48 ± 0.04	1.15 ± 0.16	1.47 ± 0.17
3.5 h	0.76 ± 0.08	0.49 ± 0.06	1.34 ± 0.19	1.69 ± 0.18
4 h	0.79 ± 0.08	0.51 ± 0.05	1.33 ± 0.17	1.76 ± 0.16
5 h	0.75 ± 0.05	0.46 ± 0.03	1.38 ± 0.22	1.88 ± 0.26
6 h	0.67 ± 0.07	0.40 ± 0.05	1.32 ± 0.23	1.90 ± 0.22
7 h	0.61 ± 0.08	0.38 ± 0.07	1.36 ± 0.16	1.91 ± 0.18
8 h	0.64 ± 0.10	0.38 ± 0.07	1.26 ± 0.22	1.71 ± 0.24
10 h	0.64 ± 0.06	0.39 ± 0.05	1.14 ± 0.19	1.52 ± 0.23
12 h	0.54 ± 0.05	0.32 ± 0.04	1.01 ± 0.17	1.03 ± 0.18
16 h	0.49 ± 0.06	0.30 ± 0.05	0.87 ± 0.16	0.75 ± 0.20
20 h	0.34 ± 0.04	0.22 ± 0.03	0.65 ± 0.18	0.47 ± 0.20
24 h	0.28 ± 0.03	0.18 ± 0.02	0.43 ± 0.13	0.27 ± 0.12
30 h	0.14 ± 0.01	0.11 ± 0.01	0.22 ± 0.07	0.13 ± 0.05
34 h	0.11 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.06 ± 0.01
48 h	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
54 h	0.04 ± 0.00	0.04 ± 0.01	0.00	0.00
72 h	0.00	0.00	0.00	0.00
96 h	0.00	0.00	0.00	0.00

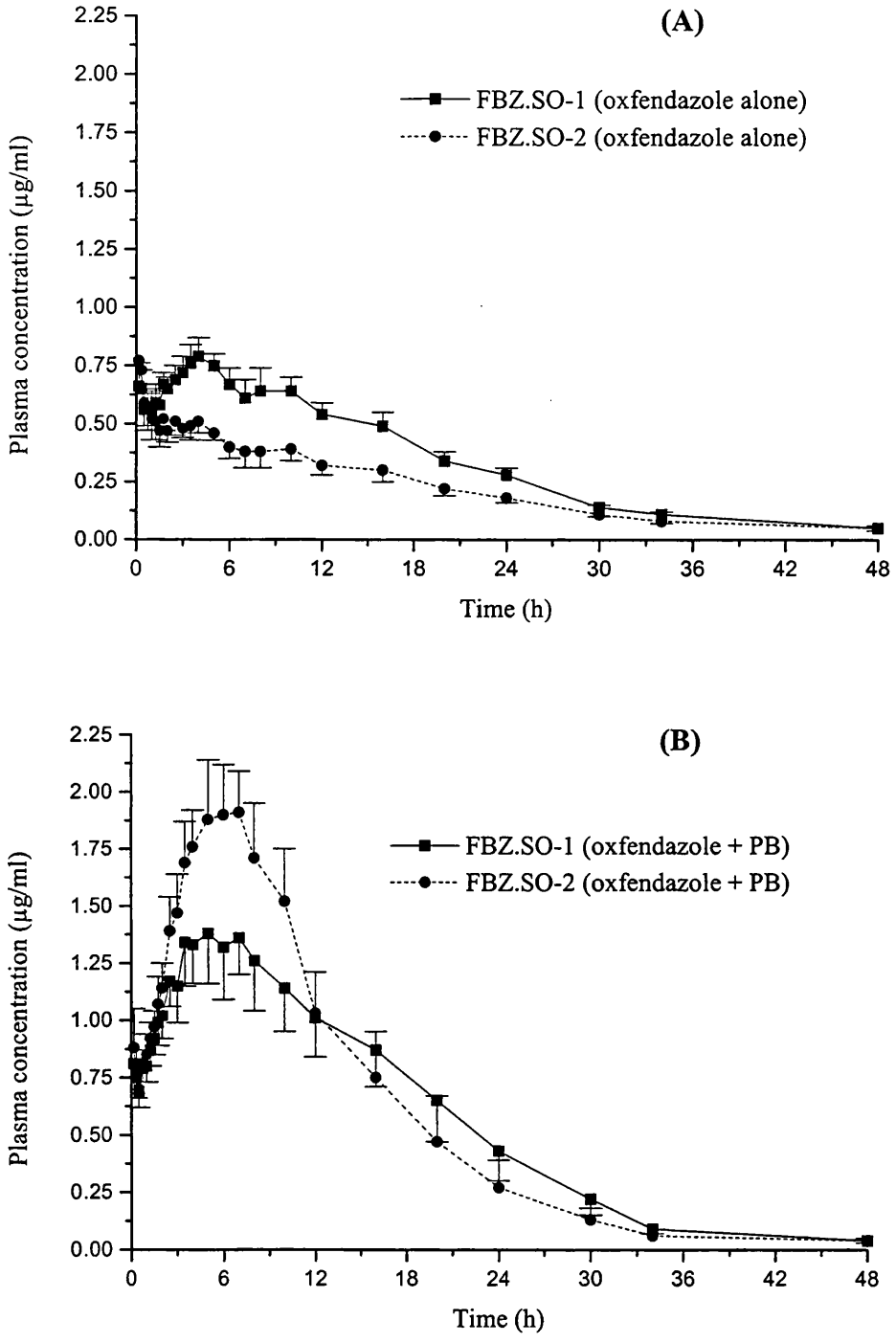


Figure 2-2-4. Mean (± SEM) plasma concentrations (µg/ml) of enantiomers (FBZ.SO-1 and FBZ.SO-2) of oxfendazole (FBZ.SO) in ponies following iv administration of oxfendazole (10 mg/kg) either alone (A) or in combination (B) with piperonyl butoxide (PB) (31 mg/kg).

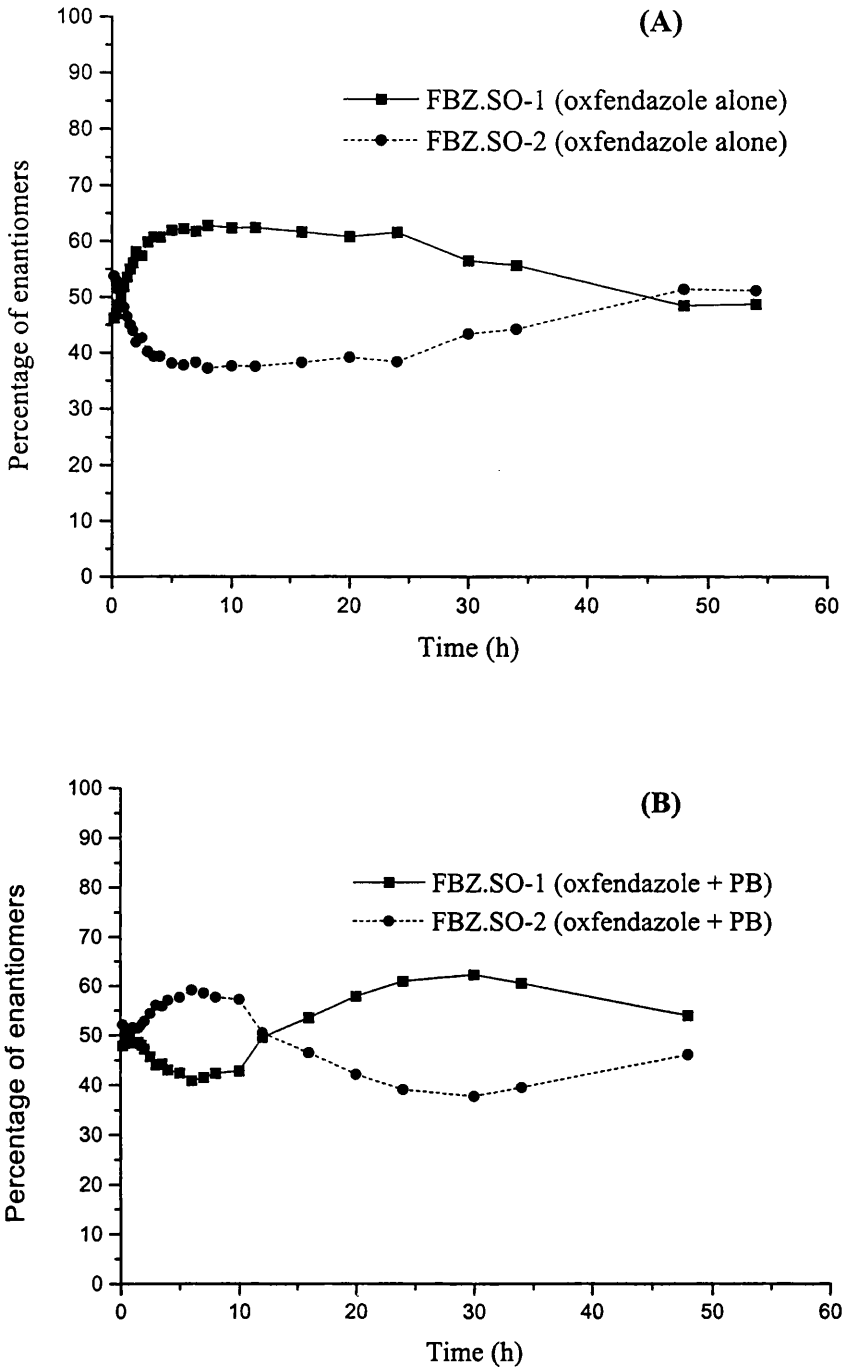


Figure 2-2-5. Ratio of the percentage of enantiomers (FBZ.SO-1 and FBZ.SO-2) (μg/ml) in ponies following iv administration of oxfendazole (10 mg/kg) either alone (A) or in combination with piperonyl butoxide (31 mg/kg) (B).

approximately 60:40 from 5 h until 24 h after drug administration and then the ratio changed towards that of a racemate between 24 h and 48 h. The co-administration of piperonyl butoxide dramatically changed the absolute and relative plasma disposition of each enantiomer. The FBZ.SO-2 enantiomer predominated from the time of administration until 10 h and the ratio approached 40:60 5 h after FBZ.SO administration. From 10 h the FBZ.SO-1 enantiomer predominated and the ratio (FBZ.SO-1:FBZ.SO-2) increased until 30 h after administration and then decreased until 48 h at which time it approached a racemate. The AUCs of FBZ.SO-1 and FBZ.SO-2 were 15.78 and 10.68 μg.h/ml, respectively whereas they increased to 26.16 and 27.45 μg.h/ml, respectively following co-administration of FBZ.SO with piperonyl butoxide.

2.2.4 Discussion

The intravenous administration of FBZ.SO generated an unusual plasma profile since an initial decline phase was followed by an incline and subsequently a plateau in the parent molecule concentration. Since the total dose was administered as a single intravenous bolus administration over a short period of approximately one minute, this should have been followed by a decline associated with distribution and elimination processes. The reasons for this concentration-time profile are unclear, however it is hypothesized that they may be related to drug solubility. It is difficult to prepare benzimidazole compounds for intravenous injection because of their poor water solubility. In this study, FBZ.SO was dissolved in dimethylsulphoxide (DMSO) for administration and a very concentrated (50% FBZ.SO) solution was prepared for practical administration purposes such that the volumes for delivery were 2 ml/100 kg. It is possible that upon delivery into the aqueous environment of the jugular blood, FBZ.SO came out of solution and particulate material was trapped at a tissue site such as the lung. Release of this reservoir over time could have accounted for the unusual increases in plasma concentration. When FBZ.SO was co-administered with piperonyl butoxide, after an initial short decline phase (similar to administration of FBZ.SO alone) the plasma concentration time curve of parent molecule followed an increase phase.

The metabolism of sulphide to sulphoxide benzimidazoles is thought to be catalysed principally by the flavine monooxygenase (FMO) system (Galtier *et al*, 1986) whereas metabolism of sulphoxide to sulphone is thought to be catalysed by hepatic cytochrome P450 (Souhaili-el-Amri *et al.*, 1988). Benchaoui and McKellar (1996) have reported that

the pharmacokinetic disposition and antinematodal potentiation of FBZ was significantly increased by the co-administration of piperonyl butoxide, an inhibitor of cytochrome P450 (Franklin, 1977) in sheep and goats. Moreover it was demonstrated recently that in fasted horses the AUC of fenbendazole was increased from 0.32±0.11 μg.h/ml (FBZ alone) to 3.51±0.40 μg.h/ml when FBZ (10 mg/kg) was administered in combination with piperonyl butoxide (63 mg/kg) (McKellar, 1997a). In the present study, the parent drug (FBZ.SO) and its sulphide metabolite (FBZ) achieved significantly greater concentrations (AUC and C_{max}) following administration of FBZ.SO with piperonyl butoxide. This was probably associated with inhibition of metabolism of the FBZ.SO to FBZ.SO_2 since the elimination rates of each moiety appeared to be the same or faster (Figure 2-2-3) when administered with piperonyl butoxide and because the drug was administered intravenously, absorption was not relevant.

The presence of both enantiomers of benzimidazole sulphoxides has been reported after administration of the prochiral sulphide parent molecules (ABZ and FBZ) in the plasma of sheep, goats and cattle (Delatour, 1990a,b; 1991a,b). The flavine containing monooxygenase that is responsible for sulphoxidation produces the (+) sulphoxide whereas the cytochrome dependent monooxygenase that is responsible for sulphonation specifically uses the (-) enantiomer as a substrate (Landoni *et al*, 1997). Both the FMO and the MFO systems (cytochrome P450 mediated mixed function oxidases) act equally in rats, and probably in other monogastrics (man, dog, horse), while the FMO system is predominant in ruminants (Delatour *et al*, 1994). In the present study, FBZ.SO displayed enantiospecific disposition in the horse since the FBZ.SO-1 enantiomer was predominant following administration of the racemate and the FBZ.SO-1:FBZ.SO-2 ratio was 60:40 throughout most of the disposition period in plasma. The co-administration of piperonyl butoxide dramatically altered the enantiospecific disposition of FBZ.SO since the FBZ.SO-2 enantiomer predominated for the first 12 h following administration and then the ratio changed in favour of the FBZ.SO-1 enantiomer.

In conclusion, co-administration of FBZ.SO with piperonyl butoxide significantly affected its plasma disposition. The increased AUC of active moieties (FBZ.SO and FBZ) following co-administration of FBZ.SO with piperonyl butoxide may improve its anthelmintic activity since this has been demonstrated previously in sheep (Benchaoui and McKellar, 1996). The pharmacokinetics of FBZ.SO was shown to be

enantioselective and the co-administration of piperonyl butoxide markedly altered the disposition of both enantiomers in the horse.

Section 3

The effect of piperonyl butoxide on the metabolism and chirality of benzimidazoles in microsome samples of equine liver: *in vitro* studies

2.3.1. Introduction

Benzimidazole anthelmintics are extensively metabolised in all animal species and the parent drug is short-lived and metabolic products predominate in plasma. The primary metabolites, usually produced by oxidation and hydrolysis, are all more polar and water soluble than the parent drug. For thioether and sulphoxide benzimidazole compounds, liver microsomal oxidation is a common metabolic pathway and they are metabolised into their sulphoxides, which in turn are oxidized into the more polar and less anthelmintically active sulphone metabolites (Lanusse and Prichard, 1993). The less oxidized moieties of benzimidazoles are thought to have greater affinity for nematode tubulin and consequently metabolic inhibitors which increase their total relative bioavailability, could be particularly useful in improving efficacy.

The present study was undertaken to determine the effect of piperonyl butoxide, a cytochrome P450 inhibitor, on the *in vitro* metabolism of oxfendazole, fenbendazole and oxibendazole using horse liver microsome samples.

2.3.2. Materials and methods

2.3.2.1. Liver microsome preparations

2.3.2.1.1 Chemicals

Sodium chloride (0.9%), potassium chloride (1.15%) (BDH Chemicals Ltd., Coole, UK) and tris buffer in 20% glycerol (Sigma Chemical Ltd., UK) were used for the isolation of microsomes from liver tissue.

The chemicals used for the cofactor solution were as follows: Trizma hydrochloride, trizma base, isocitrate dehydrogenase, nicotinamide adenine dinucleotide phosphate (NADP) and tri sodium isocitrate, all obtained from Sigma Chemical Ltd. (UK); and $MgCl_2$ from BDH Chemicals Ltd. (Poole, UK).

The standard compounds used were FBZ, FBZ.SO, FBZ.SO_2 and OH.FBZ from Hoechst Ltd. (Frankfurt, Germany), OBZ (Vetoquinol Ltd., UK) and 90% piperonyl butoxide (Aldrich Chemicals Ltd., UK). Solvents used in this experiment included varying proportions of acetonitrile (Rathburn Chemicals Ltd., UK) with water and acetic acid (BDH Chemicals Ltd., Coole, UK). Dimethylsulphoxide (DMSO) (BDH Chemicals Ltd., Coole, UK) was used to dissolve standard compounds to be added to incubation mixtures.

Microsome protein was measured using the Coomassie Blue Protein Assay Reagent (Fluka, Switzerland) with bovine serum albumin (BSA) (Sigma Chemical Ltd., UK).

2.3.2.1.2 Isolation of microsomes

Liver microsomes were prepared from livers obtained from seven horses euthanased for reasons other than hepatic diseases. After euthanasia, the liver was removed and perfused with ice-cold saline (0.9%, NaCl solution) through the hepatic veins. A piece of the liver lobe (300-400 g) was then drained of excess moisture and weighed. One hundred grams (100 g) of the liver portions were used from each animal. All procedures were performed at 0-4°C. The liver tissue was placed in 300 ml of 1.15% KCl solution and finely chopped with a sharp knife before homogenisation using a Potter-Elvehjem homogeniser. Several passages of the teflon pestle were necessary to disrupt the tissue. The liver homogenate was centrifuged for 20 minutes at 9000 *g* to clean the tissue (Removal of debris, nuclei and mitochondria). The floating fat layer was removed with a pasteur pipette, and the supernatant decanted into 6 Beckman Ultra-Clear tubes (California, USA). The tubes were centrifuged at 105000 g for 75 minutes in Beckman L8-70 refrigerated ultracentrifuge. After discarding the cytosolic fraction (supernatant), the microsomal pellet was resuspended in 60 ml of 0.1 M tris-phosphate buffer (pH 7.4) containing 20% (v/v) glycerol using an Ultra-turrax. The microsomal suspensions were then stored at -70°C until the incubation assays. The protein content was determined using the Coomassie Blue Protein Assay Reagent. Diluted (x200) microsomal suspensions were used for determination of total protein. Bovine serum albumin (BSA) was used as a standard and a standard curve was run with each assay. The assay is based on the absorbance shift from 465 to 595nm that occurs when the regent binds to proteins in an acidic solution. Cytochrome P450 concentrations were not determined in this study.

2.3.2.1.3 Drug incubation

Incubations were carried out in a shaking-water bath at 37°C for a period of 1 h. Ten-ml glass test tubes were used. One assay of an incubation mixture containing 4 mg of microsomal protein, 5 µl of test drugs (0.5 µM, 1 µM and 2.5 µM of FBZ.SO, FBZ and OBZ standards were each dissolved in DMSO) alone or with 5 µl of piperonyl butoxide (PB), and 1 ml of the co-factor solution (the precise amounts of co-factor solution are shown in Appendix C-1). Piperonyl butoxide was used at a constant concentration of 5 µM. Tubes without microsome were used as controls for possible non-enzymatic drug conversion. Incubations were conducted in triplicate. After incubation, the test tubes with the reaction mixture were placed in boiling water for 2 minutes to terminate the reaction and then immediately stored at -20°C until analysis.

2.3.2.2 Drug analysis

2.3.2.2.1 Extraction

Oxfendazole, FBZ (and their relevant metabolites) and OBZ in microsome samples were extracted in a similar fashion to the procedure outlined in section 1. Incubated blank microsome samples were used for calibration. The drug extraction method involved the use of total samples of the incubation mixture and each incubation tube was rinsed with 1 ml acetonitrile. Chloroform (6 ml) was added to each tube. The tubes were shaken on a slow rotary mixer for 10 min. After centrifugation at 1825 g for 15 min, 3 ml of the organic phase was transferred to a 10-ml glass tube for analysis of FBZ.SO, FBZ and their metabolites, and another 3 ml transferred to another tube for chiral analysis.

2.3.2.2.2 HPLC system

This was carried out as described in section 1 except for the following modifications. The column used was a Nemesis nukleosil C_{18} column (4µ, 150x4.6mm) (Phenomenex, Cheshire, UK). For FBZ.SO, FBZ and their metabolites, the mobile phase through the column utilised a gradient profile changing from 25:75 (acetonitrile:water) to 45:55 for 6 min, to 75:25 for 11 min and this changed to 25:75 which was maintained for up to 13 min for equilibration of the column. The flow rate was 1.5 ml/min. The retention times were 3.48 min (FBZ.SO), 4.68 min (OH.FBZ), 5.58 min ($FBZ.SO_2$) and 8.41 min (FBZ).

For OBZ and its unidentified metabolites a mobile phase of acetonitrile:water) with trifluoro acetic acid 0.5% (w/w) pumped as a gradient profile changing from 15:85 (acetonitrile:water) to 65:45 in 8 min and this changed to 15:85 for equilibration of column for 3 min at 1.5 ml/min flow rate. The retention times were 2.6 min for the first unidentified metabolite (M1), 3.6 min for the second unidentified metabolite (M2), 5.4 min for the third unidentified metabolite (M3), 6.2 min for the fourth unidentified metabolite (M4) and 7.4 min for OBZ. The amounts of unidentified metabolites produced in the incubation medium were estimated by using OBZ standard.

The HPLC system used for chiral analysis was as described in section 1.

2.3.2.2.3 Statistical analysis

The extent of conversion and the amount of unchanged drug, with and without metabolic inhibition were compared by one-way analysis of variance. Results were considered significant when $P < 0.05$.

2.3.3 Results

The microsomal protein content of the different equine livers used is given in Appendix C-2. Incubation done without microsomes resulted in negligible sulphonation (~1%) of the parent molecule (FBZ.SO).

Oxfendazole (FBZ.SO), FBZ and OBZ were incubated in liver microsome preparations alone or with piperonyl butoxide for 1 h. Only the sulphone (Figure 2-3-2, Appendix C-4) metabolite was detected after incubation of FBZ.SO. Sulphoxide (Figure 2-3-4 and Appendix C-6), Sulphone (Figure 2-3-5 and Appendix C-7) and hydroxy (Figure 2-3-6 and Appendix C-8) metabolites were detected after incubation of FBZ. Four unidentified metabolites (M1, M2, M3 and M4) were detected following OBZ incubation (Figures 2-3-7, 2-3-8, 2-3-9, 2-3-10 and 2-3-11, and Appendices C-9, C-10, C-11, C-12 and C-13).

The extent of metabolism was 90.0%, 87.8% and 78.7% for FBZ.SO (Figure 2-3-1 and Appendix C-3) and 88.4%, 83.2% and 68.3% for FBZ (Figure 2-3-3 and Appendix C-5) after 0.5 μM, 1 μM and 2.5 μM substrate incubation, respectively. Whereas 12.1%, 7.8% and 11.4% of FBZ.SO, and 17.6%, 20.3% and 16.9% of FBZ were metabolised in the presence of piperonyl butoxide following 0.5 μM, 1 μM and 2.5 μM drug incubation,

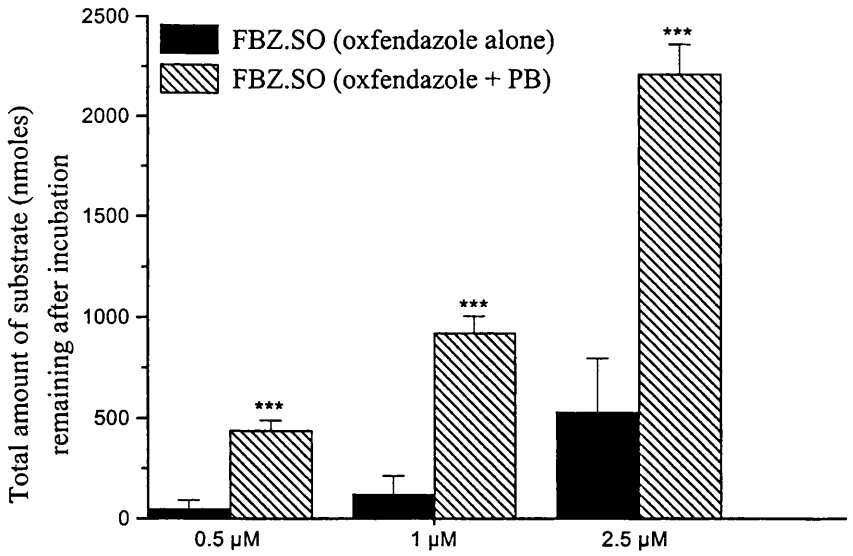


Figure 2-3-1. Total amount of unchanged oxfendazole (FBZ.SO) remaining in the microsomal reaction mixture following incubation (0.5, 1 and 2.5 µM) with (5µM) and without (control) piperonyl butoxide (PB). (***) P<0.001.

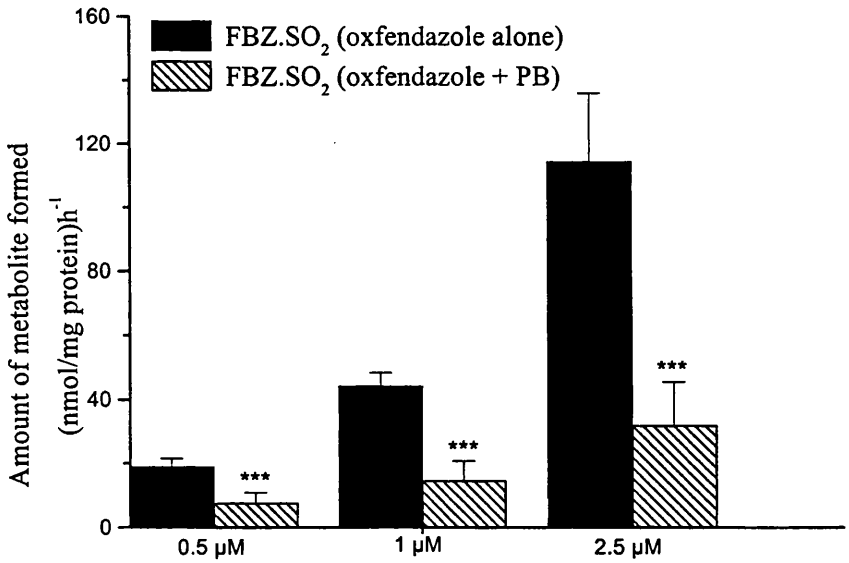


Figure 2-3-2. Amount of fenbendazole sulphone ($FBZ.SO_2$), produced in microsomal reaction mixture following oxfendazole (0.5, 1 and 2.5 µM) incubation with (5 µM) and without piperonyl butoxide (PB). (***) P<0.001.

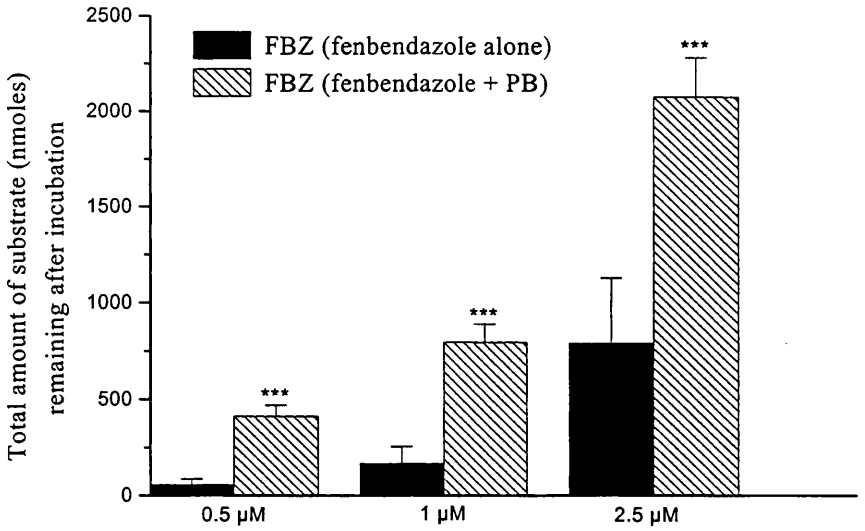


Figure 2-3-3. Total amount of unchanged fenbendazole (FBZ) remaining in the microsomal reaction mixture following incubation (0.5, 1 and 2.5 µM) with (5µM) and without (control) piperonyl butoxide (PB). (***) P<0.001.

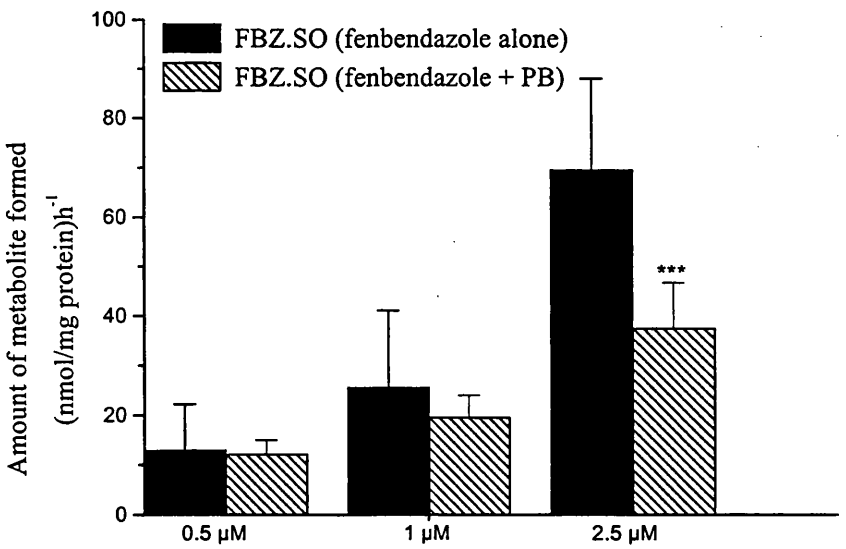


Figure 2-3-4. Amount of fenbendazole sulphoxide (FBZ.SO), produced in microsomal reaction mixture following fenbendazole (0.5, 1 and 2.5 µM) incubation with (5 µM) and without (control) piperonyl butoxide (PB). (***) P<0.001.

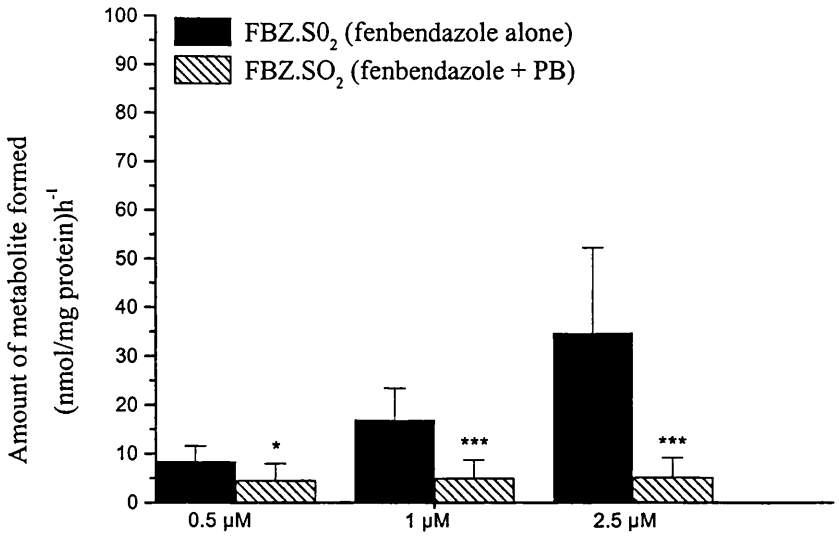


Figure 2-3-5. Amount of fenbendazole sulphone ($FBZ.SO_2$), produced in microsomal reaction mixture following fenbendazole (0.5, 1 and 2.5 µM) incubation with (5 µM) and without (control) piperonyl butoxide (PB). (*) $P<0.05$, (***) $P<0.001$.

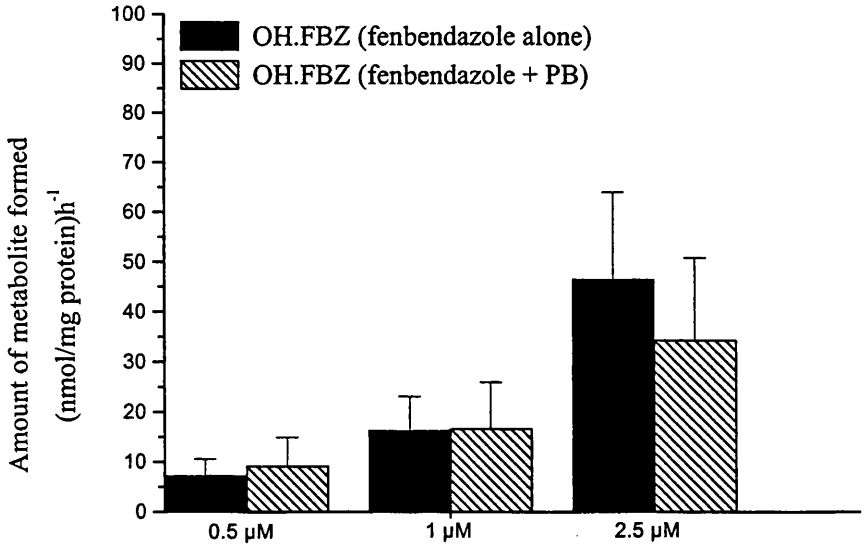


Figure 2-3-6. Amount of hydroxy fenbendazole (OH.FBZ), produced in microsomal reaction mixture following fenbendazole (0.5, 1 and 2.5 µM) incubation with (5 µM) and without (control) piperonyl butoxide (PB).

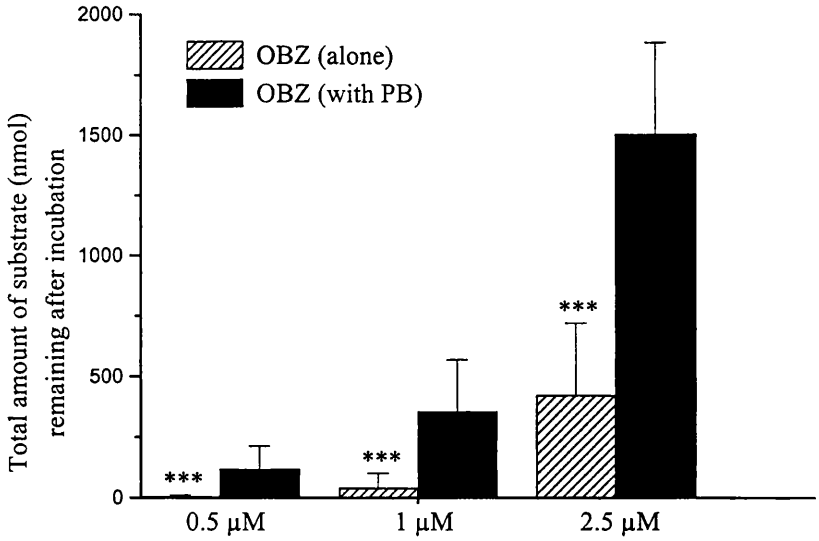


Figure 2-3-7. Total amount of unchanged oxibendazole (OBZ) remaining in the microsomal reaction mixture following incubation (0.5, 1 and 2.5 µM) with (5µM) and without (control) piperonyl butoxide (PB). (***) P<0.001.

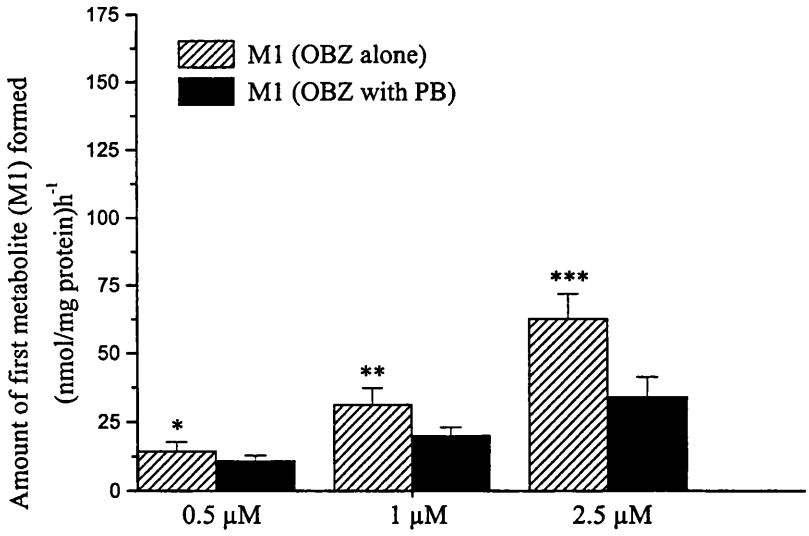


Figure 2-3-8. Amount of the first unidentified metabolite (M1) produced in microsomal reaction mixture following oxibendazole (0.5, 1 and 2.5 µM) incubation with (5 µM) and without (control) piperonyl butoxide (PB). (*) P<0.05, (**) P<0.01, (***) P<0.001.

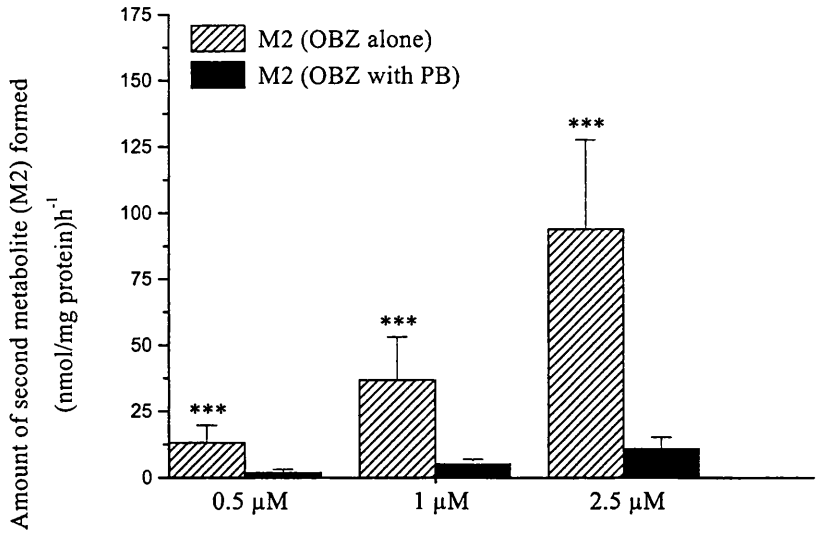


Figure 2-3-9. Amount of the second unidentified metabolite (M2) produced in microsomal reaction mixture following oxibendazole (0.5, 1 and 2.5 µM) incubation with (5 µM) and without (control) piperonyl butoxide (PB). (***) P<0.001.

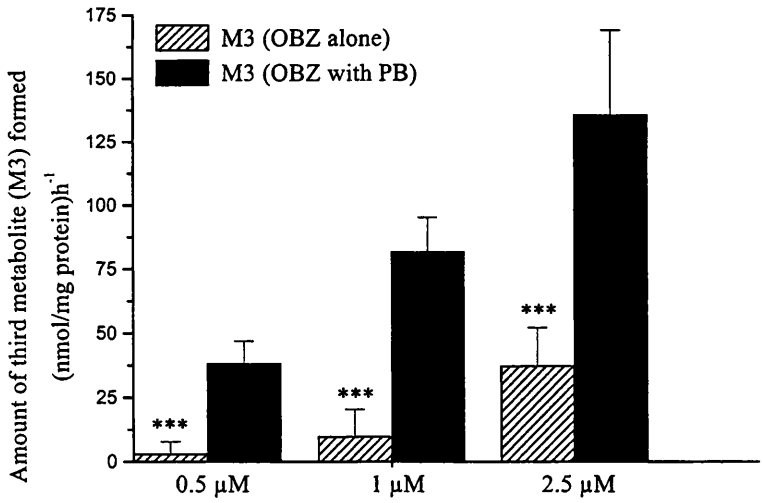


Figure 2-3-10. Amount of the third unidentified metabolite (M3) produced in microsomal reaction mixture following oxibendazole (0.5, 1 and 2.5 µM) incubation with (5 µM) and without (control) piperonyl butoxide (PB). (***) P<0.001.

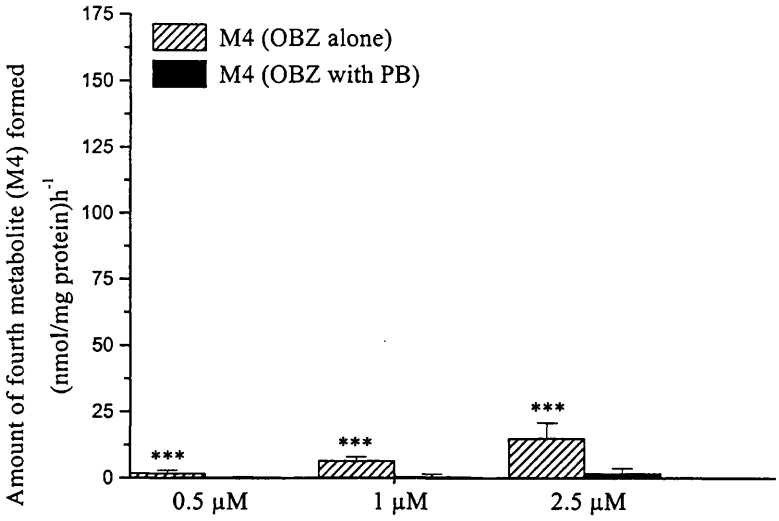


Figure 2-3-11. Amount of the fourth unidentified metabolite (M4) produced in microsomal reaction mixture following oxibendazole (0.5, 1 and 2.5 µM) incubation with (5 µM) and without (control) piperonyl butoxide (PB). (***) $P<0.001$.

respectively. The ratios between the sulphone ($FBZ.SO_2$) metabolites formed without PB and in the presence of PB after 0.5 μM, 1 μM and 2.5 μM FBZ.SO or FBZ incubation were approximately 2:1, 3:1, 5:1 and 2:1, 3:1, 7:1, respectively. This was associated with a concurrent significant ($P<0.001$) reduction in the formation of the sulphone ($FBZ.SO_2$) metabolite when the substrate was incubated with piperonyl butoxide. After FBZ incubation, the formation of the sulphoxide (FBZ.SO) metabolite was inhibited significantly ($P<0.001$) only at the highest concentration (2.5 μM) (Figure 2-3-4) in the presence of piperonyl butoxide, however the generation of the hydroxy metabolite (OH.FBZ) of FBZ was not affected by piperonyl butoxide (Figure 2-3-6).

Oxibendazole was extensively metabolised to its unidentified metabolites (M1, M2, M3 and M4) and this was also inhibited by piperonyl butoxide (Figure 2-3-7). Following 0.5 μM, 1 μM and 2.5 μM OBZ incubation, 1%, 4% and 17% of the parent drug remained in the medium, respectively whereas 24%, 36% and 60% of the substrate (OBZ) remained after OBZ incubation with piperonyl butoxide. Three unidentified metabolites (M1, M2, M4) were significantly inhibited but one of the unidentified metabolites (M3) was increased markedly after OBZ incubation with piperonyl butoxide.

The enantiospecific metabolism was determined following incubation of FBZ.SO (Figures 2-3-12, 2-3-13 and Appendices C-14, C15) as a racemate substrate and following incubation of FBZ (Figures 2-3-14, 2-3-15 and Appendices C-16, C17) with and without piperonyl butoxide). Microsomal metabolism was apparently enantiospecific since metabolism of the enantiomers was significantly different ($P<0.001$) and the ratios (FBZ.SO-1:FBZ.SO-2) of enantiomers (remaining in the incubation medium) were 7:1, 7:1 and 5:1 after incubation of 0.5 μM, 1 μM and 2.5 μM FBZ.SO (alone), respectively (Figure 2-3-12). There was a marked change in the ratio when FBZ.SO was incubated with piperonyl butoxide such that the ratios (FBZ.SO-1:FBZ.SO-2) approached 1:1 for all 3 concentration incubations (Figure 2-3-13). Fenbendazole (FBZ) metabolism to sulphoxide (FBZ.SO) was also shown to be enantiospecific since FBZ.SO-1 predominated in the reaction mixture and the ratios were 10:0, 20:1 and 10:1 after incubation of 0.5 μM, 1 μM and 2.5 μM FBZ (alone), respectively (Figure 2-3-14). Piperonyl butoxide affected the enantiospecific character of the metabolism since the ratios (FBZ.SO-1:FBZ.SO-2) were 6:1, 4:1 and 3:1 when 0.5 μM, 1 μM and 2.5 μM FBZ were incubated with piperonyl butoxide (Figure 2-3-15), respectively.

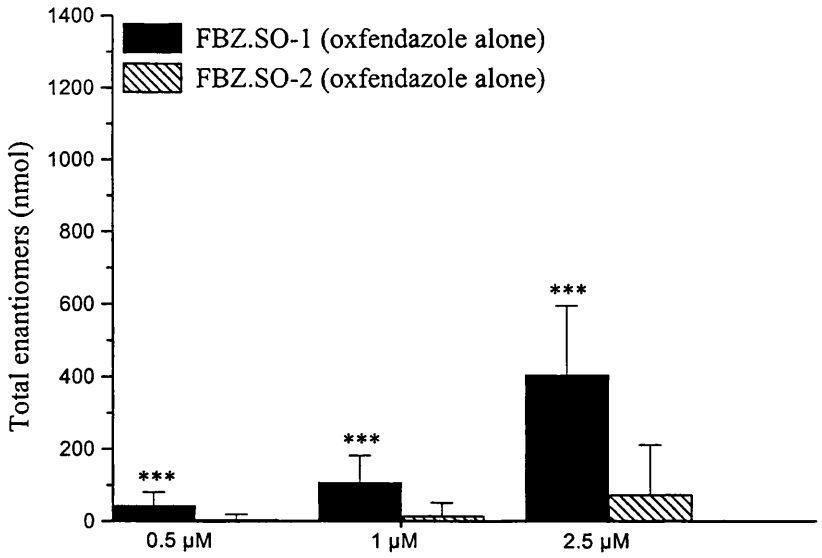


Figure 2-3-12. Total enantiomers (FBZ.SO-1 and FBZ.SO-2) remaining in microsome reaction mixture following oxfendazole (0.5, 1, 2.5 μM) incubation. (***) $P<0.001$.

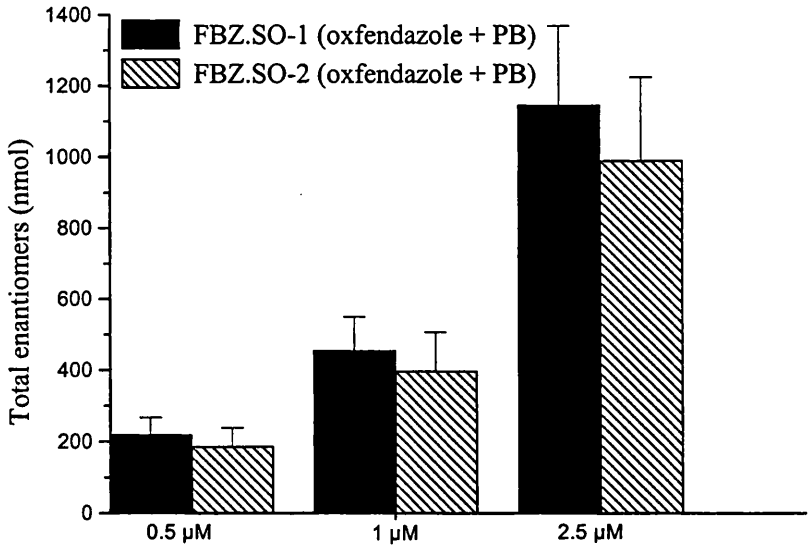


Figure 2-3-13. Total enantiomers (FBZ.SO-1 and FBZ.SO-2) remaining in microsome reaction mixture following oxfendazole (0.5, 1, 2.5 μM) incubation with (5μM) piperonyl butoxide (PB).

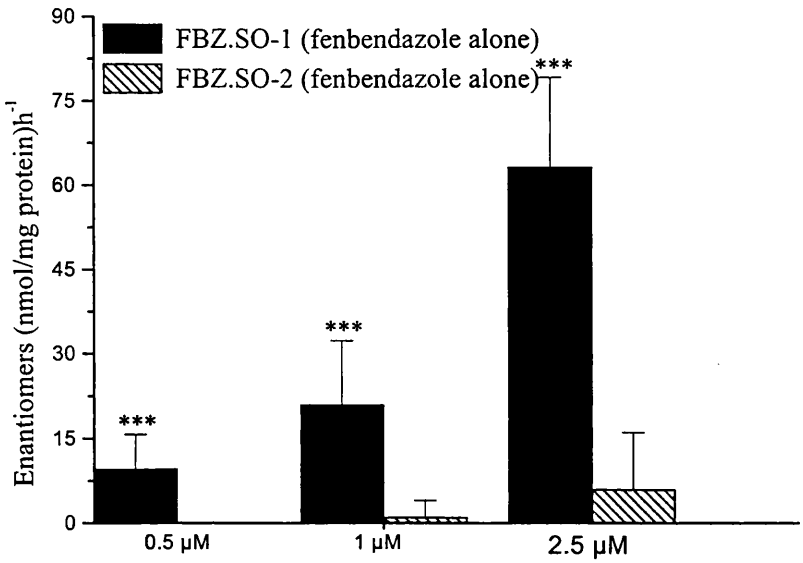


Figure 2-3-14. Enantiomers (FBZ.SO-1 and FBZ.SO-2) produced in microsome reaction mixture following fenbendazole (0.5, 1, 2.5 µM) incubation. (***) P<0.001.

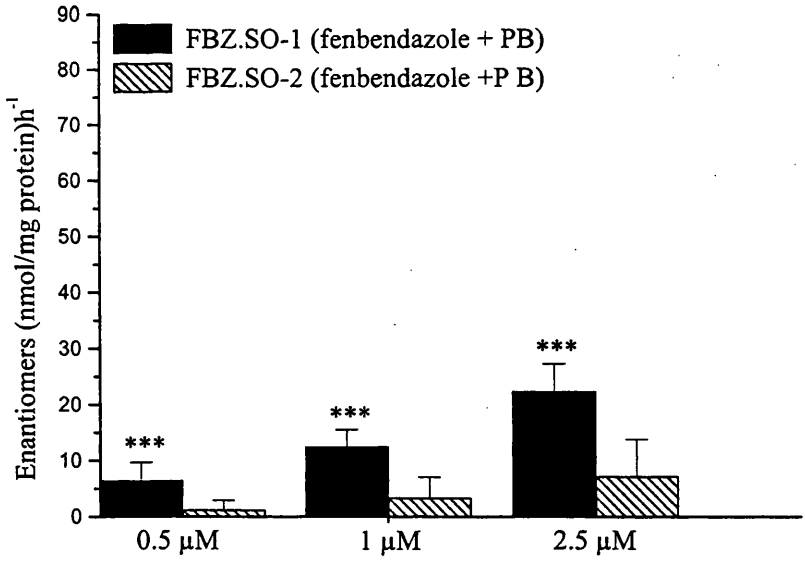


Figure 2-3-15. Enantiomers (FBZ.SO-1 and FBZ.SO-2) produced in microsome reaction mixture following fenbendazole (0.5, 1, 2.5 µM) incubation with (5µM) piperonyl butoxide (PB). P<0.001.

2.3.4 Discussion

The *in vitro* studies with liver microsome samples supported the *in vivo* studies described in section 1 and section 2. It has been demonstrated that both sulphoxidation and sulphonation of fenbendazole were inhibited by piperonyl butoxide in rat liver microsomes and hepatocytes (Benchaoui and McKellar, 1996). In the present study, piperonyl butoxide inhibited significantly ($P<0.001$) the sulphonation of FBZ.SO and the sulphoxidation and sulphonation of FBZ. The mean substrate concentrations metabolised were 85.5% and 80.0% following 3 different concentrations (0.5, 1 and 2.5 μM) of FBZ.SO and FBZ incubations, respectively, whereas only 10.4% and 18.3% of the substrates (FBZ.SO and FBZ), respectively were metabolised in the presence of piperonyl butoxide. Both FBZ.SO and FBZ extensively metabolised their sulphone metabolites as reported in the study *in vivo* in section 1 and 2. Only sulphone metabolite was detected after FBZ.SO incubation whereas sulphoxide, sulphone and hydroxy metabolites were detected after FBZ incubation. This could be due to the cytochrome P450 enzyme system which is thought to be responsible for sulphonation and this system may be more predominant than the FMO enzyme system in horse. Piperonyl butoxide did not inhibit the formation of FBZ.SO metabolite following 0.5 and 1 μM FBZ incubation but significantly inhibition occurred after the highest (2.5 μM) FBZ incubation ($P<0.001$). No significant inhibition was observed for formation of the OH.FBZ metabolite after FBZ incubation with piperonyl butoxide. These data suggest that piperonyl butoxide does not affect the enzyme system that is responsible for the hydroxylation of FBZ.

The present liver microsome study showed that OBZ was metabolised extensively to its unidentified metabolites and piperonyl butoxide significantly inhibited the metabolism of OBZ. Three unidentified metabolites (M1, M2 and M4) were significantly decreased whereas one of the unidentified metabolite (M3) was significantly increased when OBZ was incubated with piperonyl butoxide. Piperonyl butoxide not only inhibits many P450 enzyme systems but also induce some P450 metabolising enzymes (Vodicnik *et al.*, 1981; Erickson *et al.*, 1988; Adams *et al.*, 1993a, b). However, such induction is unusual after one-hour incubation since enzyme induction typically requires a much longer incubation period (Delatour, personal communication).

The chiral analysis also supported the *in vivo* study reported in section 2. It was apparent that FBZ.SO-2 was metabolised much more rapidly than FBZ.SO-1 in liver microsomes and that piperonyl butoxide altered the metabolism such that the ratio of FBZ.SO-1:FBZ.SO-2 remaining in the medium after incubation of FBZ.SO was much closer to racemate (50:50). However, FBZ.SO-1 was produced much more extensively than FBZ.SO-2 following FBZ administration and the proportion of FBZ.SO-2 was increased significantly in total enantiomers (FBZ.SO-1+FBZ.SO-2) formed after incubation with piperonyl butoxide present. The stereo-selective character of P450 (responsible for the sulphonation) and FMO (responsible for sulphoxidation) enzyme systems possibly explain the differential metabolism and generation rates of enantiomers after FBZ.SO and FBZ incubation, respectively. Furthermore, the inhibition of the P450 enzyme systems by piperonyl butoxide may alter the rates of enantiomer metabolism.

In conclusion, piperonyl butoxide inhibited the metabolism of FBZ.SO, FBZ and OBZ *in vitro*. Piperonyl butoxide also altered the metabolism of the enantiomers after FBZ.SO incubation and generation of enantiomers after FBZ incubation *in vitro* using equine liver microsomes. This finding may have important applications *in vivo* when aiming to enhance the efficacy of FBZ.SO, FBZ and OBZ.

CHAPTER 3

Pharmacokinetics and faecal excretion pattern of ivermectin, doramectin and moxidectin in horses

3.1 Introduction

Avermectins and milbemycins have been used intensively to control parasites in animals, humans and on crops worldwide. Both chemical groups are naturally derived 16-membered macrocyclic lactones (Takiguchi *et al.*, 1980) and are produced by the soil dwelling actinomycetes, *Streptomyces* spp. Avermectins and milbemycins have excellent activity against nematodes and have systemic activity against several pathogenic ectoparasites of domestic animals at low dosage rates. Due to the high activity of avermectins and milbemycins against both nematodes and arthropods, they are now classified as "endectocides" (McKellar and Benchaoui, 1996). However these drugs have no useful activity against trematodes or cestodes (Shoop *et al.*, 1995).

The pharmacokinetic behaviour of ivermectin (IVM) has been investigated more extensively than that of the other members of the macrocyclic lactones, since IVM was the first avermectin used commercially and is the most widely used endectocide across animal species. The pharmacokinetic behaviour of avermectins and milbemycins are significantly affected by route of administration, formulation of the drug, and interspecies and interindividual variation (McKellar and Benchaoui, 1996). These anthelmintics are highly lipophilic substances, and are extensively distributed throughout the body and slowly eliminated from tissues such as liver and fat (Zulalian *et al.*, 1994). For this reason a larger volume of distribution (V_d) is generally obtained for these compounds compared to other anthelmintics (Lanusse *et al.*, 1997).

In the present study, the pharmacokinetic disposition and faecal excretion of IVM, doramectin (DRM) and moxidectin (MXD) were studied in horses after oral administration.

3.2 Materials and methods

3.2.1 Animals

Twenty-four horses weighing 490-880 kg were used in this study. Animals were kept at pasture but were gathered into a corral for the period of drug administration and for 4 h thereafter. Water was available *ad libitum* during the experimental period. No invasive procedures were involved beyond blood and faecal sampling procedures. Horses were

allocated into three groups of eight such that the mean weight of animals in each group was similar and the horses were identified by unique freeze brand or natural markings.

The animals used in the study were treated with Panacur (10%) biannually however they were not treated prior to the study.

3.2.2 Drug administration and sampling procedure

The commercially available equine formulation of IVM (Eqvalan® paste, 1.87% w/v) and MXD (Equine® gel, 2% w/v), and the injectable cattle formulation of DRM (Dectomax® 1% w/v) were administered orally as a single bolus dose on the back of the tongue each at 200 µg/kg bodyweight.

Heparinized blood samples were collected by jugular venipuncture prior to drug administration and 1, 2, 4, 8, 12, 20, 24, 32, 48, 72, 96, 120 h and 8, 11, 25, 39, 66 and 80 days later. Supplementary samples were collected on day 197 following administration of MXD to cater for the longer residence time of this drug. Faecal samples (>10 g) were also collected *per rectum* throughout the blood sampling period, before drug administration and thereafter at 4, 8, 24, 32, 48, 120 h and 8, 11, 25, 39 days in order to determine the pattern of faecal excretion. Blood samples were centrifuged at 1825 g for 30 min and plasma was transferred to plastic tubes. All the plasma and faecal samples were stored at -20°C until estimation of drug concentration.

The stability of IVM, DRM and MXD in stored experimental samples was corroborated by HPLC determination of fortified plasma kept under the same conditions throughout the period of the experiment.

3.2.3 Drug analysis

The parent compounds of IVM, DRM and MXD in plasma and faeces were analyzed by high performance liquid chromatography (HPLC) with a liquid phase extraction procedure adapted from that described by Scott and McKellar (1992).

3.2.3.1 Standard preparation

Stock solutions (100 µg/ml) of pure standards of IVM (Merck, Rahway, NJ, USA), DRM (Pfizer Inc., Groton, USA) and MXD (American Cyanamid, Princeton, NJ, USA) were

prepared using acetonitrile (Rathburn Chemical Ltd., UK) as a solvent. These were diluted to give 5, 10, 100, 200 500 ng/ml and 0.5, 1, 5, 10, 50 µg/ml standard solutions for plasma and faecal samples, respectively for calibration as standard curves and to add to drug-free plasma and faecal samples to determine the recovery.

3.2.3.2 Extraction from Plasma

Drug-free plasma samples (1 ml) were spiked with either IVM, DRM or MXD standards to reach the following final concentrations: 0.5, 1, 10, 20, 50 ng/ml, and acetonitrile (1 ml) was added. After vortexing for 15 seconds, chloroform, 5 ml, (Rathburn Chemical ltd., UK) was added. The tubes were shaken on a slow rotary mixer for 15 min. After centrifugation at 1825 g for 15 min, the supernatant was removed from the tube with a pasteur pipette. The organic phase (4 ml) was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 43°C in a sample concentrator (model SC210A, Svant Instrument Inc., Holbrook, NY, USA). The dry residue was dissolved in 100 µl of N-methylimidazole (Sigma-Aldrich Co. Ltd., Gillingham, Dorset, UK) solution in acetonitrile (1:1). To initiate the derivatization, 150 µl trifluoroacetic anhydride (Sigma-Aldrich Co. Ltd., Gillingham, Dorset, UK) solution in acetonitrile (1:2) was added. Finally, 50 µl of this solution was injected into the chromatographic system.

3.2.3.3 Extraction from faeces

Wet-faecal material was mixed finely with a spatula to obtain a homogeneous sample. Drug-free wet faeces samples (0.5 g) were spiked with either IVM, DRM or MXD standards to reach the following final concentrations: 0.05, 0.1, 0.5, 1, 5 µg/g. Water (1 ml) and 4 ml acetonitrile were added to the 10 ml-ground glass tubes including 0.5 g spiked and experimental wet-faecal samples. After vortexing for 15 seconds, 6 ml chloroform was added. The tubes were shaken on a slow rotary mixer for 15 min. After centrifugation at 1825 g for 15 min, the supernatants were removed with a pasteur pipette. The organic phase (5 ml) was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 43 °C in the sample concentrator. The dry residue was dissolved in 100 µl of N-methylimidazole solution in acetonitrile (1:1). To initiate the derivatization, 150 µl trifluoroacetic anhydride solution in acetonitrile (1:2) was added. The derivatized samples were diluted appropriately with acetonitrile and filtered with

GF/C glass microfibre filter (Whatman International Ltd., Maidstone, England). This solution (50µl) was injected into the chromatographic system.

3.2.3.4 HPLC system

The mobile phase of 100% acetonitrile for IVM, acetonitrile:water (99.5:0.5) for DRM and acetonitrile:methanol (65:35) for MXD was delivered (model LC-10AS, Shimadzu, Kyoto, Japan) at a flow rate of 1.8 ml/min for IVM and DRM, and 1.2 ml/min for MXD. A Genesis C_{18}, 4 µm column (150 mm x 4.6 mm) (Crawford Scientific, Strathaven, UK) was used for IVM and MXD, and a Nova-Pak C_{18}, 4 µm column (150 x 3.9 mm) (Waters, Milford, Massachusetts, USA) was used for DRM with florescence detection (model RF-10A, Shimadzu, Kyoto, Japan) at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. Retention times were 8.90 min (IVM), 5.43 min (DRM), and 4.82 min (MXD) (Figure 3-1).

For faecal samples a solvent delivery system (Spectra Physics SP4000, Burke Electronics Ltd., Glasgow, UK) connected to a Nemesis C_{18}, 4µm column (150 mm x 4.6 mm) (Phenomenex, Cheshire, UK) and a florescence detector (Spectra Physics FL3000) at an excitation wavelength of 365 nm and an emission wavelength of 475 nm was used. The mobile phase was 100% acetonitrile for IVM, acetonitrile:water (97:3) for DRM and acetonitrile:water (96.5:3.5) for MXD.

3.2.3.5 Recovery and precision

Recovery of the three parent molecules under study was measured by comparison of the peak areas from spiked plasma samples with the areas resulting from direct injections of standards in acetonitrile carried through the derivatization procedure. The inter-assay precision of the extraction and chromatography procedures was evaluated by processing replicate aliquots of drug-free horse plasma or faecal samples containing known amounts of the drugs on different days. The limit of quantification of the assay was 0.25 ng/ml for plasma and 0.05 µg/g for faecal samples. Recoveries and coefficients of interassay variations are reported in Appendices D-1 and D-8 for plasma and faecal extractions, respectively. To determine the dry proportion of wet faecal samples, 1.0 g of wet faeces from each sample was weighed exactly into an evaporating bowl and heated in an oven at

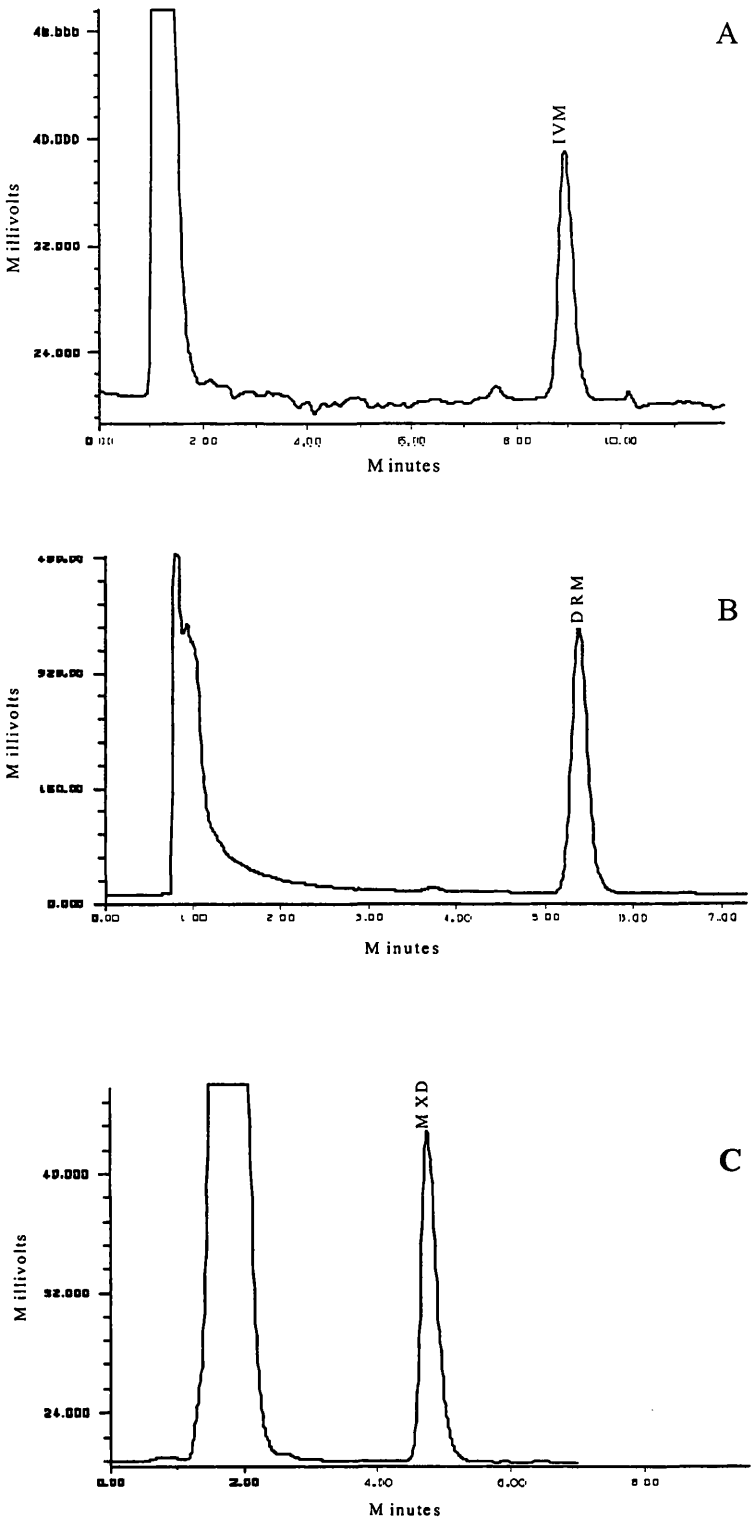


Figure 3-1. Typical chromatograms for standards of ivermectin (A), doramectin (B) and moxidectin (C).

70°C for 10 h. The weight of each was determined and the percentage of each dry sample was calculated.

3.2.4 Pharmacokinetic and statistical analysis of data

The individual data from each animal were analysed using non-compartmental model analysis with extravascular input as described in section 1. The pharmacokinetic parameters are reported as mean ± SEM. Mean pharmacokinetic parameters for IVM, DRM and MXD obtained following oral administration to horses were statistically compared by the Mann-Whitney U test. Mean values were considered significantly different at $P < 0.05$.

3.3 Results

The mean plasma concentrations of IVM, DRM and MXD are presented in Table 3-1 for mean values and individual values are shown in Appendices D-2, D-4 and D-6, respectively. The plasma concentration vs time curves are shown in Figures 3-2 and 3-3 and the pharmacokinetic parameters of IVM, DRM and MXD are presented in Table 3-2. Large variations in kinetic parameters were observed between individual animals in this study, however a similar pattern of absorption and the time to reach the peak plasma concentration (t_{max}) was observed for each substance. The peak plasma concentrations (C_{max}) and times to reach peak plasma concentrations (t_{max}) were not significantly different for IVM, DRM and MXD however the area under the curve (AUC) for MXD (86.8 ± 10.4 ng.d/ml) was significantly larger than that of IVM (46.4 ± 8.2 ng.d/ml) but not of DRM (76.5 ± 15.6 ng.d/ml). The mean residence time (MRT) of MXD (16.3 ± 2.5 day) was significantly longer than DRM (4.0 ± 1.1 day) and IVM (2.4 ± 0.2 day). Mean (±SEM) and individual dry-faecal concentrations are shown in Table 3-3 and Appendix D-9, respectively and the mean dry-faecal concentration vs time curves presented in Figure 3-4. The faecal excretion pattern of IVM, DRM and MXD were similar and no significant difference was observed for C_{max} and AUC values of any of the molecules in faeces. The highest faecal concentrations (19.5 µg/g for IVM, 20.5 µg/g for DRM and 16.6 µg/g for MXD) were determined at 24 h for all molecules.

Table 3-1. Mean (± SEM) plasma concentrations (ng/ml) of ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) following oral administration to horses at 200 μg/kg bodyweight.

	Mean ± SEM		
Time	IVM (n=8)	DRM (n=8)	MXD (n=8)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1 h	0.99 ± 0.61	1.71 ± 0.96	1.67 ± 1.07
2 h	5.08 ± 1.72	6.31 ± 2.96	8.05 ± 3.19
4 h	19.45 ± 4.03	23.62 ± 6.59	23.63 ± 5.39
8 h	19.87 ± 4.04	23.14 ± 5.46	27.26 ± 2.56
12 h	18.48 ± 3.03	22.79 ± 3.38	21.73 ± 2.94
20 h	14.40 ± 2.61	17.19 ± 1.87	16.65 ± 2.00
24 h	13.20 ± 2.23	14.86 ± 1.85	13.11 ± 1.95
32 h	10.54 ± 1.73	13.19 ± 1.85	10.15 ± 1.19
48 h	6.86 ± 0.94	10.83 ± 1.64	6.40 ± 0.69
72 h	5.61 ± 1.08	7.95 ± 1.15	4.36 ± 0.36
96 h	3.89 ± 0.70	6.52 ± 1.16	3.29 ± 0.41
120 h	2.90 ± 0.62	4.70 ± 0.74	2.46 ± 0.35
8 days	1.54 ± 0.40	3.60 ± 0.77	1.87 ± 0.23
11 days	0.08 ± 0.06	0.63 ± 0.36	1.00 ± 0.14
25 days	0.00 ± 0.00	0.26 ± 0.26	0.75 ± 0.11
39 days	0.00 ± 0.00	0.18 ± 0.18	0.65 ± 0.09
66 days	0.00 ± 0.00	0.00 ± 0.00	0.51 ± 0.07
80 days	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.08
188 days	-	-	0.00 ± 0.00
197 days	-	-	0.00 ± 0.00

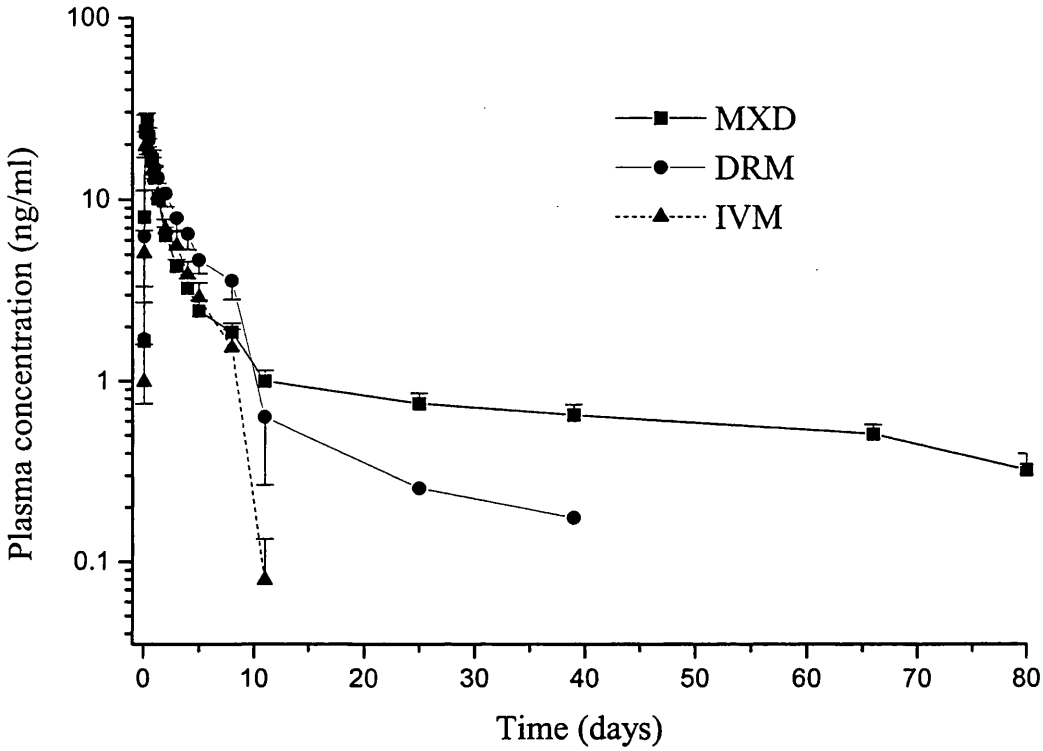


Figure 3-2. Semi log plot of mean (±SEM) plasma concentrations of ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) following oral administration to horses (n=8) at a dose rate of 200 μg/kg.

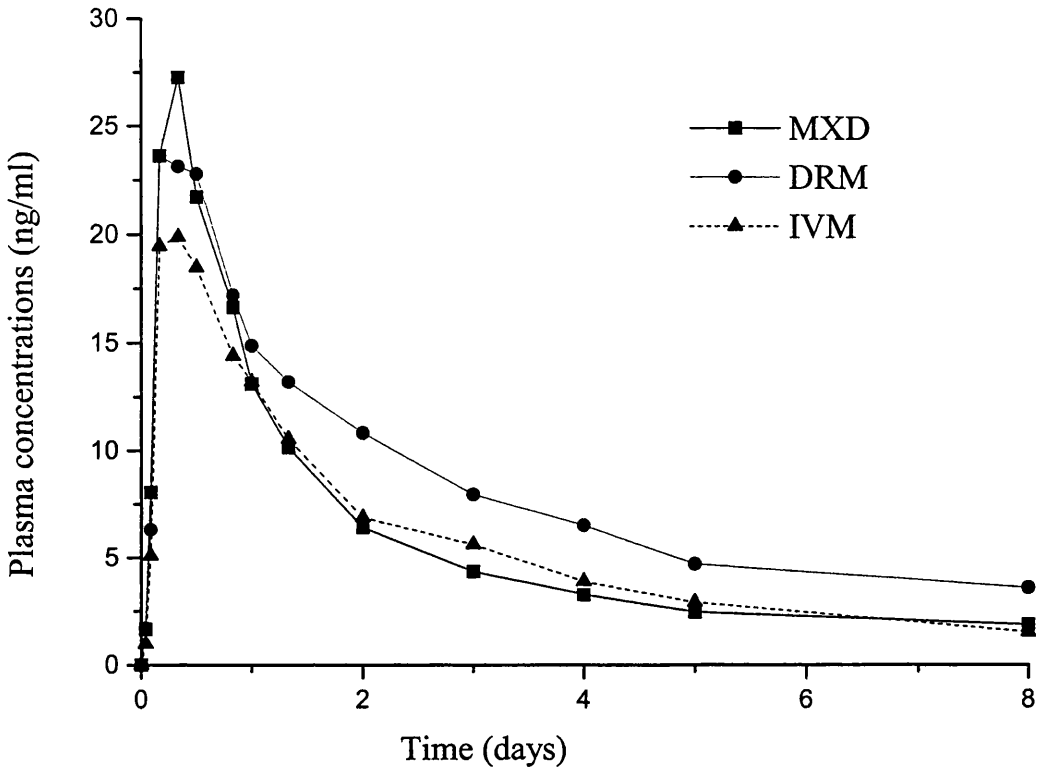


Figure 3-3. Mean (±SEM) plasma concentrations of ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) on the first 8 days following oral administration to horses (n=8) at a dose rate of 200 μg/kg.

Table 3-2. Mean (±SEM) pharmacokinetic parameters of ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) following their oral administration to horses at a dose rate of 200 μg/kg (n=8).

	Mean ± SEM		
Parameters	IVM (n=8)	DRM (n=8)	MXD (n=8)
C_{max} (ng/ml)	23.5 ± 4.15	26.98 ± 5.46	30.16 ± 4.47
t_{max} (h)	9.6 ± 2.16	10.56 ± 2.88	7.44 ± 0.96
AUC_{last} (ng.d/ml)	46.41 ± 8.2	76.54 ± 15.57	86.81 ± 10.45*
$AUMC_{last}$ (ng.d^2/ml)	117.4 ± 25.3	390.12 ± 198.1	1550 ± 336**
MRT_{last} (d)	2.4 ± 0.15	4.00 ± 1.05***	16.31 ± 2.45**

* MXD significantly different from IVM ($P < 0.05$).
** MXD significantly different from IVM and DRM ($P < 0.05$).
*** DRM significantly different from IVM ($P < 0.05$).
C_{max}: peak plasma concentration; t_{max}: time to reach peak plasma concentration; AUC_{last}: area under the (zero moment) curve from time 0 to the last detectable concentration; $AUMC_{last}$: area under the moment curve from time 0 to t last detectable concentration; MRT_{last}: mean residence time.

Table 3-3. Mean (± SEM) dry-faecal concentrations (μg/g) of ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) following their oral administration to horses (n=8) at 200 μg/kg bodyweight.

	Mean ± SEM		
Time	IVM (n=8)	DRM (n=8)	MXD (n=8)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4 h	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
8 h	1.12 ± 0.57	0.07 ± 0.05	0.11 ± 0.09
24 h	19.53 ± 3.00	20.52 ± 2.61	16.61 ± 3.74
32 h	10.28 ± 2.26	10.91 ± 2.30	5.92 ± 1.68
48 h	1.26 ± 0.28	3.89 ± 1.76	0.91 ± 0.32
120 h	0.03 ± 0.03	0.20 ± 0.12	0.01 ± 0.00
8 day	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00
11 day	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25day	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
39 day	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

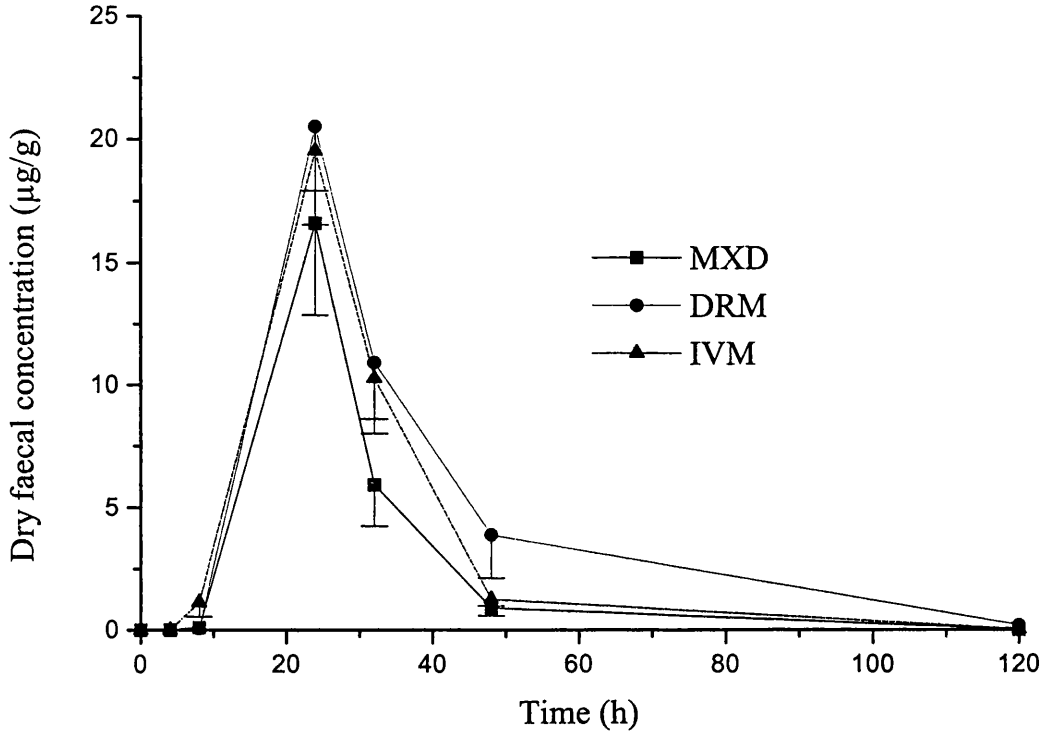


Figure 3-4. Mean (±SEM) dry-faecal concentrations (µg/g) of ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) following their oral administration to horses (n=8) at 200 µg/kg bodyweight.

3.4. Discussion

The pharmacokinetics and activity of avermectins and milbemycins are particularly influenced by the physicochemical properties of the active molecules. It has been reported that MXD is 100 times more lipophilic than IVM (Hayes, 1994) and that the water solubility of MXD (4.3 mg/L) (Lanusse and Prichard, 1993) is much higher than that of IVM (0.006-0.009 mg/L) (Fisher and Mrozik, 1989). The water solubility and lipophilicity of MXD are unusual for a drug since increased water solubility is usually directly associated with decreased lipophilicity. In cattle, the concentration of MXD in fat tissue has been shown to be ninety-fold higher than that detected in plasma 28 days following treatment (Zulalian *et al.*, 1994). Although IVM, DRM and MXD showed similar absorption patterns, the plasma decline of MXD was initially faster in comparison to IVM and DRM following oral administration in horses in the current study (Figures 3-2 and 3-3). In contrast, the MRT of MXD (16.3 ± 2.5 day) was significantly longer than the values for DRM (4.0 ± 1.1 days) and IVM (2.4 ± 0.2 days). These results may also be associated with greater proportions of MXD accumulating in fat tissue than IVM and DRM. The higher fat tissue reservoir of MXD may explain the extended persistence of that molecule compared to that of the avermectins and could confer persistent efficacy against equine parasites due to its longer retention time in plasma and excretion into the gastrointestinal tract.

There is a high correlation ($r^2 = 0.922$) between body weight and time until MXD is no longer detectable in sheep (Shoop *et al.*, 1997). A similar correlation could not be determined for IVM or MXD in the current study due to similar body weight of animals in the groups. However a correlation ($r^2 = 0.703$) was found between body weight and time until concentrations fell below the limit of detection of DRM. The lightest horse (490 kg) demonstrated zero detectable plasma concentration of DRM at day 8 whereas; the heaviest horse (880 kg) reached zero detectable plasma concentration at day 39. These results could be related to the amount of fat tissue in the animals.

The results of the present study differ substantially from those previously reported for IVM and MXD in horses (Perez *et al.*, 1999). In the previous study MXD was administered at 400 μg/kg whereas in this study it was administered at 200 μg/kg. Nevertheless the C_{max} of 70.35 ± 10.73 ng/ml obtained by Perez *et al.* (1999) was more

than double and the AUC (363.6 ± 66.0 ng.d/ml vs 86.81 ± 10.45 ng.d/ml) was approximately four times that obtained in the present study. Differences in the two studies may not be associated simply with dosage rates since IVM was given at the same dosage rate of 200 μg/kg in both studies but produced different pharmacokinetic parameters. Thus, the C_{max} 43.99 ± 23.05 ng/ml, AUC 132.7 ± 47.3 ng.d/ml and MRT 4.78 ± 0.64 d obtained by Perez *et al.* (1999) were all substantially larger than in the present study (C_{max} 23.5 ± 4.2 ng/ml, AUC 46.4 ± 8.2 ng.d/ml and MRT 2.4 ± 0.2 d). These differences may in part be due to differences in methodology although they would appear to be too large to be wholly attributed to such differences. It is unlikely that formulation differences could be responsible since both studies used Eqvalan® produced by Merck, Sharp & Dome. In the present study horses were 'yarded' for the period during and immediately (4 h) after drug administration and were then returned to a grass paddock. Feeding could therefore have caused differences in drug absorption. Parasitism could have had an effect since the horses used by Perez *et al.* (1999) were known to be infected with gastrointestinal parasites and such infections may have modest effects on absorption of anthelmintics (McKellar *et al.*, 1991). Unfortunately the parasitological status of the horses in the present study was unknown, although cyathostomes were seen in the faeces of these animals and clearly they were exposed to parasites. The most likely factor affecting the pharmacokinetics of ivermectin in the present study was the breed and size of the animals used. Perez *et al.* (1999) used Chilean criollo horses weighing 390-446 kg whereas a mixed group of thoroughbreds and hunters weighing between 560 kg and 690 kg were administered IVM in the present study.

The mode of activity of avermectins and milbemycins is not specific to parasitic nematodes and arthropods and when these agents reach the environment they may affect non-target organisms, which play an important role in the decomposition of faeces (McKellar, 1997b). Ivermectin has been shown to be excreted in high concentrations in the bile of ruminants (Bogan and McKellar, 1988) and primarily eliminated in faeces with less than 2% of the total dose being excreted in urine (Chiu *et al.*, 1990). Wet-faecal concentrations of IVM as low as 0.001 ppm are toxic to some dung-breeding insects (Strong and James, 1993). Other avermectins and milbemycins have similar ecotoxicological effects, since they share similar broad-spectrum antiparasitic activity, although the potency of different agents may make them less of a risk for specific non-

target species (McKellar, 1997b). The present study indicated that highest concentrations of all the macrocyclic lactones are found in the faeces for 48 h after drug administration, by 120 h after administration, concentrations of the anthelmintics were below the limit of detection (0.05 μg/g). This suggests that the period of greatest environmental risk is for two days following administration of these drugs to horses although it is known that very low concentrations of ivermectin (0.001 ppm) have deleterious effects on some dung-breeding organisms (Strong and James, 1993).

In conclusion, the results from this study show that the persistence of MXD in plasma is significantly greater than that of IVM and DRM and this may have a positive effect on its efficacy. No significant difference was observed for the faecal excretion patterns of IVM, DRM and MXD following their oral administration in horses.

CHAPTER 4

Pharmacokinetics and faecal excretion pattern of pyrantel embonate in horses

4.1 Introduction

Pyrantel (PYR) is an imidazothiazole derivative, which belongs to the tetrahydropyrimidine class of anthelmintics. It is available as tartrate and pamoate (syn. embonate) salts. Different salts of PYR have different pharmacokinetic properties and consequently different toxicities to the host. The pamoate salt is almost insoluble in water and poorly absorbed from the gastrointestinal tract from which most passes unchanged in the faeces (Arundel, 1983). Reduced systemic absorption of the pamoate form potentially increases availability in the lumen of the intestine (Bjorn *et al.*, 1996). The tartrate salt of PYR is soluble in water and absorbed rapidly and extensively from the intestine of monogastric animals (Faulkner *et al.*, 1972).

Pyrantel acts selectively as an agonist at synaptic and extrasynaptic nicotinic acetylcholine receptors on muscle cells of nematodes. It produces contraction and spastic paralysis, which serves to eliminate the parasites from the host (Martin, 1997). Pyrantel is highly effective (95%-97%) against small strongyles, *Parascaris equorum* and *Strongylus vulgaris*, and has moderate active against *Strongylus edentatus* (70%) and *Oxyuris equi* (65%) (Mirck, 1985). Continuous low-level daily administration of pyrantel tartrate to horses was highly effective against common gastrointestinal parasitic infections of horses, including large strongyles (*S. vulgaris, S. edentatus* and Triodontophorus spp.), adult small strongyles (Cyathostomum spp., Cylicocyclus spp., and Cylicostephanus spp.), and adult and fourth-stage *P. equorum* (Valdez *et al.*, 1995).

There are no data available in the literature on the pharmacokinetics of PYR in horses. In the present study, the pharmacokinetic disposition and faecal excretion of PYR pamoate were reported in horses after oral administration.

4.2 Materials and methods

4.2.1 Animals

Eight horses weighing 525-570 kg were used in this study. Animals were kept at pasture and water was provided *ad libitum* during the experimental period. Horses were identified by unique freeze brand or natural markings.

4.2.2 Drug administration and sampling

The commercially available equine formulation of pyrantel embonate (PYR) (Strongid-P, 43.9%, Pfizer ltd., Kent, UK) was administered orally as a single bolus dose at 13.3 mg/kg bodyweight to each animal. Heparinized blood samples were collected by jugular venipuncture prior to drug administration and 1, 2, 4, 8, 12, 20, 24, 32, 48, 72, 96 and 120 h thereafter. Faecal samples (>10 g) were also collected *per rectum* throughout the blood sampling period, before drug administration and then at 4, 8, 12, 20, 24, 32, 48, 72, 96 and 120 h in order to determine the faecal excretion of PYR. Blood samples were centrifuged at 1825 g for 30 min and the recovered plasma was transferred to plastic-stoppered tubes. All plasma and faeces samples were harvested and stored at -20 °C until estimation of drug concentration.

4.2.3 Drug analysis

The parent compound of PYR was analysed by high performance liquid chromatography (HPLC) with a liquid phase extraction procedure adapted from that described by McKellar *et al.*, (1993)

4.2.3.1 Standard preparation

Stock solutions (100 µg/ml and 1 mg/ml) of pure PYR standard (Pfizer Inc., Kent, UK) were prepared using acetonitrile (Rathburn Chemical ltd., UK) as the solvent. These were diluted to give 0.05, 0.1, 0.5, 1, 5 µg/ml and 5, 50, 500 µg/ml standard solutions for plasma and faecal samples respectively, to calibrate as standard curves and to add to drug-free plasma and faecal samples to determine the recovery.

4.2.3.2 Extraction from plasma

Briefly, 1 ml drug-free plasma samples were fortified with PYR standard to reach the following final concentrations: 0.005, 0.01, 0.05, 0.1, and 0.5 µg/ml. Morantel citrate was used as an internal standard. Sodium hydroxide (NaOH) (0.5 ml, 0.4 M) was added to tubes containing 1 ml fortified blank and experimental plasma samples. After vortexing for 15 seconds, 6 ml chloroform (Rathburn Chemical ltd., UK) was added. The tubes were stoppered and shaken for 2 minutes. After centrifugation at 1825 g for 15 min, the supernatants were removed from the tubes with pasteur pipettes. Four (4) ml of the

organic phase was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 43°C in the sample concentrator (model SC210A, Svant Instrument Inc., Holbrook, NY, USA). The dry residue was dissolved in 300 µl of mobile phase. After placing in an ultrasonic water bath for 1 minute, 100 µl of this solution was injected into the chromatographic system.

4.2.3.3 Extraction from faeces

Wet faecal material was mixed finely with a spatula to obtain a homogeneous sample. Drug-free wet faecal samples (0.5 g) were fortified with PYR standard to reach the following final concentrations: 0.5, 5, 50, 100, and 400 µg/ml. Acetonitrile (2 ml) was added to the 10 ml-ground glass tubes containing 0.5 g fortified blank faeces and experimental faecal samples. After vortexing for 15 seconds, 6 ml chloroform (Rathburn Chemical ltd., UK) was added. The tubes were stoppered and shaken for 2 min. After centrifugation at 1825 g for 15 min, the supernatants were removed from the tubes with pasteur pipettes. Four (4) ml of the organic phase was transferred to a thin-walled 10 ml-conical glass tube and evaporated to dryness at 43°C in the sample concentrator. The dry residue was dissolved in 300 µl of mobile phase and 50 µl of this solution was injected into the chromatographic system. Because of the photosensitivity of PYR all preparative processes were conducted in covered apparatus.

4.2.3.4 HPLC system

A mobile phase of acetonitrile : water (30:70) with 0.6% (v/v) triflouro acetic acid (TFA) pumped at a flow rate 1 ml/min was used for plasma samples and acetonitrile : water (15:85) with 0.6% (v/v) TFA pumped at flow rate 1.4 ml/min was used for faecal samples. Genesis nukleosil C_{18}, 4 µm column (15cm x 4.6mm) (Jhones Chromatography, Mid Glamorgan, UK) was used with ultraviolet detection (model RF-10A, Shimadzu, Kyoto, Japan) at wavelength of 322 nm. The retention times of PYR were 3.65 min for plasma (Figure 4-1) and 10.90 min for faeces.

4.2.3.5 Recovery and precision

Recovery of the drugs under study was measured by comparison of the peak areas from spiked plasma and faecal samples with the areas resulting from direct injections of standard solutions. The inter-assay precision of the extraction and chromatography

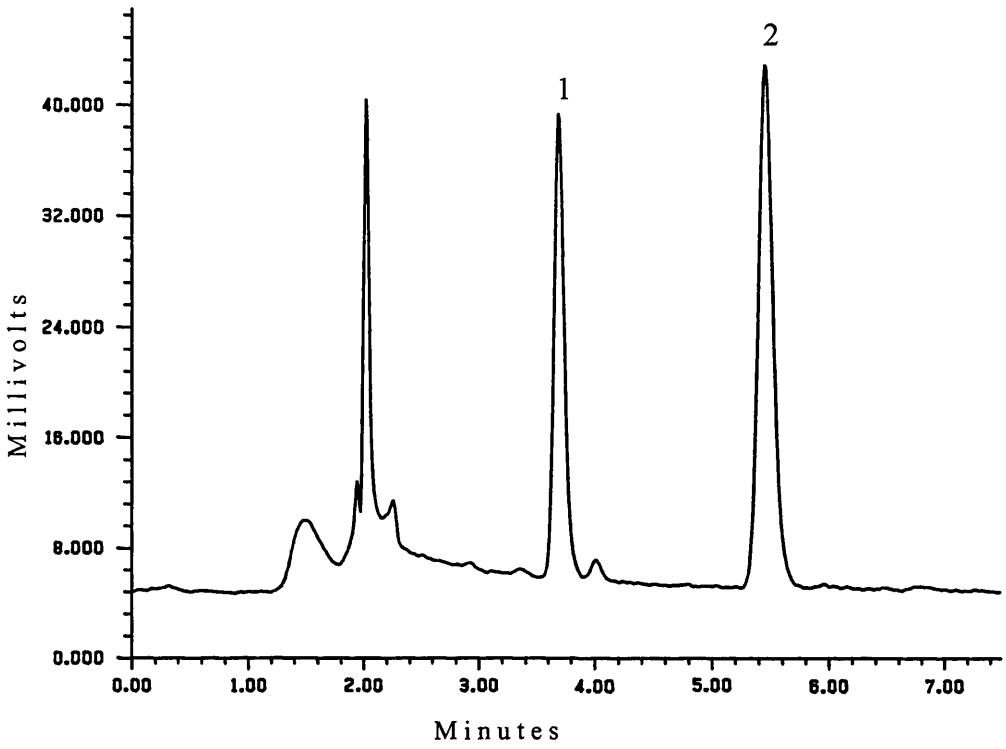


Figure 4-1. Typical chromatograph for pyrantel (1) and internal standard, morantel (2).

procedures was evaluated by processing replicate aliquots of drug-free horse plasma and faecal samples containing known amounts of the drugs on different days. The limit of quantification for the assay was 0.005 μg/ml and 0.5 μg/g for plasma and faecal samples respectively, and was determined as five times the area at the limit of detection. Mean recoveries were 97.5% (inter assay CV=8.2%) for plasma samples and 93.8% (inter assay CV=6.6%) for faecal samples.

To determine the dry weight of wet faecal samples, 1.0 g of wet faeces from each sample was weighed exactly into an evaporating bowl and heated in an oven at 70°C for 10 h. The weight of each was determined and the percentage of each dry sample was calculated.

4.2.4 Pharmacokinetic and statistic analysis of data

Data were analysed as described in chapter 2, section 2.1.2.3.6.

4.3 Results

The plasma concentration of PYR administered orally as the embonate salt is presented in Table 4-1 for mean values and, in Appendix E-2 for individual value. The plasma concentration vs time curve is shown in Figure 4-2 and the pharmacokinetic parameters of PYR are presented in Table 4-2. Pyrantel was detected in plasma between 1 h and 60 h. The maximum plasma concentration (C_{max}) was 0.09 ± 0.02 μg/ml and was achieved at 7.50±1.41 h (t_{max}). The area under the curve (AUC) and mean residence time (MRT) of PYR were 1.06 ± 0.24 μg.h/ml and 11.99 ± 1.30 h, respectively. Pyrantel was detected in faeces between 12 h and 72 h. Dry faecal concentration (Table 4-3) vs time is shown in Figure 4-3. The highest dry faecal concentration (1.034 mg/g) was determined at 24 h.

Table 4-1. Mean (± SEM) plasma concentrations (μg/ml) of pyrantel (PYR) following oral administration to horses at 13.3 mg/kg bodyweight.

	Mean ± SEM (n = 8)
Time (h)	PYR
0	0.000 ± 0.000
1	0.005 ± 0.001
2	0.022 ± 0.006
4	0.064 ± 0.005
8	0.091 ± 0.008
12	0.047 ± 0.005
20	0.017 ± 0.002
24	0.011 ± 0.002
32	0.005 ± 0.001
48	0.001 ± 0.000
72	0.000 ± 0.000
96	0.000 ± 0.000
120	0.000 ± 0.000

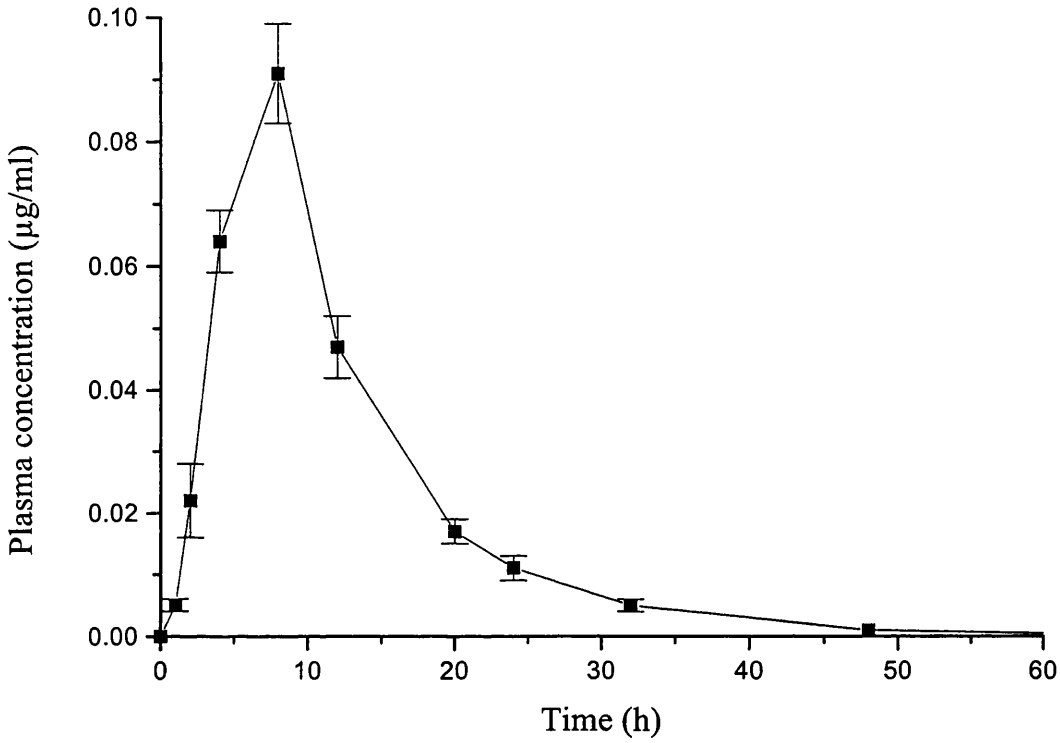


Figure 4-2. Mean (±SEM) plasma concentration of pyrantel (PYR) following oral administration to horses (n=8) at 13.3 mg/kg bodyweight.

Table 4-2. Mean (±SEM) pharmacokinetic parameters of pyrantel (PYR) following oral administration to horses (n=8) at 13.3 mg/kg bodyweight.

	Mean ± SEM (n = 8)
Pharmacokinetic parameters	(PYR)
C_{max} (μg/ml)	0.09 ± 0.02
t_{max} (h)	7.50 ± 1.41
AUC_{last} (μg.h/ml)	1.06 ± 0.24
$AUMC_{last}$ (μg.h^2/ml)	12.72 ± 3.26
MRT_{last} (h)	11.99 ± 1.30

Table 4-3. Mean (±SEM) dry-faecal concentration (mg/g) of pyrantel (PYR) following oral administration to horses (n=8) at a dose rate of 13.3 mg/kg.

	Mean ± SEM (n=8)
Time (h)	PYR
0	0.000 ± 0.00
4	0.000 ± 0.00
12	0.000 ± 0.00
20	0.705 ± 0.19
24	1.034 ± 0.06
32	0.730 ± 0.07
48	0.106 ± 0.02
72	0.000 ± 0.00
96	0.000 ± 0.00
120	0.000 ± 0.00

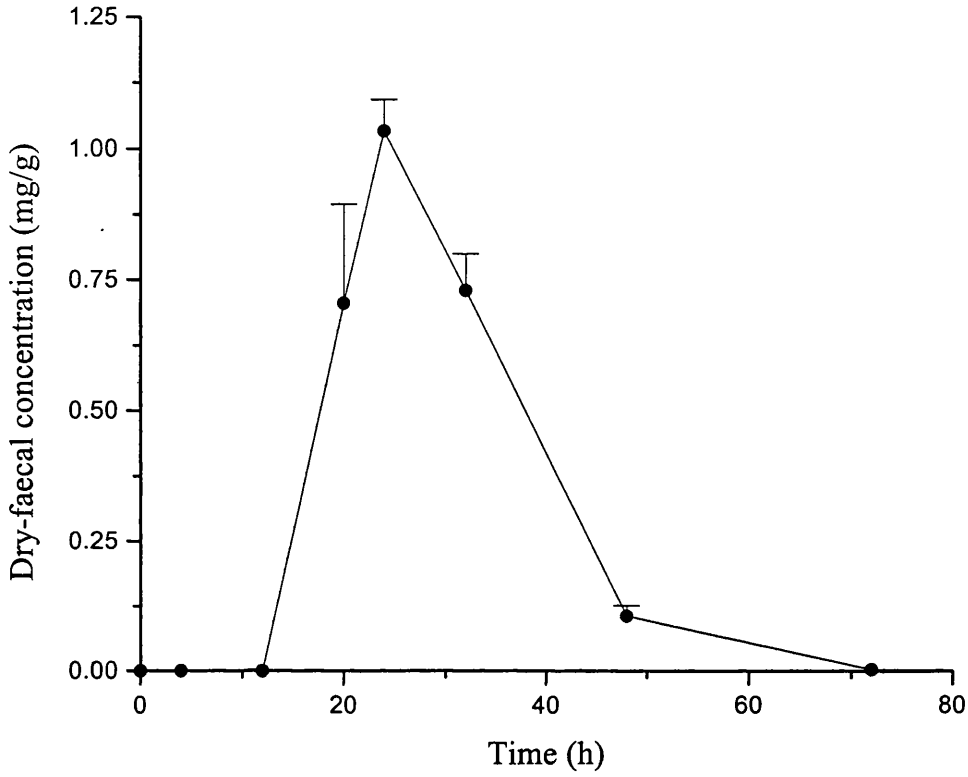


Figure 4-3. Mean (±SEM) dry-faecal concentration (mg/g) of pyrantel (PYR) following oral administration to horses (n=8) at a dose rate of 13.3 mg/kg bodyweight.

4.4 Discussion

The present results indicate that following oral administration in horses, plasma levels of the parent drug were very low. It is likely that the poor solubility of the embonate reduces its absorption and provides a safer dosage formulation and higher concentrations in the intestines than more soluble formulations.

The pharmacokinetic disposition of PYR has been determined after intravenous and oral administration in pigs (Bjorn *et al.*, 1996). Pyrantel citrate was extensively distributed (2.74L/kg) and rapidly cleared (1.09 L/kg.h) following intravenous administration. After oral administration at a dose rate of 32.84 mg/kg bodyweight, t_{max} (3.26 h) was significantly longer, C_{max} (0.23 μg/ml) lower and AUC (3.18 μg.h/kg) smaller for pyrantel pamoate (syn. embonate) than pyrantel citrate (t_{max}: 1.51 h, C_{max}: 1.92 μg/ml, AUC: 8.42 μg.h/kg) (Bjorn *et al.*, 1996) given at dose rate 22.00 mg/kg bodyweight. Although a significantly greater quantity of pyrantel citrate was absorbed (mean bioavailability of 41%) than pyrantel pamoate (mean bioavailability of 16%), the rapid clearance of the citrate resulted in a shorter MRT (4.92 h) compared to the pamoate form (11.74 h). In the previous study PYR pamoate was administered at 32.84 mg/kg in pigs whereas in this study it was administered at 13.3 mg/kg in horses. The lower concentrations of PYR pamoate in the plasma of horses than pigs may reflect different doses administered between two species and in gastrointestinal pH or transit time. Pyrantel is not known to have any substantial effect on faecal invertebrates however it is apparent that faeces from treated horses will have high concentrations of PYR for at least 48 h.

The present study demonstrates limited absorption of PYR following administration as an embonate in horses and it seems likely that a substantial component of its dynamic effect will be associated with PYR retained in the gastrointestinal tract where the adult stages of most parasitic nematodes reside.

CHAPTER 5

General Discussion

The results of the pharmacokinetic studies of FBZ and FBZ.SO in the horse suggest that FBZ.SO would produce greater anthelmintic activity than FBZ since FBZ.SO has greater systemic bioavailability than FBZ. Thus, at same dose rate, FBZ.SO could be more potent for treatment of migrating stages of strongyles and lungworm. The activity of benzimidazole anthelmintics has been associated with their persistence in the body (Lacey, 1988) and the more reduced moieties of the benzimidazole sulphides have greater activity for binding to nematode tubulin than their oxidised metabolites (Lubega and Prichard, 1991) and consequently may have greater activity *in vivo*. Furthermore, FBZ is known to have greater affinity for nematode tubulin than FBZ.SO and therefore is likely to have greater inherent potency. It is clear from this study that clinical data should be obtained for these two compounds at equivalent doses and under similar experimental conditions in order to relate the pharmacokinetic data to dynamic activity.

It is apparent from the results reported here that the plasma levels of the parent OBZ were very low following oral administration in horses and the liver microsome study showed that OBZ was metabolised extensively to unidentified metabolites. Gottschall and Wang (1996) reported that in swine OBZ was quickly metabolised and liver was the only tissue which contained significant residue following oral administration at a level of 15 mg/kg bodyweight. It was reported that OBZ has little or no effect against migrating stages of *S. vulgaris, T. axei, H. muscae* and *D. megastoma* (Kates *et al.*, 1975; Nawaliski and Theodorides, 1976, 1977). High gastrointestinal concentration of OBZ observed from the faecal study could be effective for adult stages of most parasitic nematodes that inhabit in the gastrointestinal tract but low plasma concentrations of OBZ are unlikely to be effective against migrating larval and tissue stages of the nematodes. It is likely that the high first-pass metabolism decreases OBZ bioavailability in horses. The present *in vitro* liver microsome study showed that piperonyl butoxide significantly inhibited the metabolism of OBZ. It is possibly that bioavailability of OBZ after oral administration at same dose rate can be increased by co-administration of piperonyl butoxide and this may improve its efficacy against migrating larval and tissue stages of the nematodes.

This study showed that in horses, the pharmacokinetic behaviour of benzimidazole anthelmintics was substantially different from ruminant species and this may cause misleading extrapolation of information from ruminants to horses.

In the present studies horses were 'yarded' for the period during and immediately (4 h) after drug administration and were then returned to a grass paddock. The maximum faecal excretions were observed at 24 h for the benzimidazoles, macrocyclic lactones and PYR and this possibly reflects the gastrointestinal transit times in horses for the present study. Grass based feeding could increase the gastrointestinal transit time and thus decrease the absorption time and systemic bioavailability of anthelmintics drugs. Similarly, lower plasma concentrations of benzimidazoles are achieved in ruminants on pasture compared to those fed a hay-based diet and these have been correlated with more rapid gastrointestinal transit time in the pasture fed animals (Taylor *et al.*, 1992, 1993). It was also shown that the feeding regimen affected markedly the pharmacokinetics of the active moieties and thus their potential efficacy. The relative bioavailability of benzimidazoles increased in dogs (McKellar *et al.*, 1993a) when the benzimidazoles were administered with food, whereas bioavailability decreased in sheep (Ali and Hennessy, 1993) and pigs (Lanusse *et al*, 1994) with food. In fasted cattle, significantly higher and AUC values were obtained for ABZ.SO compared to fed animals following intraruminal ABZ (10 mg/kg) administration (Lanusse at al, 1993). Recently, the effect of feeding on the bioavailability of benzimidazoles was investigated in horses with and without the potentiating agent, piperonyl butoxide (McKellar, 1997a). The area under curve of FBZ was 0.32±0.11 μg.h/ml in fasted horses and 0.44±0.1 μg.h/ml in fed horses following oral administration of FBZ (10 mg/kg) although this difference was not significant. This finding was further investigated with piperonyl butoxide whereby the AUC of parent FBZ increased from 3.51±0.40 μg.h/ml to 5.81±1.96 μg.h/ml in unfed and fed horses. It is possible that the food increased the absorption of the piperonyl butoxide thus improving its dynamic effects on liver metabolism. As well as feeding or fasting feed type could therefore have caused differences in absorption and thus, systemic bioavailability and efficacy of the anthelmintic drugs in ruminants and monogastrics.

It is likely that metabolic inhibition will be more effective than simply increasing the dose rate for improving the efficacy, since the pharmacokinetics of benzimidazoles in some monogastrics are dose-independent and absolute bioavailability can not be increased by increasing the dose (McKellar *et al.*, 1993a). The potentiation of the activity of benzimidazoles and pro-benzimidazoles has been achieved by the co-administration of metabolic inhibitors, which are thought to act principally by improving the

pharmacokinetic profiles of the active moieties of the administered drug. Parbendazole improves the bioavailability and anthelmintic efficacy of oxfendazole by decreasing its hepatic metabolism and biliary secretion (Hennessey *et al.*, 1992). Methimazole, which inhibits flavine-containing monooxygenase, improves the bioavailability and disposition of ABZ.SO following co-administration with the pro-benzimidazole netobimin, in sheep (Lanusse and Prichard, 1992a,) and cattle (Lanusse and Prichard, 1992b). Cimetidine inhibits cytochrome P450 by complexing the cytochrome haeme active site. It has thus been shown to inhibit ABZ metabolism in rats (Wen *et al*, 1996) and to improve the clinical efficacy of the ABZ against hepatic hydatid cysts in man (Wen *et al*, 1994). It was shown that piperonyl butoxide, an inhibitor of P450 inhibited the sulphoxidation and sulphonation of FBZ in an *in vitro* study using microsomal preparations from rat liver and increased significantly the bioavailability and efficacy of active moieties (FBZ and FBZ.SO) of FBZ in sheep and goats (Benchaoui and McKellar, 1996).

Fenbendazole, ABZ and their sulphoxide metabolites, which have a chiral centre about the sulphur atom, undergo enantioselective biotransformation in ruminant species (Delatour *et al.*, 1990a, b; 1991a, b). Following administration of the parent sulphide albendazole, the plasma concentration of the enantiomers generated is never a racemate; at zero time the +/- ratio is 75/25 and this changed in favour of the (+) enantiomer to reach a value of 96/4 at 36 h (Delatour *et al.*, 1990a). When the prochiral FBZ and ABZ were administered orally to sheep, the AUCs of the FBZ.SO-1 and FBZ.SO-2 enantiomers were 26% and 74% after FBZ treatment and (-) and (+) enantiomers were 14% and 86% after ABZ treatment, respectively (Delatour *et al.*, 1990b).

There have been no studies carried out to determine enantiospecific dispositions of benzimidazole sulphoxides in horse. In the present study, enantiospecific pharmacokinetics of FBZ.SO enantiomers is demonstrated firstly in the horse. The *in vivo* studies (section 1 and section 2) and *in vitro* study (section 3) clearly demonstrates that the plasma disposition and microsomal metabolism of FBZ.SO are enantiospecific and the first enantiomer (FBZ.SO-1) is predominant either in the plasma or in incubation medium. In addition, after prochiral FBZ incubation the FBZ.SO-1 enantiomer was produced more rapidly than FBZ.SO-2. This suggests that the cytochrome P450 system, which is responsible for the sulphonation, uses selectively FBZ.SO-2 and the flavine system, which is responsible for the sulphoxidation of FBZ.SO produces selectively

FBZ.SO-1. The identification (R or S) of the enantiomers of FBZ.SO is still unclear but the FBZ.SO-2 enantiomer is likely to be R (-) and the FBZ.SO-1 to be S (+) form since it is thought that the enanitioselectivity of the flavine system produces principally S (+) sulphoxide whereas the cytochrome P450 enzyme systems specifically use R (-) substrate. Furthermore an enantiospecific metabolic effect of the piperonyl butoxide was also demonstrated. In the *in vivo* study (section 2), the co-administration of piperonyl butoxide significantly altered the enantiospecific dispositions of FBZ.SO. Thus, the FBZ.SO-1 enantiomer predominated in the plasma following administration of FBZ.SO alone whereas the FBZ.SO-2 predominated for the first 12 h during which piperonyl butoxide was probably present at high concentrations and the ratio of AUCs of the enantiomers (FBZ.SO-1:FBZ.SO-2) was 3:2 when FBZ.SO was administered alone whereas the same ratio altered to 1:1 when FBZ.SO was co-administered with piperonyl butoxide. These findings were also supported by the *in vitro* studies after FBZ.SO incubation with piperonyl butoxide. The second enantiomer was metabolised more rapidly than FBZ.SO-1 after FBZ.SO incubation alone and piperonyl butoxide inhibited the metabolism of both enantiomers such that remaining enantiomers in the medium were close to racemate. The characterization of the chiral behaviour of the sulphoxide benzimidazoles may have a significant impact on their pharmacology and clinical use. The eudismic (potency) ratio or the binding affinity to parasite and mammalian tubulin for each enantiomer of sulphoxide benzimidazoles are unclear, however the alterations in enantiomer use and generation together with the alteration in achiral metabolism of benzimidazoles by piperonyl butoxide could have a major impact on the efficacy of benzimidazole sulphides and sulphoxides in the horse.

The present studies demonstrate the very great potential for synergy of benzimidazole sulphides and sulphoxides when combined with piperonyl butoxide. It is anticipated that the synergistic effects demonstrated in the present studies could have major positive benefits for the treatment of equine cyathostomiasis and benzimidazole resistant parasites.

Anthelmintic activity is generally related to the presence of concentrations of an active drug or metabolites at the site of action for a minimum period of time during which the parasite is exposal to the drug. The antiparasitic spectrum and efficacy pattern for the different endectocide molecules are similar; however differences in physicochemical

properties among them may account for differences in formulation flexibility, pharmacokinetic behaviour and persistence of their anthelmintic activity. In the current study, IVM, MXD and DRM showed similar absorption patterns but the plasma decline of MXD was initially faster in comparison to IVM and DRM following oral administration in horses. In contrast, the MRT value of MXD (16.31 days) was significantly longer than the values for DRM (4.0 days) and IVM (2.4 days). The plasma pharmacokinetic results may be associated with greater proportions of MXD accumulating in fat tissue than IVM and DRM. The higher fat tissue reservoir of MXD may explain the extended persistence of that molecule compared to that of the avermectins.

It was shown that ivermectin is degraded to some extent by the ruminal microflora. In sheep, the bioavailability of IVM after intraruminal administrations was 75% lower than following intra-abomasal administration (Chiu *et al.*, 1990) and *in vitro* incubation of ivermectin in ruminal fluid was followed by its gradual disappearance from the incubate (Prichard et al., 1985). A similar biodegradation probably occurs in the horse and may partly explain the higher bioavailability of IVM after subcutaneous than oral administration. Marriner and co-workers reported that following subcutaneous administration IVM (200 μg/kg) in horses C_{max} was 60.7 ng/ml and the AUC was 550.4 ng.d/ml whereas in this study, after oral administration at the same dose rate, C_{max} and AUC of IVM were 23.5 ng/ml and 46.41ng.d/ml, respectively. A substantial difference in pharmacokinetics between the subcutaneous and oral route has also were reported for MXD in cattle (Miller *et al.*, 1994). Lower C_{max} (6.5 ng/ml) and shorter mean residence time were observed after oral dose (200 μg/ml) compared to subcutaneous injection (C_{max}: 75 ng/ml) at a same dose rate.

Large interindividual variation was observed for the endectocides in the present study. The reasons for such differences are unclear. Several factors could affect drug absorption and metabolism such as parasite burden, feeding, breed, animal size and pathological changes of liver. The parasitological status of the horses in the present study was unknown, although adult cyathostomes were observed in the faeces of these animals. There is a paucity of data on the effect of parasitism on the pharmacokinetics of anthelmintics in horses. The effects of parasitism on the pharmacokinetics of anthelmintics has been investigated in some ruminant species. It has been shown that

parasitic infections with *O. circumcincta* and *Trichostrongylus colubriformis* significantly decreased the bioavailability of benzimidazoles in ruminant (Marriner *et al*, 1985; Debackere *et al*., 1993). Helminthiasis can cause hyperplasic changes in the abomasum (Anderson *et al*, 1988), which possibly affects the absorption and the disposition of FBZ and increased gastric pH (Mostafa and McKellar, 1989) which causes reduced dissolution of fenbendazole in the gastrointestinal tract. In contrast, the bioavailability of netobimin, ABZ, LEV and IVM was not altered significantly by intestinal parasitism (*Nematodirus battus*) in ruminants following parenteral and oral administration (McKellar *et al*, 1991) and this is possibly due to the degree of parasitism or different pathophysiological changes compared to *T. colubriformis* (McKellar *et al*, 1991, 1993b). The pharmacokinetics and metabolism of anthelmintics could be altered by helminth infections and this would potentially affect the drug efficacy.

It was reported that there was a high correlation ($r^2 = 0.922$) between body weight and time until MXD is no longer detectable in sheep (Shoop *et al*., 1997). That is, the lightest lamb (20.9 kg) reached zero detectable plasma concentration of MXD at day 24, while the heaviest lamb (38.6 kg) still possessed detectable plasma concentrations at day 60. A similar correlation could not be determined for IVM or MXD in the current study because of the similar body weight of animals in the groups. However a correlation ($r^2 = 0.703$) was found between body weight and time until concentrations fell below the limit of detection of DRM. The lightest horse (490 kg) demonstrated zero detectable plasma concentration of DRM at day 8 whereas the heaviest horse (880 kg) reached zero detectable plasma concentration at day 39. These results could be related to the amount of fat tissue in the animals. Moxidectin could confer persistent efficiency against equine parasites due to its longer retention time in plasma, although the minimum effective plasma concentration would need to be defined or clinical efficacy studies undertaken to confirm this. The extraordinary persistence of moxidectin in the horse may make this drug very useful for parasite control but also has implications for withdrawal period if treated horses are likely to enter the food chain. The plasma persistence is not clearly related to substantial faecal excretion and the faecal excretion studies suggest that the environmental consequences of administration of the avermectins may be similar - in relation to persistence - if not activity, against drug dwelling/feeding arthropods.

The present results indicate that following oral administration of PYR, plasma levels of the parent drug were very low in horses. It is likely that the poor solubility of the embonate reduces its absorption and provides a safer dosage formulation and higher concentrations in the intestines than more soluble formulations. The present study demonstrates limited absorption of PYR following administration as an embonate in horses and it seems likely that a substantial component of its dynamic effect will be associated with PYR retained in the gastrointestinal tract where the adult stages of most parasitic nematodes reside.

Anthelmintics are available with broad-spectrum activity against most of the important equine parasites. Unfortunately some developmental stages of the target parasites are relatively (early fourth (EL_4) stages of cyathostomes) and totally (EL_3) unaffected by the available drugs, and widespread resistance has developed to phenothiazine and the benzimidazole group of anthelmintics (Bennett, 1983; Condor and Campbell, 1995). The indiscriminate use of anthelmintics has inevitably resulted in the development of anthelmintic resistance and anthelmintics in horses are frequently used without consideration of the epidemiology of the parasites, the disposition and residence times of the active drug molecules, or the best clinical practice to extent the lifespan of the anthelmintics and delay the selection of resistant parasitic populations. Where the anthelmintic must be used, the most appropriate strategy to delay the development of resistance is to use combination anthelmintic products with different modes of action but similar residence times (Anderson *et al*, 1988, 1991).

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Appendices A

A-1 Recovery and coefficients of variation for oxfendazole (FBZ.SO) and its metabolites following liquid phase extraction from plasma.

	Added concentration (μg/ml)	Recovery (%)	Coefficient of variation (%) (Between assay)
FBZ.SO	0.01	94.66 (n=13)	9.99
	0.05	94.02 (n=14)	10.76
	0.1	92.41 (n=15)	9.72
	0.5	92.21 (n=16)	11.71
	1	93.13 (n=14)	9.85
Mean		93.28 (n=72)	10.40
FBZ	0.01	87.99 (n=15)	9.22
	0.05	87.16 (n=13)	9.57
	0.1	90.60 (n=15)	7.83
	0.5	90.19 (n=15)	9.39
	1	89.77 (n=13)	7.60
Mean		89.14 (n=71)	8.72
$FBZ.SO_2$	0.01	90.97 (n=15)	8.92
	0.05	96.21 (n=14)	10.78
	0.1	97.93 (n=16)	5.85
	0.5	95.27 (n=15)	5.09
	1	94.73 (n=14)	6.09
Mean		95.02 (n=74)	7.35

A-2. Plasma concentration (μg/ml) of oxfendazole (FBZ.SO) following oral administration of oxfendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.11	0.08	0.08	0.12	0.25	0.00	0.05	0.10
2	0.14	0.09	0.17	0.00	0.26	0.06	0.12	0.11
4	0.21	0.14	0.69	0.05	0.21	0.25	0.15	0.18
8	0.10	0.06	0.58	0.01	0.06	0.49	0.06	0.23
12	0.06	0.20	NS	0.01	0.02	0.09	0.07	0.09
20	0.22	0.25	0.06	0.01	0.02	0.01	0.32	0.01
24	0.04	0.15	0.01	0.01	0.02	0.00	0.53	0.02
32	0.01	0.04	0.00	0.01	0.02	0.01	0.14	0.00
48	0.02	0.02	0.00	0.01	0.02	0.02	0.04	0.00
72	0.01	0.01	0.00	NS	0.01	0.00	0.00	0.00
96	NS	0.00	0.00	NS	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	NS	0.00	0.00	0.00	0.00

A-3. Pharmacokinetic parameters of oxfendazole (FBZ.SO) following oral administration of oxfendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
C_{max} (μg/ml)	0.21	0.32	0.58	0.10	0.25	0.57	0.53	0.23
t_{max} (h)	4.00	20.00	4.00	2.00	1.00	8.00	24.00	8.00
AUC_{last} (μg.h/ml)	3.13	6.26	6.67	0.86	2.85	4.10	8.41	2.56
$AUMC_{last}$ ($\mu g.h^2/ml$)	64.40	131.14	50.29	10.61	52.20	31.85	191.68	20.38
MRT_{last} (h)	20.59	20.95	7.54	12.28	18.30	7.78	22.79	7.97

A-4. Plasma concentration (μg/ml) of fenbendazole (FBZ) following oral administration of oxfendazole a at dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00
4	0.03	0.03	0.02	0.02	0.03	0.00	0.05	0.00
8	0.06	0.04	0.08	0.03	0.02	0.04	0.07	0.05
12	0.05	0.09	NS	0.04	0.03	0.10	0.09	0.09
20	0.03	0.16	0.05	0.02	0.03	0.03	0.09	0.04
24	0.03	0.12	0.02	0.02	0.03	0.00	0.17	0.01
32	0.01	0.02	0.00	0.01	0.02	0.00	0.13	0.00
48	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
72	0.00	0.00	0.00	NS	0.00	0.00	0.00	0.00
96	NS	0.00	0.00	NS	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	NS	0.00	0.00	0.00	0.00

A-5. Pharmacokinetic parameters of fenbendazole (FBZ) following oral administration of oxfendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
C_{max} (μg/ml)	0.06	0.16	0.08	0.04	0.03	0.10	0.17	0.09
t_{max} (h)	8.00	20.00	8.00	12.00	12.00	12.00	24.00	12.00
AUC_{last} (μg.h/ml)	1.06	2.57	1.09	0.65	0.79	0.87	3.10	1.04
$AUMC_{last}$ ($\mu g.h^2/ml$)	17.70	46.64	13.39	9.62	13.22	10.73	62.32	14.01
MRT_{last} (h)	16.77	18.17	12.25	14.72	16.80	12.33	20.08	13.50

A-6. Plasma concentration (μg/ml) of fenbendazole sulphone ($FBZ.SO_2$) following oral administration of oxfendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.20	0.17	0.16	0.24	0.20	0.05	0.07	0.13
2	0.31	0.31	0.40	0.35	0.27	0.17	0.14	0.34
4	0.70	0.45	1.07	0.46	0.47	0.50	0.22	0.47
8	0.77	0.32	1.22	0.21	0.45	0.98	0.16	0.90
12	0.27	0.38	NS	0.17	0.35	0.90	0.15	0.65
20	0.14	0.52	0.36	0.09	0.19	0.34	0.26	0.37
24	0.36	0.63	0.08	0.07	0.13	0.22	0.50	0.17
32	0.00	0.16	0.05	0.02	0.05	0.17	0.66	0.04
48	0.02	0.02	0.00	0.01	0.02	0.02	0.07	0.01
72	0.00	0.00	0.00	NS	0.01	0.02	0.04	0.00
96	NS	0.00	0.00	NS	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	NS	0.00	0.00	0.00	0.00

A-7. Pharmacokinetic parameters of fenbendazole sulphone ($FBZ.SO_2$) following oral administration of oxfendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
C_{max} (μg/ml)	0.77	0.63	1.22	0.46	0.47	0.98	0.66	0.90
t_{max} (h)	8.00	24.00	8.00	4.00	4.00	8.00	32.00	8.00
AUC_{last} (μg.h/ml)	10.63	14.53	17.29	5.29	8.96	17.17	16.84	13.35
$AUMC_{last}$ ($\mu g.h^2/ml$)	129.93	266.34	167.80	58.17	134.81	276.96	477.45	170.47
MRT_{last} (h)	12.23	18.34	9.71	11.01	15.05	16.14	28.35	12.77

A-8. Recovery and coefficients of variation for oxfendazole (FBZ.SO) and its metabolites following liquid phase extraction from wet faeces.

	Added concentration (μg/ml)	Recovery (%)	Coefficient of variation (%) (Between assays)
FBZ.SO	1	95.24 (n=16)	2.99
	5	94.86 (n=16)	3.57
	50	91.25 (n=16)	4.27
	100	93.29 (n=16)	4.19
	200	91.38 (n=16)	5.97
Mean		93.20 (n=80)	4.20
FBZ	1	93.38 (n=16)	7.08
	5	94.29 (n=16)	6.67
	50	89.60 (n=16)	8.91
	100	87.34 (n=16)	6.12
	200	82.79 (n=14)	3.85
Mean		89.48 (n=78)	6.52
$FBZ.SO_2$	1	97.66 (n=8)	3.39
	5	95.87 (n=8)	2.19
	50	94.23 (n=8)	3.00
	100	94.70 (n=8)	1.66
	200	93.24 (n=8)	4.76
Mean		95.14 (n=40)	3.00

A-9. Dry faecal concentrations (mg/ml) of oxfendazole (FBZ.SO) and its metabolites (FBZ, FBZ.SO_2) following oral administration of oxfendazole at a dose rate of 10 mg/kg bodyweight.

FBZ.SO	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	NS	0.00	0.00	NS	0.00	0.00
12	NS	0.00	NS	NS	0.00	NS	NS	NS
20	NS	NS	0.47	NS	1.07	NS	NS	0.75
24	1.05	0.91	0.49	1.18	1.38	0.66	0.25	2.25
32	NS	0.36	0.35	0.73	0.73	0.41	0.72	0.41
48	0.04	0.08	0.05	0.07	0.01	0.09	0.09	0.02
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NS
96	0.00	NS	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0	0.00	0.00	0.00	0.00	0.00	0.00

FBZ	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	NS	0.00	0.00	NS	0.00	0.00
12	NS	0.00	NS	NS	0.00	NS	NS	NS
20	NS	NS	0.13	NS	0.14	NS	NS	0.14
24	0.20	0.18	0.11	0.19	0.18	0.15	0.11	0.51
32	NS	0.12	0.08	0.16	0.10	0.10	0.11	0.17
48	0.04	0.02	0.02	0.03	0.01	0.04	0.05	0.02
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NS
96	0.00	NS	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FBZ.SO_2	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	NS	0.00	0.00	NS	0.00	0.00
12	NS	0.00	NS	NS	0.00	NS	NS	NS
20	NS	NS	0.01	NS	0.01	NS	NS	0.01
24	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.03
32	NS	0.01	0.01	0.01	0.01	0.01	0.01	0.01
48	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NS
96	0.00	NS	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

A-10. Plasma concentrations (μg/ml) of fenbendazole (FBZ) following oral administration of fenbendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	9	10	11	12	13	14	15	16
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.02	0.01	0.00	0.01	0.01	0.01	0.01	0.00
2	0.05	0.03	0.01	0.02	0.01	0.03	0.02	0.00
4	0.04	0.02	0.02	0.01	0.01	0.07	0.05	0.03
8	0.02	0.01	0.01	0.01	0.01	0.03	0.05	0.04
12	NS	NS	0.01	NS	0.01	0.04	0.02	NS
20	0.03	0.01	0.01	0.04	0.01	0.01	0.00	0.01
24	0.02	0.01	0.01	0.04	0.01	0.01	0.00	0.01
32	0.01	0.01	0.00	0.03	0.01	0.00	0.00	0.00
48	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

A-11. Pharmacokinetic parameters of fenbendazole (FBZ) following oral administration of fenbendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	9	10	11	12	13	14	15	16
C_{max} (μg/ml)	0.05	0.03	0.02	0.04	0.01	0.07	0.05	0.04
t_{max} (h)	2.00	2.00	4.00	20.00	20.00	4.00	4.00	8.00
AUC_{last} (μg.h/ml)	0.77	0.41	0.25	1.18	0.34	0.85	0.56	0.48
$AUMC_{last}$ (μg.h^2/ml)	10.10	5.45	3.19	28.37	6.38	11.34	4.81	4.98
MRT_{last} (h)	13.06	13.24	12.59	24.04	18.60	13.31	8.57	10.30

A-12. Plasma concentrations (μg/ml) of fenbendazole sulphoxide (FBZ.SO) following oral administration of fenbendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	9	10	11	12	13	14	15	16
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
2	0.02	0.01	0.00	0.01	0.00	0.01	0.01	0.00
4	0.01	0.01	0.01	0.01	0.00	0.02	0.01	0.01
8	0.01	0.00	0.00	0.01	0.00	0.01	0.01	0.01
12	NS	NS	0.00	NS	0.01	0.01	0.02	NS
20	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
24	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
32	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

A-13. Pharmacokinetic parameters of fenbendazole sulphoxide (FBZ.SO) following oral administration of fenbendazole at dose a rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	9	10	11	12	13	14	15	16
C_{max} (μg/ml)	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.01
t_{max} (h)	2.00	2.00	4.00	32.00	12.00	4.00	12.00	8.00
AUC_{last} (μg.h/ml)	0.21	0.08	0.05	0.25	0.16	0.21	0.20	0.16
$AUMC_{last}$ ($\mu g.h^2/ml$)	2.61	0.70	0.48	4.60	3.06	2.19	2.38	2.07
MRT_{last} (h)	12.38	9.32	9.26	18.46	18.79	10.50	11.91	12.57

A-14. Plasma concentrations (μg/ml) of fenbendazole sulphone ($FBZ.SO_2$) following oral administration of fenbendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	9	10	11	12	13	14	15	16
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.00
2	0.04	0.03	0.00	0.02	0.00	0.04	0.03	0.00
4	0.07	0.03	0.03	0.04	0.00	0.12	0.06	0.04
8	0.06	0.04	0.03	0.02	0.00	0.12	0.09	0.08
12	NS	NS	0.02	NS	0.02	0.09	0.02	NS
20	0.07	0.02	0.01	0.04	0.02	0.04	0.01	0.03
24	0.06	0.02	0.01	0.05	0.01	0.02	0.02	0.02
32	0.01	0.01	0.00	0.05	0.00	0.01	0.01	0.01
48	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
72	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

A-15. Pharmacokinetic parameters of fenbendazole sulphone ($FBZ.SO_2$) following oral administration of fenbendazole at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	9	10	11	12	13	14	15	16
C_{max} (μg/ml)	0.07	0.04	0.03	0.05	0.02	0.12	0.09	0.08
t_{max} (h)	4.00	8.00	8.00	32.00	12.00	4.00	8.00	8.00
AUC_{last} (μg.h/ml)	1.68	1.00	0.59	1.11	0.30	1.95	0.99	1.32
$AUMC_{last}$ (μg.h^2/ml)	25.30	16.91	12.51	21.65	5.09	25.05	14.21	19.60
MRT_{last} (h)	15.08	16.94	21.35	19.56	17.03	12.84	14.32	14.88

A-16. Dry faecal concentrations (mg/ml) of fenbendazole (FBZ) and its sulphoxide metabolite (FBZ.SO) following oral administration of fenbendazole at a dose rate of 10 mg/kg bodyweight.

FBZ	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	NS	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	NS	NS	0.00	NS	NS	NS	NS	NS
20	1.36	NS	0.09	0.14	0.75	NS	NS	NS
24	1.47	1.06	0.79	1.07	1.10	0.86	0.62	0.78
32	0.91	0.69	NS	1.26	0.37	0.37	2.00	0.65
48	0.40	0.33	0.16	NS	NS	0.02	0.27	0.03
72	0.03	0.00	0.00	0.00	0.00	0.00	0.01	NS
96	0.00	NS	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FBZ.SO	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	NS	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	NS	NS	0.00	NS	NS	NS	NS	NS
20	0.01	NS	0.00	0.00	0.00	NS	NS	NS
24	0.01	0.02	0.01	0.05	0.01	0.01	0.01	0.02
32	0.02	0.01	NS	0.02	0.01	0.02	0.01	0.02
48	0.01	0.01	0.01	NS	NS	0.00	0.00	0.00
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NS
96	0.00	NS	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

A-17. Recovery and coefficients of variation for oxibendazole (OBZ) following liquid phase extraction from plasma.

	Added concentration (μg/ml)	Recovery (%)	Coefficient variation (%) (Between assay)
OBZ	0.005	94.54 (n=8)	4.78
	0.010	93.85 (n=8)	2.19
	0.020	89.95 (n=8)	5.72
	0.050	95.87 (n=8)	5.04
	0.100	98.58 (n=8)	3.17
Mean		94.56 (n=40)	4.18

A-18. Plasma concentrations (μg/ml) of oxibendazole (OBZ) following oral administration at a dose rate of 10 mg/kg bodyweight.

	Animal numbers							
Time (h)	17	18	19	20	21	22	23	24
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.000	0.004	0.005	0.006	0.004	0.006	0.006	0.004
2	0.000	0.000	0.005	0.000	0.000	0.028	0.004	0.000
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
32	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
48	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
72	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

A-19. Recovery and coefficients of variation for oxibendazole following liquid phase extraction from wet faeces.

	Added concentration (μg/ml)	Recovery (%)	Coefficient variation (%) (Between assay)
OBZ	0.50	93.94 (n=8)	4.10
	5.00	97.50 (n=8)	4.93
	50.00	93.63 (n=8)	5.73
	100.00	94.14 (n=8)	1.48
	300.00	88.98 (n=8)	6.65
Mean		93.64 (n=40)	4.58

A-20. Dry faecal concentrations (mg/ml) of oxibendazole (OBZ) following oral administration at a dose rate of 10 mg/kg bodyweight.

OBZ	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.12	0.03	0.03	0.00	0.00	0.00
16	0.00	0.14	0.51	0.30	0.35	0.08	0.04	0.15
24	0.01	0.60	0.22	0.56	1.39	0.82	0.17	0.48
32	0.08	0.15	0.15	0.46	0.77	0.18	0.18	0.73
48	0.04	0.00	3.16	0.05	0.03	0.04	0.05	0.03
72	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

A-21. Recovery and coefficients of variation for enantiomers (FBZ.SO-1 and FBZ.SO-2) of oxfendazole following liquid phase extraction from plasma.

	Added concentration (μg/ml)	Recovery (%)	Coefficient variation (%) (Between assay)
FBZ.SO-1	0.025	84.69 (n=5)	3.62
	0.050	85.73 (n=6)	8.16
	0.250	94.52 (n=6)	6.72
	0.500	85.31 (n=6)	11.47
	2.500	91.51 (n=6)	9.80
Mean		88.35 (n=29)	7.96
FBZ.SO-2	0.025	80.23 (n=6)	6.51
	0.050	81.98 (n=6)	13.03
	0.250	93.65 (n=6)	8.66
	0.500	90.59 (n=6)	11.88
	2.500	96.52 (n=6)	11.64
Mean		88.60 (n=30)	10.35

A-22. Plasma concentrations (μg/ml) of the enantiomer (FBZ.SO-1) of oxfendazole (FBZ.SO) following oral administration of oxfendazole (10 mg/kg) in horse.

	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.032	0.019	0.014	0.051	0.098	0.000	0.012	0.030
2	0.059	0.031	0.021	0.054	0.153	0.024	0.028	0.059
4	0.050	0.046	0.110	0.026	0.123	0.090	0.040	0.063
8	0.046	0.029	0.103	0.012	0.052	0.275	0.029	0.136
12	0.026	0.082	NS	0.009	0.023	0.059	0.033	0.051
20	0.035	0.102	0.011	0.006	0.010	0.006	0.145	0.009
24	0.021	0.039	0.005	0.005	0.015	ULQ	0.248	0.007
32	0.000	0.000	ULQ	0.000	0.000	0.000	0.054	0.000
48	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
72	0.000	0.000	0.000	NS	0.000	0.000	0.000	0.000
96	NS	0.000	0.000	NS	0.000	0.000	0.000	0.000
120	0.000	0.000	0.000	NS	0.000	0.000	0.000	0.000

A-23. Plasma concentrations (μg/ml) of the enantiomer (FBZ.SO-2) of oxfendazole (FBZ.SO) following oral administration of oxfendazole (10 mg/kg) in horse.

	Animal numbers							
Time (h)	1	2	3	4	5	6	7	8
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.035	0.037	0.021	0.053	0.085	0.000	0.020	0.039
2	0.059	0.052	0.031	0.047	0.103	0.026	0.030	0.072
4	0.058	0.079	0.190	0.019	0.078	0.072	0.031	0.060
8	0.026	0.039	0.161	0.000	0.012	0.132	0.013	0.070
12	0.018	0.119	NS	0.000	0.007	0.016	0.016	0.020
20	0.025	0.215	0.008	0.000	0.000	0.000	0.099	0.000
24	0.008	0.090	0.000	0.000	0.007	0.000	0.123	0.000
32	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000
48	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
72	0.000	0.000	0.000	NS	0.000	0.000	0.000	0.000
96	NS	0.000	0.000	NS	0.000	0.000	0.000	0.000
120	0.000	0.000	0.000	NS	0.000	0.000	0.000	0.000

Appendices B

B-1. Recovery and coefficients of variation for oxfendazole (FBZ.SO) and its metabolites (in combination with piperonyl butoxide or alone) following liquid phase extraction from plasma.

	Added concentration (μg/ml)	Recovery (%)	Coefficient of variation (%) (Between assay)
FBZ.SO	0.01	95.48 (n=6)	9.55
	0.05	98.75 (n=6)	11.12
	0.1	96.20 (n=6)	9.48
	1	97.78 (n=6)	11.29
	2	96.64 (n=6)	7.66
Mean		96.97 (n=30)	9.82
FBZ	0.01	95.89 (n=6)	12.43
	0.05	95.07 (n=6)	12.05
	0.1	92.95 (n=6)	12.61
	1	98.58 (n=6)	10.67
	2	94.51(n=6)	11.33
Mean		95.40 (n=30)	11.82
$FBZ.SO_2$	0.01	96.91 (n=6)	5.87
	0.05	99.63 (n=6)	11.68
	0.1	90.39 (n=6)	11.19
	1	95.49 (n=6)	13.39
	2	98.56 (n=6)	7.84
Mean		96.20 (n=30)	10.00

B-2. Plasma concentrations (μg/ml) of oxfendazole (FBZ.SO) following iv administration of oxfendazole (10 mg/kg) alone in ponies.

	Animals					
Time	A	B	C	D	E	F
10 min	1.85	0.97	1.16	1.02	1.37	1.48
20 min	1.32	1.05	1.32	1.36	0.96	1.14
30 min	1.42	1.16	1.05	1.11	0.83	1.28
45 min	0.93	0.90	1.07	0.95	0.76	1.27
60 min	1.05	1.02	1.05	1.02	0.87	1.20
75 min	1.27	1.32	1.06	0.87	0.71	0.82
90 min	1.15	1.05	1.15	0.99	0.52	0.86
105 min	1.24	1.19	1.13	0.79	0.62	1.23
120 min	1.36	1.26	1.08	0.83	0.77	1.20
2.5 h	0.89	1.25	1.12	0.79	0.92	1.28
3 h	0.92	1.30	1.21	0.95	0.98	1.38
3.5 h	1.28	1.11	1.28	0.92	0.98	1.32
4 h	1.27	1.25	1.30	1.14	0.88	1.50
5 h	1.43	0.72	1.31	0.94	0.63	0.98
6 h	1.38	0.83	1.19	0.87	0.81	1.32
7 h	1.25	1.15	1.26	0.77	0.67	1.08
8 h	1.49	1.23	1.21	0.73	2.95	1.40
10 h	1.18	1.12	1.23	0.86	0.80	1.29
12 h	1.01	0.88	1.10	0.70	2.16	1.25
16 h	0.98	0.80	1.04	0.47	0.74	1.11
20 h	0.81	0.84	0.75	0.36	0.49	0.62
24 h	0.23	0.43	0.69	0.32	0.38	0.56
30 h	0.00	0.44	0.70	0.32	0.20	0.28
34 h	0.00	0.24	0.43	0.18	0.18	0.20
48 h	0.08	0.15	0.36	0.19	0.12	0.14
54 h	0.14	0.12	0.27	0.16	0.05	0.10
72 h	0.11	0.03	0.29	0.15	0.02	0.04
96 h	0.03	0.00	0.20	0.08	0.06	0.02

B-3. Plasma concentrations (μg/ml) of oxfendazole (FBZ.SO) following iv administration of oxfendazole (10 mg/kg) in combination with piperonyl butoxide (PB) (31 mg/kg) in ponies.

	Animals					
Time	A	B	C	D	E	F
10 min	1.66	1.39	1.83	1.66	1.03	1.47
20 min	1.58	1.39	1.56	1.11	1.20	1.15
30 min	1.18	1.20	1.18	1.20	1.16	1.29
45 min	1.52	1.29	1.76	1.05	1.70	1.24
60 min	1.48	1.68	1.81	1.28	1.71	1.25
75 min	1.86	1.75	1.84	1.69	2.04	1.35
90 min	1.90	1.97	2.12	1.32	2.08	1.25
105 min	2.22	2.11	2.14	1.26	1.61	1.57
120 min	2.18	2.28	2.30	1.79	2.11	1.41
2.5 h	1.96	2.31	2.98	2.12	2.84	1.96
3 h	2.68	2.68	3.11	2.64	2.76	2.12
3.5 h	3.74	3.36	2.06	3.19	2.48	2.47
4 h	3.45	3.24	4.00	3.22	2.45	2.03
5 h	4.19	4.08	4.01	2.46	2.30	2.12
6 h	3.56	3.87	4.82	2.75	2.35	2.61
7 h	4.62	4.61	5.11	2.77	2.35	2.67
8 h	3.88	1.14	4.16	2.94	2.65	3.14
10 h	3.02	3.48	3.70	1.92	2.01	2.16
12 h	2.12	0.89	3.48	1.00	1.48	1.90
16 h	1.38	1.89	2.89	1.02	1.03	1.29
20 h	1.00	1.05	1.93	0.41	0.40	0.42
24 h	0.65	0.59	1.01	0.22	0.33	0.31
30 h	0.35	0.29	0.32	0.13	0.15	0.31
34 h	0.17	0.15	0.21	0.07	0.11	0.13
48 h	0.09	0.09	0.11	0.04	0.10	0.09
54 h	0.06	0.08	0.10	0.03	0.12	0.05
72 h	0.04	0.04	0.06	0.02	0.04	0.04
96 h	0.02	0.02	0.03	0.01	0.05	0.03

B-4. Plasma concentrations (μg/ml) of fenbendazole (FBZ) following iv administration of oxfendazole (10 mg/kg) alone in ponies.

	Animals					
Time	A	B	C	D	E	F
10 min	0.02	0.02	0.00	0.02	0.01	0.00
20 min	0.00	0.01	0.02	0.04	0.00	0.00
30 min	0.01	0.02	0.01	0.03	0.02	0.00
45 min	0.01	0.01	0.03	0.01	0.00	0.00
60 min	0.02	0.02	0.02	0.00	0.01	0.00
75 min	0.01	0.02	0.02	0.00	0.00	0.00
90 min	0.01	0.01	0.00	0.00	0.00	0.00
105 min	0.00	0.00	0.00	0.00	0.00	0.00
120 min	0.03	0.00	0.00	0.00	0.00	0.00
2.5 h	0.00	0.00	0.01	0.00	0.00	0.00
3 h	0.00	0.00	0.04	0.00	0.00	0.00
3.5 h	0.01	0.02	0.07	0.00	0.00	0.00
4 h	0.00	0.02	0.01	0.00	0.01	0.00
5 h	0.01	0.01	0.04	0.00	0.00	0.00
6 h	0.02	0.01	0.04	0.00	0.03	0.03
7 h	0.02	0.01	0.08	0.00	0.02	0.03
8 h	0.04	0.01	0.05	0.01	0.03	0.04
10 h	0.04	0.01	0.04	0.03	0.04	0.04
12 h	0.03	0.01	0.03	0.01	0.05	0.06
16 h	0.05	0.02	0.02	0.03	0.04	0.08
20 h	0.04	0.02	0.02	0.01	0.04	0.05
24 h	0.00	0.03	0.02	0.02	0.09	0.05
30 h	0.00	0.02	0.03	0.01	0.03	0.07
34 h	0.00	0.01	0.01	0.00	0.09	0.01
48 h	0.00	0.00	0.00	0.00	0.03	0.00
54 h	0.00	0.00	0.00	0.00	0.00	0.00
72 h	0.00	0.00	0.00	0.00	0.00	0.00
96 h	0.00	0.00	0.00	0.00	0.00	0.00

B-5. Plasma concentrations (μg/ml) of fenbendazole (FBZ) following iv administration of oxfendazole (10 mg/kg) in combination with piperonyl butoxide (PB) (31 mg/kg) in ponies.

	Animals					
Time	A	B	C	D	E	F
10 min	0.01	0.02	0.05	0.03	0.00	0.05
20 min	0.02	0.05	0.04	0.01	0.00	0.01
30 min	0.01	0.02	0.02	0.02	0.01	0.04
45 min	0.03	0.02	0.04	0.01	0.02	0.01
60 min	0.01	0.01	0.03	0.01	0.01	0.01
75 min	0.02	0.01	0.00	0.02	0.00	0.01
90 min	0.02	0.01	0.05	0.02	0.00	0.01
105 min	0.01	0.02	0.02	0.01	0.00	0.01
120 min	0.01	0.02	0.01	0.01	0.02	0.03
2.5 h	0.01	0.02	0.01	0.01	0.03	0.01
3 h	0.02	0.04	0.01	0.02	0.01	0.02
3.5 h	0.02	0.03	0.01	0.03	0.01	0.02
4 h	0.02	0.01	0.02	0.04	0.02	0.04
5 h	0.03	0.05	0.02	0.04	0.02	0.04
6 h	0.04	0.04	0.06	0.06	0.03	0.05
7 h	0.05	0.05	0.06	0.08	0.04	0.04
8 h	0.05	0.04	0.06	0.09	0.07	0.04
10 h	0.06	0.07	0.07	0.11	0.08	0.07
12 h	0.07	0.05	0.10	0.08	0.05	0.09
16 h	0.07	0.08	0.09	0.13	0.10	0.13
20 h	0.09	0.11	0.10	0.07	0.05	0.06
24 h	0.07	0.06	0.10	0.05	0.06	0.05
30 h	0.09	0.09	0.10	0.05	0.04	0.03
34 h	0.04	0.06	0.08	0.02	0.01	0.02
48 h	0.01	0.02	0.07	0.00	0.00	0.01
54 h	0.01	0.01	0.00	0.00	0.00	0.01
72 h	0.00	0.00	0.00	0.00	0.00	0.00
96 h	0.00	0.00	0.00	0.00	0.00	0.00

B-6. Plasma concentrations (μg/ml) of fenbendazole sulphone ($FBZ.SO_2$) following iv administration of oxfendazole (10 mg/kg) alone in ponies.

	Animals					
Time	A	B	C	D	E	F
10 min	0.40	0.22	0.22	0.17	0.23	0.26
20 min	0.39	0.31	0.35	0.30	0.30	0.31
30 min	0.51	0.43	0.35	0.32	0.34	0.52
45 min	0.44	0.38	0.44	0.45	0.35	0.60
60 min	0.57	0.51	0.53	0.55	0.48	0.67
75 min	0.75	0.73	0.59	0.48	0.43	0.54
90 min	0.75	0.65	0.70	0.62	0.38	0.62
105 min	0.86	0.80	0.00	0.58	0.39	0.90
120 min	1.01	0.90	0.00	0.69	0.60	0.89
2.5 h	0.63	1.01	0.82	0.74	0.80	1.05
3 h	0.88	1.15	0.98	0.97	0.90	1.15
3.5 h	1.35	0.97	0.98	0.92	0.89	1.19
4 h	1.26	1.21	1.20	1.08	0.94	1.40
5 h	1.64	0.79	1.30	1.09	0.70	1.00
6 h	1.89	0.97	1.30	1.08	1.01	1.43
7 h	1.71	1.59	1.45	0.93	0.86	1.14
8 h	2.15	1.77	1.46	1.11	0.82	1.53
10 h	1.84	1.71	1.61	1.22	1.05	1.50
12 h	1.67	1.44	1.50	0.88	1.10	1.57
16 h	1.72	1.40	1.48	0.76	1.42	1.73
20 h	1.44	1.64	1.25	0.57	1.21	1.28
24 h	0.08	0.95	1.03	0.56	1.15	1.30
30 h	0.00	1.18	0.81	0.24	0.83	0.95
34 h	0.00	0.80	0.66	0.46	0.67	0.72
48 h	0.03	0.45	0.32	0.19	0.30	0.42
54 h	0.09	0.28	0.28	0.12	0.18	0.27
72 h	0.05	0.11	0.11	0.04	0.08	0.09
96 h	0.02	0.03	0.06	0.03	0.07	0.05

B-7. Plasma concentrations (μg/ml) of fenbendazole sulphone ($FBZ.SO_2$) following iv administration of oxfendazole (10 mg/kg) in combination with piperonyl butoxide (PB) (31 mg/kg) in ponies.

	Animals					
Time	A	B	C	D	E	F
10 min	0.30	0.26	0.23	0.26	0.15	0.18
20 min	0.41	0.39	0.27	0.21	0.17	0.31
30 min	0.34	0.37	0.25	0.29	0.17	0.39
45 min	0.50	0.44	0.42	0.29	0.22	0.46
60 min	0.48	0.52	0.44	0.38	0.19	0.49
75 min	0.60	0.51	0.44	0.52	0.20	0.55
90 min	0.60	0.53	0.47	0.40	0.19	0.52
105 min	0.63	0.60	0.46	0.37	0.13	0.70
120 min	0.57	0.62	0.47	0.39	0.19	0.54
2.5 h	0.47	0.58	0.55	0.60	0.26	0.68
3 h	0.60	0.64	0.56	0.76	0.22	0.63
3.5 h	0.78	0.78	0.34	0.91	0.20	0.72
4 h	0.71	0.74	0.69	0.98	0.23	0.53
5 h	0.82	0.97	0.49	0.79	0.23	0.54
6 h	0.78	0.95	0.80	0.99	0.26	0.75
7 h	1.04	1.02	0.83	1.01	0.32	0.68
8 h	0.90	1.38	0.69	1.24	0.43	0.85
10 h	0.95	1.13	0.73	1.03	0.56	0.66
12 h	1.09	1.38	0.89	0.87	0.30	0.88
16 h	1.39	1.71	1.43	1.72	0.85	1.30
20 h	1.81	1.93	1.73	1.20	0.80	0.62
24 h	1.64	1.78	1.76	1.00	1.05	0.81
30 h	1.39	1.66	1.40	0.96	0.62	0.78
34 h	0.92	1.02	1.11	0.48	0.43	0.44
48 h	0.45	0.39	0.29	0.20	0.15	0.16
54 h	0.21	0.27	0.20	0.14	0.09	0.12
72 h	0.10	0.10	0.07	0.08	0.03	0.06
96 h	0.04	0.05	0.03	0.03	0.05	0.01

B-8. Plasma concentrations (μg/ml) of the enantiomer (FBZ.SO-1) of oxfendazole (FBZ.SO) in ponies following iv administration of oxfendazole (10 mg/kg) alone.

	Animals					
Time	A	B	C	D	E	F
10 min	0.98	0.37	0.86	0.53	0.81	0.44
20 min	0.89	0.32	0.71	0.62	0.74	0.59
30 min	0.71	0.30	0.73	0.55	0.61	0.46
45 min	0.71	0.28	0.71	0.54	0.71	0.43
60 min	0.74	0.26	0.64	0.58	0.71	0.41
75 min	0.80	0.31	0.64	0.59	0.79	0.43
90 min	0.78	0.32	0.54	0.66	0.72	0.46
105 min	0.78	0.66	0.67	0.62	0.81	0.46
120 min	0.80	0.63	0.65	0.63	0.75	0.46
2.5 h	0.81	0.68	0.65	0.68	0.86	0.47
3 h	0.80	0.72	0.57	0.77	0.94	0.50
3.5 h	1.00	0.76	0.76	0.81	0.79	0.43
4 h	0.89	0.79	0.86	0.69	1.02	0.47
5 h	0.85	0.79	0.76	0.75	0.84	0.52
6 h	0.87	0.83	0.47	0.71	0.67	0.47
7 h	0.91	0.74	0.58	0.60	0.54	0.30
8 h	0.88	0.00	0.80	0.75	0.50	0.26
10 h	0.78	0.70	0.59	0.68	0.74	0.36
12 h	0.63	0.00	0.55	0.58	0.58	0.34
16 h	0.61	0.50	0.45	0.47	0.69	0.23
20 h	0.44	0.32	0.37	0.42	0.35	0.17
24 h	0.35	0.24	0.30	0.32	0.35	0.13
30 h	0.18	0.13	0.16	0.15	0.12	0.09
34 h	0.12	0.08	0.13	0.11	0.14	0.06
48 h	0.05	0.05	0.06	0.05	0.07	0.00
54 h	0.00	0.00	0.05	0.00	0.00	0.00
72 h	0.00	0.00	0.00	0.00	0.00	0.00
96 h	0.00	0.00	0.00	0.00	0.00	0.00

B-9. Plasma concentrations (μg/ml) of the enantiomer (FBZ.SO-2) of oxfendazole (FBZ.SO) in ponies following iv administration of oxfendazole (10 mg/kg) alone.

	Animals					
Time	A	B	C	D	E	F
10 min	1.07	0.43	1.07	0.58	0.99	0.50
20 min	0.91	0.31	0.88	0.70	0.89	0.67
30 min	0.66	0.25	0.96	0.48	0.72	0.47
45 min	0.69	0.24	0.87	0.53	0.67	0.37
60 min	0.62	0.23	0.69	0.44	0.78	0.35
75 min	0.60	0.25	0.66	0.45	0.79	0.34
90 min	0.58	0.24	0.54	0.44	0.68	0.36
105 min	0.56	0.43	0.63	0.50	0.68	0.34
120 min	0.54	0.42	0.51	0.38	0.67	0.31
2.5 h	0.57	0.44	0.57	0.50	0.70	0.30
3 h	0.52	0.44	0.47	0.52	0.63	0.31
3.5 h	0.66	0.47	0.57	0.45	0.55	0.25
4 h	0.59	0.49	0.57	0.46	0.67	0.29
5 h	0.55	0.48	0.46	0.48	0.50	0.31
6 h	0.59	0.50	0.28	0.41	0.39	0.26
7 h	0.63	0.50	0.33	0.33	0.31	0.16
8 h	0.61	ND	0.48	0.38	0.28	0.14
10 h	0.58	0.46	0.32	0.36	0.38	0.21
12 h	0.47	0.00	0.32	0.31	0.34	0.18
16 h	0.44	0.30	0.28	0.26	0.41	0.13
20 h	0.31	0.19	0.25	0.24	0.22	0.10
24 h	0.23	0.16	0.18	0.19	0.23	0.07
30 h	0.15	0.11	0.12	0.09	0.09	0.07
34 h	0.11	0.07	0.11	0.07	0.11	0.05
48 h	0.06	0.00	0.06	0.00	0.08	0.00
54 h	0.05	0.00	0.05	0.00	0.05	0.00
72 h	0.00	0.00	0.00	0.00	0.00	0.00
96 h	0.00	0.00	0.00	0.00	0.00	0.00

B-10. Plasma concentrations (μg/ml) of the enantiomer (FBZ.SO-1) of oxfendazole (FBZ.SO) in ponies following iv administration of oxfendazole (10 mg/kg) in combination with piperonyl butoxide (PB) (31 mg/kg).

	Animals					
Time	A	B	C	D	E	F
10 min	0.85	1.05	1.11	0.98	0.49	0.36
20 min	0.81	0.92	0.96	0.89	0.48	0.45
30 min	0.74	0.87	0.87	0.71	0.52	0.43
45 min	0.84	0.89	0.91	0.78	0.81	0.53
60 min	0.90	1.02	0.93	0.74	0.63	0.56
75 min	0.98	1.10	0.94	0.80	0.75	0.67
90 min	1.08	1.16	0.96	0.78	0.82	0.75
105 min	1.18	1.20	1.25	0.78	0.66	0.85
120 min	1.29	1.28	1.10	0.80	0.81	0.84
2.5 h	1.38	1.55	1.25	0.87	1.09	0.87
3 h	1.44	1.74	1.24	0.80	0.83	0.87
3.5 h	1.55	2.16	1.31	1.00	0.97	1.02
4 h	1.58	1.95	1.51	0.89	1.12	0.93
5 h	1.86	2.06	1.64	0.85	0.99	0.90
6 h	1.71	2.11	1.54	0.87	0.62	1.05
7 h	1.34	2.03	1.51	0.91	1.25	1.09
8 h	0.00	2.04	1.59	0.88	0.97	0.82
10 h	0.00	1.77	1.48	0.78	0.83	0.84
12 h	0.65	1.60	1.40	0.63	0.80	0.97
16 h	1.11	1.19	1.33	0.61	0.54	0.42
20 h	0.75	0.84	1.37	0.44	0.27	0.23
24 h	0.43	0.49	1.01	0.35	0.17	0.13
30 h	0.21	0.28	0.53	0.18	0.07	0.05
34 h	0.12	0.13	0.12	0.12	0.05	0.00
48 h	0.05	0.06	0.05	0.05	0.00	0.00
54 h	0.00	0.00	0.00	0.00	0.00	0.00
72 h	0.00	0.00	0.00	0.00	0.00	0.00
96 h	0.00	0.00	0.00	0.00	0.00	0.00

B-11. Plasma concentrations (μg/ml) of the enantiomer (FBZ.SO-2) of oxfendazole (FBZ.SO) in ponies following iv administration of oxfendazole (10 mg/kg) in combination with piperonyl butoxide (PB) (31 mg/kg).

	Animals					
Time	A	B	C	D	E	F
10 min	1.08	1.07	1.35	1.00	0.47	0.30
20 min	0.84	0.86	1.06	0.95	0.42	0.44
30 min	0.63	0.87	0.90	0.86	0.48	0.47
45 min	0.87	0.90	0.97	0.87	0.69	0.58
60 min	0.99	1.13	0.93	0.82	0.56	0.67
75 min	1.04	1.10	0.97	1.00	0.61	0.80
90 min	1.07	1.16	0.98	1.11	0.69	0.84
105 min	1.18	1.27	1.26	1.15	0.53	1.02
120 min	1.38	1.38	1.13	1.23	0.70	1.03
2.5 h	1.55	1.88	0.00	1.43	0.99	1.11
3 h	1.72	1.98	1.39	1.70	0.86	1.16
3.5 h	1.94	0.00	2.13	1.89	1.03	1.46
4 h	1.96	2.38	1.80	1.76	1.31	1.35
5 h	2.47	2.66	2.20	1.50	1.15	1.29
6 h	2.27	2.71	2.10	1.50	1.30	1.53
7 h	1.86	2.66	2.15	1.61	1.47	1.70
8 h	0.00	2.45	2.20	1.62	1.21	1.09
10 h	0.00	2.15	2.07	1.36	0.98	1.06
12 h	0.42	1.47	0.00	0.78	1.01	1.45
16 h	0.91	0.79	1.65	0.43	0.45	0.28
20 h	0.46	0.45	1.42	0.19	0.19	0.13
24 h	0.23	0.27	0.84	0.14	0.10	0.07
30 h	0.12	0.16	0.36	0.07	0.05	0.05
34 h	0.09	0.09	0.07	0.06	0.00	0.00
48 h	0.05	0.05	0.05	0.00	0.00	0.00
54 h	0.00	0.00	0.00	0.00	0.00	0.00
72 h	0.00	0.00	0.00	0.00	0.00	0.00
96 h	0.00	0.00	0.00	0.00	0.00	0.00

Appendices C

C-1. Quantities of components of the NADPH-generating cofactor solution for ten assays.

Components	Amount required per ten assays
Tris buffer, pH 7.4, 0.1 M	8.5 ml
$MgCl_2$, 0.15 M	1.0 ml
Nicotinamide, 0.5 M	1.0 ml
Trisodium isicitrate	40.0 mg
Isocitrate dehydorgenase	2.0 units
$NADP^+$	8.0 mg

N.B. - 1.0 ml of the above solution is usually required per assay.
- The components should be thoroughly mixed and dissolved prior to use.
- The mixed solution should not be allowed stand for more than a few minutes; otherwise the generated NADPH will break down.

C-2. Calculated amounts of microsomal protein used.

	Animals						
	T	U	V	W	X	Y	Z
Microsomal protein (mg/g of liver)	7.22	10.11	4.5	6.00	4.95	10.50	8.40
Microsomal protein (mg/ml) of microsome suspension)	12.04	16.84	7.50	16.60	8.20	17.50	14.00
Weight of liver used (g)	100	100	100	100	100	100	100
Final volume of microsome suspension (ml)	60	60	60	60	60	60	60
Volume of microsome suspension (ml) per 4 mg protein	0.332	0.237	0.533	0.240	0.487	0.228	0.285

C-3. Amount of oxfendazole (FBZ.SO) (nmol) remaining in the microsomal reaction mixture after oxfendazole incubation with and without piperonyl butoxide (PB).

		FBZ.SO (nmol)							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	468	427	475	339	431	449	490	440 ± 50
	1 (n=7)	928	911	974	788	881	920	1054	922 ± 81
	2.5 (n=7)	2149	2307	2135	1973	2183	2352	2393	2213 ± 147
Without PB	0.5 (n=7)	98	18	119	32	38	15	30	50 ± 41
	1 (n=7)	205	55	295	70	79	53	95	121 ± 93
	2.5 (n=7)	623	708	986	287	304	284	526	531 ± 264

C-4. Amount of fenbendazole sulphone ($FBZ.SO_2$) (nmol/mg protein).h^{-1}) formed in the microsomal reaction mixture after oxfendazole incubation with and without piperonyl butoxide (PB).

		$FBZ.SO_2$ (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	3	13	7	9	8	10	5	8 ± 3
	1 (n=7)	5	24	11	16	17	18	11	15 ± 6
	2.5 (n=7)	13	54	22	31	35	43	25	32 ± 14
Without PB	0.5 (n=7)	20	15	21	18	21	18	21	19 ± 2
	1 (n=7)	40	48	44	37	46	47	46	44 ± 4
	2.5 (n=7)	86	148	109	91	121	130	116	114 ± 21

C-5. Amount of fenbendazole (FBZ) (nmol) remaining in the microsomal reaction mixture after fenbendazole incubation with and without piperonyl butoxide (PB).

		FBZ (nmol)							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	446	336	446	409	330	446	473	412 ± 57
	1 (n=7)	834	633	859	782	718	904	849	797 ± 94
	2.5 (n=7)	2037	1692	2216	2190	1934	2252	2215	2077 ± 205
Without PB	0.5 (n=7)	105	19	63	60	43	78	39	58 ± 28
	1 (n=7)	297	64	255	184	96	177	104	168 ± 86
	2.5 (n=7)	1234	533	1222	948	606	474	532	793 ± 335

C-6. Amount of fenbendazole sulphoxide (FBZ.SO) (nmol/mg protein).h^{-1}) formed in the microsomal reaction mixture after incubation with fenbendazole with and without piperonyl butoxide (PB).

		FBZ.SO (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	9	17	9	13	12	12	14	12 ± 3
	1 (n=7)	13	25	14	20	21	20	24	20 ± 4
	2.5 (n=7)	28	45	23	41	47	35	44	38 ± 9
Without PB	0.5 (n=7)	16	7	20	30	8	4	6	13 ± 9
	1 (n=7)	29	16	40	52	17	10	15	26 ± 16
	2.5 (n=7)	64	68	79	105	65	46	60	70 ± 18

C-7. Amount of fenbendazole sulphone ($FBZ.SO_2$) (nmol/mg protein).h^{-1}) formed in the microsomal reaction mixture after incubation with fenbendazole with and without piperonyl butoxide (PB).

		$FBZ.SO_2$ (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	0	6	4	8	7	7	0	5 ± 3
	1 (n=7)	0	6	5	7	8	8	0	5 ± 4
	2.5 (n=7)	0	6	5	8	9	9	0	5 ± 4
Without PB	0.5 (n=7)	4	13	6	9	8	10	9	8 ± 3
	1 (n=7)	8	29	11	17	18	18	17	17 ± 7
	2.5 (n=7)	14	64	17	27	44	44	33	35 ± 18

C-8. Amount of hydroxy fenbendazole (OH.FBZ) (nmol/mg protein).h^{-1}) formed in the microsomal reaction mixture after incubation with fenbendazole with and without piperonyl butoxide (PB).

		OH.FBZ (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	6	21	3	12	9	6	8	9 ± 6
	1 (n=7)	11	33	6	22	19	11	15	17 ± 9
	2.5 (n=7)	26	59	11	47	44	24	30	34 ± 16
Without PB	0.5 (n=7)	8	12	6	11	6	4	4	7 ± 3
	1 (n=7)	18	27	13	24	14	9	11	16 ± 7
	2.5 (n=7)	46	79	27	55	50	33	36	47 ± 17

C-9. Amount of oxibendazole (OBZ) (nmol) remaining in the microsomal reaction mixture after its incubation with and without piperonyl butoxide (PB).

		OBZ (nmol)							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	4	3	0	1	14	0	15	5 ± 6
	1 (n=7)	9	5	2	7	16	78	165	40 ± 61
	2.5 (n=7)	257	325	126	329	243	739	937	422 ± 297
Without PB	0.5 (n=7)	110	125	11	34	91	170	296	119 ± 95
	1 (n=7)	296	350	96	218	320	425	779	355 ± 214
	2.5 (n=7)	1324	1688	848	1406	1434	1867	1970	1505 ± 378

C-10. Amount of the first metabolite (M1) (nmol/mg protein. h^{-1}) formed in the microsomal reaction mixture after oxibendazole incubation with and without piperonyl butoxide (PB).

		M1 (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	10	14	12	15	12	18	19	15 ± 3
	1 (n=7)	24	32	27	33	26	40	38	31 ± 6
	2.5 (n=7)	53	62	66	68	51	78	62	63 ± 9
Without PB	0.5 (n=7)	10	12	11	14	10	13	8	11 ± 2
	1 (n=7)	21	23	23	23	17	18	17	20 ± 3
	2.5 (n=7)	41	39	43	35	29	28	24	34 ± 7

C-11. Amount of the second metabolite (M2) (nmol/mg protein. h^{-1}) formed in the microsomal reaction mixture after oxibendazole incubation with and without piperonyl butoxide (PB).

		M2 (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	4	14	8	23	10	16	18	13 ± 6
	1 (n=7)	14	39	25	64	30	46	41	37 ± 16
	2.5 (n=7)	44	103	85	153	78	111	84	94 ± 34
Without PB	0.5 (n=7)	2	2	2	3	2	3	0	2 ± 1
	1 (n=7)	4	5	5	9	5	5	5	5 ± 2
	2.5 (n=7)	8	11	13	19	11	7	8	11 ± 4

C-12. Amount of the third metabolite (M3) (nmol/mg protein. h^{-1}) formed in the microsomal reaction mixture after oxibendazole incubation with and without piperonyl butoxide (PB).

		M3 (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	0	0	0	4	1	4	13	3 ± 5
	1 (n=7)	2	2	3	15	4	13	31	10 ± 11
	2.5 (n=7)	34	14	42	47	27	37	60	37 ± 15
Without PB	0.5 (n=7)	31	46	31	53	39	38	30	38 ± 9
	1 (n=7)	87	94	78	91	74	93	57	82 ± 13
	2.5 (n=7)	186	164	152	128	119	113	88	136 ± 33

C-13. Amount of the fourth metabolite (M4) (nmol/mg protein. h^{-1}) formed in the microsomal reaction mixture after oxibendazole incubation with and without piperonyl butoxide (PB).

		M4 (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5 (n=7)	1	3	2	2	2	2	0	2 ± 1
	1 (n=7)	4	9	5	7	7	7	6	6 ± 1
	2.5 (n=7)	14	23	18	18	16	5	12	15 ± 6
Without PB	0.5 (n=7)	0	0	0	1	1	0	0	0 ± 0
	1 (n=7)	0	1	2	1	1	0	0	1 ± 1
	2.5 (n=7)	0	4	4	3	3	0	0	2 ± 2

C-14. Total enantiomer (FBZ.SO-1) remaining in microsome reaction mixture following oxfendazole (0.5, 1, 2.5 μM) incubation with and without piperonyl butoxide.

		FBZ.SO-1 (nmol)						
	Conc. (μM)	T	U	V	W	X	Y	Mean ± SD
With PB	0.5	277	199	148	238	199	259	220 ± 47
	1	536	462	278	458	462	539	456 ± 95
	2.5	1442	1191	828	944	1191	1277	1146 ± 224
Without PB	0.5	93	88	21	21	15	25	44 ± 36
	1	218	182	44	54	52	88	107 ± 75
	2.5	640	561	238	285	192	516	405 ± 190

C-15. Total enantiomer (FBZ.SO-2) remaining in microsome reaction mixture following oxfendazole (0.5, 1, 2.5 μM) incubation with and without piperonyl butoxide.

		FBZ.SO-2 (nmol)						
	Conc. (μM)	T	U	V	W	X	Y	Mean ± SD
With PB	0.5	187	185	125	133	240	251	187 ± 52
	1	437	428	266	263	478	518	398 ± 109
	2.5	998	1118	822	608	1185	1209	990 ± 235
Without PB	0.5	0	33	0	0	0	0	6 ± 13
	1	0	87	0	0	0	0	15 ± 36
	2.5	104	341	0	0	0	0	74 ± 137

C-16. Enantiomers (FBZ.SO-1) produced in microsome reaction mixture following fenbendazole (0.5, 1, 2.5 μM) incubation with and without piperonyl butoxide.

		FBZ.SO-1 (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5	0	10	8	5	9	8	6	6 ± 3
	1	8	17	10	10	15	13	13	12 ± 3
	2.5	22	23	21	17	31	17	26	22 ± 5
Without PB	0.5	17	5	20	9	8	5	5	10 ± 6
	1	39	12	35	17	18	11	15	21 ± 11
	2.5	88	50	74	42	72	53	63	63 ± 16

C-17. Enantiomers (FBZ.SO-2) produced in microsome reaction mixture following fenbendazole (0.5, 1, 2.5 μM) incubation with and without piperonyl butoxide.

		FBZ.SO-2 (nmol/mg protein).h^{-1}							
	Conc. (μM)	T	U	V	W	X	Y	Z	Mean ± SD
With PB	0.5	0	3	0	0	0	2	4	1 ± 2
	1	4	7	0	0	0	3	9	3 ± 4
	2.5	11	11	6	0	0	6	18	7 ± 7
Without PB	0.5	0	0	0	0	0	0	0	0 ± 0
	1	8	0	0	0	0	0	0	1 ± 3
	2.5	27	7	8	0	0	0	0	6 ± 10

Appendices D

D-1 Recovery and coefficients of variation for ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) following liquid phase extraction from plasma.

	Added concentration (ng/ml)	Recovery (%)	Coefficient of variation (%) (Between assay)
IVM	0.50	86.00 (n=7)	9.03
	1.00	84.64 (n=8)	12.44
	5.00	93.08 (n=7)	8.82
	10.00	85.40 (n=7)	8.37
	50.00	88.18 (n=7)	6.54
Mean		87.46 (n=36)	9.04
DRM	0.50	89.43 (n=8)	3.93
	1.00	90.40 (n=8)	6.22
	10.00	91.90 (n=8)	4.62
	20.00	88.68 (n=8)	5.90
	50.00	88.31 (n=8)	3.27
Mean		89.75 (n=40)	4.79
MXD	0.50	95.44 (n=8)	9.22
	1.00	96.19 (n=7)	3.74
	10.00	93.25 (n=8)	8.54
	20.00	95.20 (n=7)	9.07
	50.00	96.51 (n=8)	9.47
Mean		95.32 (n=38)	8.01

D-2. Plasma concentration (ng/ml) of ivermectin (IVM) following oral administration at a dose rate of 200 µg/kg bodyweight.

	Animal numbers							
Time	25	26	27	28	29	30	31	32
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1 h	0.28	3.30	0.29	0.00	0.00	0.00	0.00	4.04
2 h	5.77	10.76	2.41	1.34	0.65	1.41	4.41	13.88
4 h	28.77	28.70	20.59	8.53	13.74	6.52	10.87	37.93
8 h	39.48	31.37	17.35	11.91	17.02	7.47	8.52	25.85
12 h	28.04	27.34	17.67	10.11	NS	7.56	13.03	25.62
20 h	25.37	21.89	10.39	8.54	8.18	5.03	16.55	19.23
24 h	18.95	19.12	9.05	7.37	9.63	5.07	22.14	14.29
32 h	17.33	16.75	7.92	6.09	8.76	3.73	13.21	10.56
48 h	10.07	9.50	5.65	3.62	5.49	3.38	8.84	8.36
72 h	10.71	7.14	3.73	2.57	4.00	1.81	6.85	8.05
96 h	6.55	5.35	2.23	1.78	3.74	1.13	5.41	4.89
120 h	5.54	3.91	1.55	1.01	2.07	0.87	4.32	3.95
8 days	3.03	1.85	0.70	0.45	0.82	0.28	3.13	2.04
11 days	0.42	0.00	0.00	0.00	0.00	0.00	0.25	0.00
25 days	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39 days	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
66 days	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80 days	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

D-3. Pharmacokinetic parameters of ivermectin (IVM) following oral administration at a dose rate of 200 µg/kg bodyweight.

	Animal numbers							
Time (h)	25	26	27	28	29	30	31	32
C_{max}	39.48	31.37	20.59	11.91	17.02	7.56	22.14	37.93
t_{max} (h)	8.00	8.00	4.00	8.00	8.00	12.00	24.00	4.00
AUC_{last} (µg.h/ml)	81.70	65.30	33.21	22.46	33.87	16.02	58.35	60.37
$AUMC_{last}$ ($\mu g.h^2/ml$)	232.48	147.66	67.42	46.49	79.78	33.89	188.20	143.07
MRT_{last} (h)	2.85	2.26	2.03	2.03	2.36	2.12	3.23	2.37

D-4. Plasma concentration (ng/ml) of doramectin (DRM) following oral administration at a dose rate of 200 µg/kg bodyweight.

	Animal numbers							
Time	33	34	35	36	37	38	39	40
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1 h	0.27	0.00	3.12	0.81	7.79	0.00	1.39	0.30
2 h	3.87	0.26	7.89	2.90	25.96	0.00	3.28	6.30
4 h	22.82	3.38	34.56	14.99	49.39	6.60	8.30	48.90
8 h	17.55	8.25	32.22	16.88	40.58	11.02	9.72	48.86
12 h	18.38	10.78	26.43	19.78	28.58	NS	15.99	39.60
20 h	14.25	10.75	15.51	14.75	23.92	NS	16.06	25.05
24 h	13.57	6.71	13.90	11.91	23.06	13.58	14.51	21.63
32 h	13.01	6.70	NS	8.61	18.16	10.52	13.84	21.52
48 h	8.79	6.84	12.91	6.72	15.85	8.30	8.07	19.14
72 h	7.58	4.25	7.97	5.59	11.07	4.73	8.67	13.77
96 h	4.42	2.56	7.98	4.37	11.49	4.55	5.87	10.90
120 h	3.90	2.19	7.50	4.40	6.75	2.43	3.51	6.90
8 days	3.11	2.09	6.43	1.86	6.10	1.81	1.44	5.99
11 days	0.00	0.33	3.08	0.30	0.85	0.00	0.00	0.50
25 days	0.00	0.00	2.05	0.00	0.00	0.00	0.00	0.00
39 days	0.00	0.00	1.41	0.00	0.00	0.00	0.00	0.00
66 days	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
80 days	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

D-5. Pharmacokinetic parameters of doramectin (DRM) following oral administration at a dose rate of 200 µg/kg bodyweight.

	Animal numbers							
Time (h)	33	34	35	36	37	38	39	40
C_{max} (µg/ml)	22.82	10.78	34.56	19.78	49.39	13.58	16.07	48.91
t_{max} (h)	4.00	12.00	4.00	12.00	4.00	24.00	20.00	4.00
AUC_{last} (µg.h/ml)	55.58	34.91	154.91	49.82	108.72	40.87	51.08	116.44
$AUMC_{last}$ (µg.h^2/ml)	151.5	117.99	1754.5	151.13	349.55	108.61	134.45	353.07
MRT_{last} (h)	2.73	3.38	11.33	3.03	3.22	2.66	2.63	3.03

D-6. Plasma concentration (ng/ml) of moxidectin (MXD) following oral administration at a dose rate of 200 μg/kg bodyweight.

	Animal numbers							
Time	41	42	43	44	45	46	47	48
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1 h	0.83	0.53	0.29	9.05	0.92	0.26	1.50	0.00
2 h	6.67	8.68	3.62	29.58	7.11	1.27	4.66	2.79
4 h	15.26	30.69	16.44	57.10	17.92	13.31	27.94	10.36
8 h	31.25	33.48	19.54	37.03	17.84	30.32	28.95	19.70
12 h	22.80	31.12	10.93	29.03	14.98	32.53	15.21	17.19
20 h	19.20	20.78	7.77	24.07	10.26	20.75	16.52	13.84
24 h	16.22	20.53	7.39	20.91	7.29	11.13	9.09	12.32
32 h	9.68	12.98	NS	15.24	5.06	10.88	7.49	9.70
48 h	6.60	7.45	5.74	8.99	2.71	8.36	5.45	5.88
72 h	4.12	3.73	3.77	5.79	NS	5.87	3.55	3.66
96 h	2.96	3.50	2.97	4.48	1.20	5.01	3.48	2.73
120 h	2.13	3.20	2.62	3.63	1.02	2.70	3.32	1.10
8 days	1.06	1.61	2.48	2.72	1.27	2.51	1.97	1.34
11 days	0.70	1.25	0.91	1.21	0.88	0.59	1.82	0.67
25 days	0.41	1.13	0.70	0.61	0.55	0.70	1.29	0.65
39 days	0.40	0.62	0.83	0.58	NS	NS	1.08	0.40
66 days	NS	0.53	0.49	0.27	NS	0.58	0.82	0.39
80 days	NS	0.39	0.25	0.25	NS	0.42	0.64	0.00
188 days	NS	0.00	NS	NS	0.00	ULQ	NS	0.00
197 days	0.00	NS	0.00	0.00	NS	0.00	ULQ	0.00

D-7. Pharmacokinetic parameters of moxidectin (MXD) following oral administration at a dose rate of 200 μg/kg bodyweight.

	Animal numbers							
Time (h)	41	42	43	44	45	46	47	48
C_{max} (μg/ml)	31.25	33.48	19.54	57.10	17.92	32.53	28.95	19.70
t_{max} (h)	8.00	8.00	8.00	4.00	4.00	12.00	8.00	8.00
AUC_{last} (μg.h/ml)	60.69	110.62	84.59	110.87	38.33	101.06	122.65	65.66
$AUMC_{last}$ (μg.h^2/ml)	427.6	2097.7	1933.2	1498.2	252.0	2013.0	3122.4	1058.1
MRT_{last} (h)	7.04	18.96	22.85	13.51	6.58	19.92	25.46	16.11

D-8. Recovery and coefficients of variation for ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) following liquid phase extraction from wet faeces.

	Added concentration (ng/ml)	Recovery (%)	Coefficient of variation (%) (Between assay)
IVM	0.05	99.92 (n=6)	7.22
	0.10	93.03 (n=7)	4.48
	0.50	97.78 (n=8)	4.02
	1.00	94.61 (n=8)	3.64
	5.00	94.78 (n=8)	6.24
Mean		96.02 (n=37)	5.12
DRM	0.05	96.52 (n=6)	9.91
	0.10	92.39 (n=8)	7.70
	0.50	96.57 (n=8)	10.83
	1.00	103.01 (n=8)	11.61
	5.00	98.98 (n=6)	5.59
Mean		97.49 (n=36)	9.13
MXD	0.05	93.77 (n=6)	7.56
	0.10	80.82 (n=8)	5.97
	0.50	85.67 (n=8)	8.60
	1.00	89.55 (n=8)	12.67
	5.00	92.14 (n=6)	9.45
Mean		88.39 (n=36)	8.85

D-9. Dry faecal concentrations (μg/ml) of ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) following oral administration at a dose rate of 200 μg/kg bodyweight.

IVM	Animal numbers							
Time	25	26	27	28	29	30	31	32
0 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8 h	0.0	0.4	0.6	0.0	4.6	2.4	0.9	0.0
24 h	20.1	15.1	8.6	30.0	16.6	19.8	12.5	33.6
32 h	16.9	7.7	3.0	15.6	6.3	13.7	1.5	17.5
48 h	2.8	1.3	0.4	1.1	0.9	1.6	0.8	NS
120 h	0.0	0.0	0.2	0.0	0.0	0.0	0.0	NS
8 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

DRM	Animal numbers							
Time	33	34	35	36	37	38	39	40
0 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8 h	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3
24 h	18.8	21.6	34.3	16.4	NS	19.3	NS	12.8
32 h	13.3	NS	19.2	14.1	15.4	9.1	4.5	0.7
48 h	NS	0.8	1.5	4.7	13.6	2.1	NS	0.6
120 h	0.0	0.1	0.0	0.9	0.0	0.4	0.0	NS
8 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
11 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

MXD	Animal numbers							
Time	41	42	43	44	45	46	47	48
0 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8 h	0.0	0.7	0.0	0.0	0.0	0.1	0.0	0.0
24 h	27.8	11.5	NS	7.4	6.2	8.4	23.7	31.2
32 h	NS	3.8	NS	3.9	3.6	3.9	NS	14.4
48 h	NS	0.2	NS	NS	NS	0.2	1.2	2.1
120 h	0.0	0.0	0.0	0.0	0.0	0.0	NS	0.0
8 days	NS	NS	0.0	0.0	0.0	0.0	0.0	0.0
11 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendices E

E-1. Recovery and coefficients of variation for pyrantel (PYR) following liquid phase extraction from plasma.

	Added concentration (μg/ml)	Recovery (%)	Coefficient of variation (%) (Between assay)
PYR	0.005	90.81 (n=7)	12.12
	0.010	92.82 (n=8)	7.49
	0.050	96.68 (n=8)	7.52
	0.100	92.85 (n=8)	11.16
	0.500	91.17 (n=8)	6.00
Mean		92.16 (n=39)	8.86

E-2. Plasma concentration (μg/ml) of pyrantel (PYR) following oral administration at a dose rate of 13.3 mg/kg bodyweight.

	Animal numbers							
Time (h)	49	50	51	52	53	54	55	56
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	ULQ	0.008	0.000	0.000	0.005	0.008	0.007	0.009
2	0.011	0.038	0.005	ULQ	0.025	0.039	0.014	0.042
4	0.040	0.070	0.062	0.058	0.060	0.087	0.081	0.058
8	0.089	0.063	0.075	0.070	0.082	0.117	0.122	0.107
12	0.033	0.035	0.039	NS	0.065	0.062	0.052	0.043
20	0.012	0.027	0.014	0.010	0.017	0.020	NS	0.021
24	0.007	0.008	0.009	0.006	NS	0.021	0.012	0.014
32	ULQ	0.006	ULQ	ULQ	0.005	0.008	0.007	0.007
48	ULQ	0.000	ULQ	0.000	0.000	ULQ	NS	ULQ
72	ULQ	0.000	0.000	0.000	0.000	0.000	0.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

E-3. Pharmacokinetic parameters of pyrantel (PYR) following oral administration at a dose rate of 13.3 mg/kg bodyweight.

	Animal numbers							
Time (h)	49	50	51	52	53	54	55	56
C_{max} (μg/ml)	0.09	0.07	0.08	0.07	0.08	0.12	0.12	0.11
t_{max} (h)	8.00	4.00	8.00	8.00	8.00	8.00	8.00	8.00
AUC_{last} (μg.h/ml)	0.87	0.96	0.89	0.75	1.08	1.46	1.24	1.21
$AUMC_{last}$ ($\mu g.h^2/ml$)	12.50	10.66	10.77	7.81	12.27	18.36	13.64	15.75
MRT_{last} (h)	14.40	11.15	12.15	10.42	11.34	12.55	10.96	13.00

E-4. Recovery and coefficients of variation for pyrantel (PYR) following liquid phase extraction from faeces.

	Added concentration (µg/ml)	Recovery (%)	Coefficient of variation (%) (Between assay)
PYR	0.5	94.35 (n=8)	6.36
	5.0	94.85 (n=8)	3.39
	10.0	94.87 (n=8)	7.97
	100.0	93.15 (n=8)	5.75
	400.0	85.86 (n=7)	0.71
Mean		92.62 (n=39)	4.84

E-5. Dry faecal concentrations (mg/ml) of pyrantel (PYR) following oral administration at a dose rate of 13.3 mg/kg bodyweight.

	Animal numbers							
Time (h)	49	50	51	52	53	54	55	56
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
12	NS	NS	0.000	NS	0.000	NS	NS	NS
20	1.519	0.277	0.364	1.016	NS	NS	0.949	0.106
24	0.721	1.021	NS	1.006	1.099	1.023	1.155	1.215
32	0.542	0.603	NS	0.748	0.867	0.560	1.062	NS
48	0.023	0.111	0.148	0.067	0.193	NS	NS	0.095
72	0.000	0.004	0.016	0.000	0.000	0.000	0.004	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	NS