MEASUREMENT OF BENZIMIDAZOLES AND THEIR METABOLITES IN ANIMAL TISSUES

A THESIS
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OF
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BY

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OBJECTIVES

1) Development of a high performance liquid chromatographic method for the separate detection of benzimidazoles and their principal metabolites.

2) Evaluation of the reproducibility of the method using spiked tissue, milk and plasma samples.

3) Investigation of benzimidazole concentrations in plasma, tissues and milk of animals treated, from the day of administration until residues can no longer be detected.

4) To assess if the recommended withdrawal periods are adequate and, by means of an abattoir survey, if benzimidazole residues pose a problem to the consumer.

SUMMARY

A high performance liquid chromatographic method for the separate detection of benzimidazole anthelmintics and their principal metabolites has been developed, and an evaluation was made of the reproducibility of the method using spiked tissue, milk and plasma samples. In addition, an investigation of benzimidazole concentrations in tissues, milk and plasma of treated animals was carried out, from the day of administration until residues could no longer be detected, to assess if the recommended withdrawal periods were adequate and, by means of an abattoir survey, if benzimidazole residues pose a problem to the consumer. The animals used were cattle and sheep, and the drugs used were febantel (a pro-benzimidazole), and the benzimidazoles, albendazole, fenbendazole, oxfendazole and thiabendazole.

For all of the drugs studied residues were found in tissues. Relatively high concentrations of benzimidazoles and metabolites were found in liver compared with plasma or other tissues. Detectable residues of febantel, fenbendazole, oxfendazole and thiabendazole in liver persisted beyond the recommended withdrawal periods. In muscle, residues of all the drugs studied were low, being similar to those found in plasma.

None of the sulphide benzimidazoles (albendazole and fenbendazole) were detectable in milk at any time after administration to cows and only sulphoxides (oxfendazole and albendazole sulphoxide) and sulphones (oxfendazole sulphone and albendazole sulphone) were

detectable.

In an abattoir survey of cattle and sheep carcasses, no residues of any of these drugs were detected in one hundred livers analysed.

The relevance of these results to the safety of benzimidazoles in humans is discussed.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

The Ministry of Agriculture, Fisheries and Food in the United Kingdom is charged through the Veterinary Products Committee under the Medicines Act with the duty of ensuring that food does not contain residues of drugs or other contaminants which may be harmful to the consumer. Currently in the EEC there is a programme for monitoring antibiotic residues in milk, and programmes are being developed for the detection of antibiotics and non-natural hormones in meat.

Apart from antibiotics and hormones, anthelmintics are the most commonly used drugs in cattle and sheep in the United Kingdom, with animals being treated on average two to three times a year.

This study was designed to devise methods for the measurement of residues of benzimidazoles and pro-benzimidazoles and also to assess any residue problem that may exist.

The benzimidazoles and pro-benzimidazoles are a major group of anthelmintics, with sales which account for more than fifty per cent of the total UK market for anthelmintics (1984). It may be argued that residues of these anthelmintics would not pose a problem, as it is unlikely that any farmer would dose his animals at some expense (more than one pound per head of cattle and around fifteen pence per sheep) if they were to be slaughtered soon after treatment. However, there are always casualty animals, and also some residues have been found to be long-lived (see later) and therefore, residues may pose a problem. Possibly a greater problem would be residues of other drugs such as salicylanilide flukicides, which may persist for as long as three

months (Mohammed-Ali, 1985). In addition, the use of anthelmintics in boluses, either slow-release or pulsed release, enhances the risk of an animal at slaughter having measurable residues. Indeed, a pulsed release oxfendazole bolus with five pulses at twenty-one day intervals has recently been licenced (1986).

Other groups of anthelmintics available include the avermectins (ivermectin), the imidazothiazoles (levamisole, morantel and pyrantel), the salicylanilides and substituted phenols (closantel, rafoxanide, oxyclozanide, nitroxynil and brotianide), piperazines (diethylcarbamazine) and others (arecoline, bunamidine and praziquantel).

BENZIMIDAZOLES AND PRO-BENZIMIDAZOLES - BACKGROUND

Thiabendazole was introduced almost twenty-five years ago (Brown et al., 1961). It was the first broad-spectrum anthelmintic, which was later used as an agricultural fungicide and also as an anthelmintic in man.

The introduction of thiabendazole resulted in a considerable amount of research being carried out with benzimidazoles. As a result, a series of new benzimidazoles and pro-drugs appeared on the market.

The next benzimidazole to appear on the market was parbendazole (Actor et al., 1967), followed by cambendazole (Hoff et al., 1970), mebendazole (Brugman et al., 1971), oxibendazole (Theodorides et al.,

1973), fenbendazole (Baeder et al., 1974), oxfendazole (Averkin et al., 1975) and albendazole (Theodorides et al., 1976).

The earlier benzimidazoles were thiabendazole analogues and the more recent ones are benzimidazole carbamates (Table 1.1). They are all used as veterinary anthelmintics except carbendazim (the parent benzimidazole carbamate, i.e. unsubstituted on the benzene ring), ciclobendazole (which is not commercially available) and oncodazole (which is an experimental anti-cancer drug).

Halogenated benzimidazole analogues have been reported which have flukicidal activity. However, none of these compounds are used commercially, with the exception of triclabendazole which has recently appeared on the market (Boray et al., 1983).

Numerous pro-drugs can also lead to the formation of benzimidazoles via hydrolytic or cyclization processes. For example, benomyl (Watkins, 1976) is hydrolysed to carbendazim and similarly there is a pro-drug which hydrolyses to oxfendazole (Hennessy et al., 1983). Ketal derivatives of flubendazole and mebendazole have also been reported (Michiels et al., 1982).

Benzimidazole carbamate synthesis usually involves a cyclization reaction. This reaction has been utilised in the design of pro-drugs which may be converted chemically and/or metabolically to the anthelmintically active benzimidazole. Originally, the thiophanates were developed for use as fungicides, but the ethyl analogue is also

anthelmintically active. Cyclization is the process by which the thiophanates are metabolically converted to carbendazim or its ethyl analogue. The mechanism of this cyclization reaction is poorly understood. According to Douch (1974) this reaction is mono-oxygenase dependent. McDougall et al., (1985) suggest that netobimin (a novel anthelmintic which is a pro-drug of albendazole) undergoes cyclization to albendazole in the liver and is converted (oxidized) to albendazole sulphoxide in the rumen. However, it has been observed (Bogan and Marriner, unpublished) that cyclization occurs in the rumen to a greater extent, with only very small amounts of albendazole sulphoxide being present compared to the amount of albendazole. The rumen is considered to be a purely reductive medium (Prins, 1985) and it has been demonstrated (see later) that albendazole sulphoxide is reduced to albendazole to a great extent in the rumen. A pro-drug of mebendazole has also been reported (Dawson et al., 1983) which is absorbed unchanged from the gastro-intestinal tract of the rat, resulting in higher blood concentrations of mebendazole than those achieved when mebendazole is administered at the same dose rate.

Febantel (a commercially available pro-benzimidazole anthelmintic) follows a similar pattern to that of the thiophanates. Hydrolysis results in the removal of a methoxyacetyl group and this is followed by cyclization to a benzimidazole (fenbendazole) (Delatour et al., 1982b).

There are many examples of compounds which convert metabolically and/or hydrolytically to active benzimidazoles. The more complex

pro-drug, netobimin, undergoes metabolic reduction of a nitro group to an amino group before cyclizing to a benzimidazole (albendazole).

MODE OF ACTION OF BENZIMIDAZOLES

Several mechanisms of action have been proposed for benzimidazole anthelmintics : inhibition of the uptake of low molecular weight compounds (including glucose) by susceptible parasites (Van den Bossche, 1972; Van den Bossche and de Nollin, 1973): inhibition of fumarate reductase (Kohler and Bachmann, 1978; Prichard, 1973) and disruption of cytoplasmic microtubules in intestinal cells (Borgers et al., 1975; Van den Bossche, 1976).

Other studies have demonstrated that benzimidazoles are active against parasite embryos. However, the mode of embryotoxic action has not been determined (Coles and McNeillie, 1977; Samizadeh-Yazd and Todd, 1978).

The use of competitive colchicine binding studies and turbidimetric techniques has shown that benzimidazoles have an inhibitory effect on the polymerisation of tubulin into microtubules. These studies have been carried out on tubulin from rat brain (Hoebeke et al., 1976), sheep brain (Ireland et al., 1979), calf brain (Friedman and Platzer, 1978; Laclette et al., 1980; Lee et al., 1980) and pig brain (Kohler and Backmann, 1981). Benzimidazoles bind to the same site on the protein as colchicine does. Pre-incubation of tubulin in the presence of benzimidazoles inhibits the binding of colchicine

(Barrowman et al., 1984a). Inhibition of colchicine binding is generally competitive, although cambendazole shows non-competitive binding (Friedman and Platzer, 1978). Binding of benzimidazoles to tubulin is also saturable and reversible, with one binding site per tubulin dimer. This is also true for colchicine. Pre-formed microtubules are not affected in vitro by benzimidazoles (Hoebeke et al., 1976). Benzimidazoles also bind to nematode tubulin, which has a greater affinity (384 times greater) for benzimidazoles than mammalian tubulin derived from bovine brain (Friedman and Platzer, 1980). However, other investigators have only shown the binding of benzimidazoles to be two-fold greater than binding to pig brain tubulin (Kohler and Bachman, 1981).

A great deal of evidence has accumulated to suggest that inhibition of fumarate reductase or interference with glucose uptake are not the modes of action common to all benzimidazoles (Coles, 1977; Van den Bossche, 1978). It seems likely that microtubule inhibition is the major effect; inhibition of fumarate reductase and interference with glucose uptake occurring as a result of microtubule inhibition (Barrowman et al., 1984b).

It has been postulated that all the benzimidazole anthelmintics have the same mode of action and that differences in efficacy and spectrum of activity between different benzimidazoles are due to differences in the pharmacokinetic behaviour of the drugs (Prichard et al., 1978). This view is strengthened by the fact that the newer, more potent benzimidazoles (albendazole, fenbendazole and oxfendazole) are

much more slowly absorbed and eliminated than the earlier benzimidazoles (i.e. thiabendazole). For example, after oral administration of thiabendazole to sheep, its maximum plasma concentration is reached after four hours (Tocco et al., 1964). In comparison, after oral administration of fenbendazole and oxfendazole to sheep, maximum plasma concentrations occur after twenty-four hours (Marriner and Bogan, 1981a, 1981b) and after oral administration of albendazole to sheep, maximum concentrations occur at fifteen hours (Marriner and Bogan, 1980). It has also been shown (Prichard et al., 1978) that thiabendazole is effective against inhibited larvae of Ostertagia ostertagi when it is continuously infused over a long period of time, whereas thiabendazole as a single dose has previously been shown to be ineffective against inhibited larvae (Armour, 1969; Armour et al., 1975; Anderson, 1977). Thus it has been suggested that variations in efficacy are due not only to the concentration of anthelmintic to which the parasite is exposed but also to the duration of exposure of the parasite to a toxic concentration.

TOXICITY OF BENZIMIDAZOLES

Benzimidazole anthelmintics have an unusually low degree of acute toxicity to mammals for drugs which have such a broad spectrum of activity against parasitic helminths. Even with the more acutely toxic benzimidazoles (e.g. albendazole), the no-effect level is six times the normal therapeutic dosage (Johns and Philip, 1977), and therefore, in field use, toxicity has never been recorded. In experimental animals receiving repeated doses, toxic effects are non-specific

(i.e. anorexia, lethargy). Data on the acute toxicity of some of the benzimidazoles is difficult to find, since most of it is "in house" data, and since there are no published reports of accidental toxicity in field use.

The greatest toxicity risk of benzimidazoles is teratogenicity, which is known to occur with parbendazole, cambendazole, oxfendazole and albendazole, at dose rates lower than those which produce acute toxicity.

Parbendazole is teratogenic in the rat and the sheep, although in laboratory studies, its combination with SKF-525A (an inhibitor of microsomal oxidation) shows that parbendazole itself is relatively non-toxic (Delatour, 1983). It is a hydroxy-metabolite which is responsible for its toxicity (Di Cuollo et al., 1974).

Cambendazole has also been shown to be teratogenic in the rat and the sheep (Delatour et al., 1975).

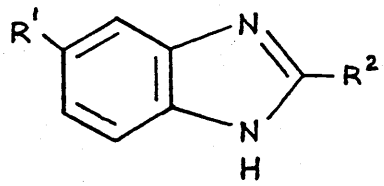
Fenbendazole is not teratogenic in any species, probably due to the fact that it is very insoluble and therefore, no matter how much is administered, not enough can dissolve to produce blood concentrations high enough to cause teratogenicity. Its sulphoxide metabolite (oxfendazole) has been identified in the rat (Becker, 1976), in the milk of cows (Delatour, 1983) and in sheep (Marriner and Bogan, 1981b) treated with fenbendazole. Oxfendazole is teratogenic in the rat and the sheep.

Albendazole has been extensively studied with regard to its teratogenic potential (Delatour et al., 1984). Metabolic studies have allowed the isolation of numerous metabolites (Gyurik et al., 1981), all of which have been investigated in the rat for their embryotoxicity. Both albendazole itself and its sulphoxide metabolite are teratogenic in the rat. Treatment with albendazole combined with SKF-525A eliminates the teratogenic effects of albendazole. Martin (1980) suggested that the product responsible for the teratogenicity of albendazole is the sulphoxide metabolite or a close derivative which is not the sulphone metabolite. Although treatment with albendazole combined with SKF-525A eliminates the teratogenic effects of albendazole, this may be due to decreased bioavailability of albendazole in the presence of SKF-525A, rather than inhibition of enzymatic S-oxidation (Delatour et al., 1984).

Febantel (a pro-benzimidazole) is teratogenic in the rat. Studies have shown that in vivo, febantel cyclizes to fenbendazole and oxfendazole. The combination of febantel with SKF-525A inhibits febantel toxicity in the rat. Therefore, the embryotoxicity of febantel is probably caused by its metabolite oxfendazole (Delatour et al., 1982).

Febantel is not teratogenic in the sheep at therapeutic doses, but since its metabolism is similar in the rat and the sheep, it is likely that it would be teratogenic in the sheep at higher dose levels.

TABLE 1.1 Benzimidazole anthelmintics and related compounds



GENERIC NAME	R^1	R^2
Thiabendazole	H	4-thiazolyl
Cambendazole	$(CH_3)_2CH$-O-CO-NH-	"
Carbendazim	H	$-NH-CO_2CH_3$
Albendazole	$CH_3CH_2CH_2$-S-	"
Ciclobendazole	Cyclopropyl-CO-	"
Fenbendazole	C_6H_5-S-	"
Flubendazole	F-C_6H_4-CO-	"
Mebendazole	C_6H_5-CO-	"
Oncodazole	2-thienyl-CO-	"
Oxfendazole	C_6H_5-SO-	"
Oxibendazole	$CH_3CH_2CH_2$-O-	"
Parbendazole	$CH_3CH_2CH_2CH_2$-	"

CHAPTER 2

AN ASSESSMENT OF THE RISK OF RESIDUES

Risk assessment is the scientific process by which the toxic properties of a substance are identified and evaluated. This process determines the likelihood that humans exposed to the substance will be adversely affected, and characterizes the nature of the effects they may experience. Using this information, the regulatory authorities can then make a judgement about whether exposure to the substance under certain defined conditions can be considered acceptable or safe.

For all substances (drugs and other environmental substances), the first step in risk assessment is the collection of toxicity information. This data includes basic knowledge about the properties of the substance and its effects in various biological systems. The necessary information comes from a variety of sources, but much of it is derived from studies in animals.

If any toxic effects noted in animals at various dose rates are deemed relevant to those which might occur in humans, the next step in risk assessment generally involves identifying the highest dose at which no adverse effects are observed in the animals, and dividing that dose by a safety factor (usually 100). This calculation yields an exposure level called the acceptable daily intake (ADI), which is considered to be a safe level of exposure for humans.

An ADI is defined as the amount of toxicant in milligrams per kilogram body weight per day (or in milligrams per day for a 70 kg person) which is not anticipated to result in any adverse effects after chronic exposure to the general population of humans, including

sensitive sub-groups. Adverse effects are considered as functional impairment or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (U.S. EPA, 1980). Operationally, ADIs are calculated by dividing a NOEL, NOAEL or LOAEL (derived from human or animal toxicity studies) by one or more uncertainty factors (safety factors).

$$ADI = \frac{\text{"no-effect" level}}{\text{uncertainty factor}}$$

NOEL (no-observed-effect level) is that dose of a chemical at which there are no statistically or biologically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

NOAEL (no-observed-adverse-effect level) is that dose of a chemical at which there are no statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects are produced at this dose, but they are not considered to be adverse.

LOAEL (lowest-observed-adverse-effect level) is the lowest dose of a chemical in a study or group of studies which produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Scientific guidelines and recommendations on the use of ADIs have been adopted by several international bodies including the Food and Drug Administration (FDA), the U.S. Environmental Protection Agency (U.S. EPA) and the joint Food and Agricultural Organisation/World Health Organisation (FAO/WHO) Food Standards Programme. Initial publications in this area of regulation suggest that ADIs for food additives or contaminants should be derived from a chronic animal NOEL or NOAEL (measured in mg/kg of diet) by dividing by a 100-fold uncertainty factor (Lehman and Fitzhugh, 1954). These workers reasoned that this factor accounted for several areas of uncertainty: intra-(human) or inter-(animal to human) species variability in response to the toxicity of a chemical, allowance for sensitive human sub-populations due to illness as compared to healthy experimental animals, and possible synergistic action of any one of the many intentional or unintentional food additives or contaminants in the human diet.

Similar areas of uncertainty have been addressed by other authors. Bigwood (1973) justified the 100-fold uncertainty factor for food additives on the basis of differences in body size of the laboratory animal versus that of man, differences in food requirements varying with age, sex, muscular expenditure and environmental conditions within a species, differences in water balance of exchange between the body and its environment among species, and differences in susceptibility to the toxic effect of a given contaminant among species. Other workers (Vettorazzi, 1976 and 1980; Lu, 1979) substantiated the use of the 100-fold uncertainty factor by discussing

differences in susceptibility between animals and humans to toxicants, variations in sensitivities in the human population, the fact that the number of animals tested is small compared with the size of the human population that may be exposed, the difficulty in estimating human intake, and the possibility of synergistic action among chemicals within the human diet.

Although the specific areas of uncertainty described by these authors (Lehman and Fitzhugh, 1954; Bigwood, 1973; Vettorazzi, 1976 and 1980) to support a 100-fold uncertainty factor differ somewhat, they can be generally viewed as being due to intra- or interspecies variability. Depending on the adverse effects found, the safety factor will be higher or lower than 100. For minor effects, 100 is used. For serious adverse effects (e.g. teratogenicity), a higher safety factor is used.

BENZIMIDAZOLE TOXICITY

Thiabendazole was introduced in 1961 and was recognized as having very low acute toxicity. No problems were encountered in its field usage and it was only after the introduction of parbendazole in 1967 that the toxicity of benzimidazoles was first recognized. It was found to be teratogenic at doses lower than those causing acute toxicity and as a result all new benzimidazoles have been extensively studied with regard to their potential teratogenicity and other genotoxic effects.

TERATOGENICITY OF BENZIMIDAZOLES

In sheep, many studies have been carried out with parbendazole, at dosages ranging from 25 mg/kg to 60 mg/kg. Teratogenicity of parbendazole was first demonstrated in sheep (Lapras et al., 1973a; Lemon and Hancock, 1974; Middleton et al., 1974; Saunders et al., 1974; Shone et al., 1974 and Szabo et al., 1974). The most commonly observed effects were skeletal malformations. These effects were later observed for cambendazole (Delatour et al., 1975a), oxfendazole (Delatour et al., 1977), albendazole (Johns and Philip, 1977) and febantel (Chement, 1982), all in sheep.

The benzimidazoles which are teratogenic in sheep do not appear to be so in cattle. With parbendazole (Miller et al., 1974) fenbendazole (Becker, 1975), oxibendazole (Theodorides et al., 1977), oxfendazole (Piercy et al., 1979) and albendazole, no malformations were observed after treatment.

Regarding teratogenicity, pigs have been reported to be insensitive to parbendazole (Hancock and Poulter, 1974), oxfendazole (Morgan, 1982), mebendazole (Van den Bossche et al., 1982) and fenbendazole (Anonymous, 1984).

In the horse, teratogenicity was not observed for fenbendazole (Becker, 1975), oxfendazole (Anonymous, 1978), oxibendazole (Anonymous, 1980) or mebendazole (Van den Bossche et al., 1982). With cambendazole, limb malformations were observed in three colts after the treatment of mares at an oral dose of 20 mg/kg (Drudge et al., 1983).

The rat is the animal species which has been most studied with regard to the teratogenicity of benzimidazoles. Embryolethal and teratogenic effects have been reported for parbendazole (Lapras et al., 1973b; Duncan and Lemon, 1974 and Mercier-Parot, 1976), mebendazole and cambendazole (Delatour et al., 1974; Fave and Maillet, 1975), ciclobendazole and carbendazim (Delatour and Richard, 1976b; Minta and Biernacki, 1982), oxfendazole (Delatour et al., 1977), albendazole (Delatour et al., 1981), febantel (Delatour et al., 1982b), ethyl 1H-benzimidazol-2-yl carbamate (Lemarchands, 1984) and netobimin (a pro-drug of albendazole) (Delatour et al., 1985b).

Fenbendazole (Baeder et al., 1974) and oxibendazole (Delatour et al., 1976c) do not appear to be teratogenic in the rat. Thiabendazole does not appear to be teratogenic in the rat, although it has been reported that minor abnormalities have occurred (retardation of ossification of the sternum) at a dose of 500 mg/kg (Khera et al., 1979).

In the mouse, parbendazole is teratogenic (Lapras et al., 1973c). The same abnormalities occur as in the rat, although double the dose is required. Thiabendazole has been reported to cause various abnormalities, but only at very high doses of between 700 mg/kg and 2400 mg/kg (Ogata et al., 1982 and 1984; Kubo et al., 1982).

Methyl thiophanate (which is metabolised to carbendazim) is not teratogenic in the mouse at a dose of 10 mg/kg/day (Makita et al., 1973).

In the hamster, parbendazole is not teratogenic at a dose rate of 100 mg/kg (Duncan and Lemon, 1974; Lemon and Hancock, 1974). However, it has been demonstrated that carbendazim is teratogenic in the hamster (Minta and Biernacki, 1982).

In the rabbit, parbendazole and other benzimidazoles are not teratogenic (Lapras et al., 1973c). Fenbendazole is embryotoxic but not teratogenic in rabbits at a dose rate of 63 mg/kg (Anonymous, 1984).

According to Minta and Biernacki (1982), carbendazim is teratogenic in rabbits, but this was not confirmed by Janardhan et al., (1984).

GENOTOXICITY OF BENZIMIDAZOLES

Some benzimidazoles have been tested for their possible genotoxicity, in bacteria and in rats and mice. These studies have involved mostly benomyl and carbendazim, which are considered toxicologically important because of their widespread use as agricultural fungicides (Sherman et al. 1975). Furthermore, carbendazim is a major residue found in plants after benomyl treatment (Clemons and Sisler, 1969). The anthelmintics commonly used in veterinary medicine have not been studied with respect to their genotoxic effects, possibly because some workers consider that human exposure to these compounds is of minor importance (Seiler, 1976).

The Dominant Lethal Test has been found to be consistently

negative for the benzimidazoles and their pro-drugs. This test has been carried out using carbendazim in mice at a dosage of 1280 mg/kg (Hofman and Peh, 1973), in rats at a dosage of 280 mg/kg (Rideg and Imre, 1982), mebendazole in mice up to a dosage of 640 mg/kg (Leonard et al., 1974), benomyl (a pro-drug of carbendazim) added to feed (Sherman et al., 1975), cypendazole (Machemer, 1974) and methyl thiophanate up to a dosage of 500 mg/kg (Makita et al., 1973).

A positive result to the Mammalian Spot Test (which looks for induced genetic alterations in melanoblastic cells) was observed for carbendazim in mice at a dose rate of 200 mg/kg on the tenth day after conception, although a dose of 100 mg/kg had no effect (Fahrig and Seiler, 1979).

Carbendazim and benomyl have been found to be mutagenic in Salmonella typhimurium, producing a two to five-fold increase in the frequency of mutants in the hisG46 and TA1530 strains (Seiler, 1972 and 1973). This was confirmed by Kappas et al., (1976). These results were controversial because, systematic study of the genotoxicity of benomyl and carbendazim showed only low activity on hisG46 strains at 100 ug/spot of carbendazim (Ficsor et al., 1978). Using TA1535, TA1536, TA1537 and TA1538 strains, benomyl was also found to be inactive at 20 ug/spot (Carere et al., 1978). Using TA98, TA100, TA1535 and TA1538 strains, mebendazole was found to be inactive at 1000 ug/plate (Paulikova and Dobias, 1981).

Using the Salmonella microsome test, carbendazim and benomyl

remained active (Ficsor et al., 1978). Using the same test, thiabendazole had no effect (Fujita and Haraga, 1980a, 1980b and 1981). According to Batzinger et al., (1978), mebendazole was active using this test, but other workers have shown it to be inactive (Paulikova and Dobias, 1981; Cortinas de Nava et al., 1983).

Carbendazim and benomyl were not mutagenic when used in a Rodent Host-Mediated Assay in rats and mice (Ficsor et al., 1978). The same applied to mebendazole in mice at a dose rate of 125 mg/kg (Paulikova and Dobias, 1981) although this has been contradicted (Batzinger et al., 1978; Bueding et al., 1979).

In vivo, carbendazim has been shown to induce chromosome abnormalities in mouse and hamster bone marrow (Seiler, 1976a), although its pro-drug methyl thiophanate does not (Makita et al., 1973). In rats and mice, thiabendazole does not induce chromosomal abnormalities (Yoshida et al., 1979). The Micronucleus Test has been shown to be positive in mice, using carbendazim at doses of 100 mg/kg to 1000 mg/kg (Seiler, 1975a), with a dose of 50 mg/kg having no effect. Using the Spermatocyte Test and the F1 Translocation Test, mebendazole has been shown to give a negative result in mice at dosages of 640 mg/kg and 160 mg/kg respectively (Leonard et al., 1974). Similar results have been obtained for methyl thiophanate in rats up to a dosage of 1000 mg/kg/day (Makita et al., 1973).

It has been suggested that benzimidazoles are mutagenic in eukaryotic cells by virtue of their anti-tubule activity. This may lead

to chromosomal disturbances during mitotic or meiotic anaphase, which may lead to numerical aberrations (Schreiner and Holden, 1983). In bacteria, benzimidazoles act like abnormal purine bases, which are incorporated by RNA-polymerase into the nucleic acids of E.coli, replacing guanine (Seiler, 1975c, 1976b and 1978).

ANTIMITOTIC EFFECTS OF BENZIMIDAZOLES

In vivo, cambendazole, mebendazole and parbendazole have been shown to be active against ascites carcinoma and leukaemia in mice (Lapras et al., 1975; Delatour et al., 1976a; Delatour and Richard, 1976b). Oncodazole was also active in these conditions (Atassi and Tagnon, 1975). In dogs, at an oral dose of 50 mg/kg/day, cambendazole was active against lymphoid leukosis and mammary adenocarcinoma (Delatour et al., 1975b), although tolerance of the drug was low. In man, clinical trials were carried out with parbendazole and oncodazole, which showed a decrease in the number of malignant cells and resulted in the disappearance of ascitic fluid in a patient with carcinoma of the stomach (De Brabander et al., 1975a).

In vitro, several models have been used to investigate the antimitotic effects of benzimidazoles. These include continuous-line cell cultures, primary explant cultures and leucocyte cultures.

Oxfendazole has been shown to have no effect on leucocytes (Delatour and Richard, 1976b), a result confirmed by other workers (Holden et al., 1980). Oxibendazole has been shown to block leucocyte

division (Delatour and Richard, 1976b). It has also been demonstrated that thiabendazole inhibits proliferation of KB and AGMK cells (Mochida et al., 1983), although only at high concentrations.

Oncodazole was tested using embryonic human and murine cutaneous fibroblasts, B16 melanoma cells, KB cells, and MO and MO4 cells. Using concentrations between 0.04 µg/ml and 10 µg/ml, the cells became rounded, the organelles became randomly dispersed and the microtubules gradually disappeared (De Brabander et al., 1975a, 1975b and 1976). The disappearance of microtubules was later confirmed using parbendazole in Vero cells (Havercroft et al., 1981).

OTHER TOXIC EFFECTS OF BENZIMIDAZOLES

Carbendazim has been shown to produce testicular degeneration in rats, with abnormalities in spermatogenesis (Styles and Garner, 1974; World Health Organisation, 1974; Rideg and Imre, 1982). Mebendazole has been shown to cause irreversible testicular degeneration in the rat (Anonymous, 1974). Testicular damage did not occur in rats after the administration of thiophanates (Makita et al., 1973; Thomas and Schein, 1974). After the administration of fenbendazole, oxfendazole or oxibendazole to stallions, it was noted that spermatogenesis was not affected (Squires et al., 1978; Anonymous, 1979; Anonymous, 1980).

Many other effects of benzimidazoles have been demonstrated and include decrease in milk production (Patton, 1974), decreased cholinesterase synthesis (Watts et al., 1982) and alopecia, which has

been observed in sheep after the administration of albendazole (Johns and Philip, 1977) and cambendazole (Guilhon and Barnabe, 1973). Alopecia has also been noted in man after mebendazole treatment (Kern et al., 1979; Miskovitz and Javitt, 1980; Braithwaite et al., 1982).

BENZIMIDAZOLE RESIDUES : A TOXICOLOGICAL EVALUATION

In all tests of toxicity described, the benzimidazole being assessed is freely available in the food of the animal or is freely dissolved in the in vitro test. However, in any assessment of the risk of residues, consideration must also be given to whether the residue is "bio-available", i.e. is readily absorbed when the food containing such a residue is consumed.

There is some confusion in the description of residues. Residues readily extractable by organic solvents are often called extractable residues. Residues which are non-extractable are often called tissue-bound residues. However, this may not equate with the likelihood of absorbability of residues, for example, a residue could be lightly bound, non organic extractable, but on digestion of tissue may or may not become free for absorption.

Tissue-bound residues have proven difficult to measure, and as a result, indirect methods of toxicological evaluation have been used. These are relay-bioavailability (Gallo-Torres, 1975) and relay-toxicity (Truhaut and Ferrando, 1975) studies. Relay-bioavailability evaluates the absorption of bound residues in animals fed tissues from treated

animals. Studies with cambendazole (Baer et al., 1977) and albendazole (Scott and Dicuollo, 1980) showed that the bioavailability of total residues was very low. Relay embryotoxicity studies were carried out in pregnant rats which were fed livers containing residues. With cambendazole (Baer et al., 1977) and albendazole (Delatour et al., 1981), relay embryotoxicity did not occur.

Because the binding of residues to tissues is considered to be irreversible (Rico and Burgat-Sacaze, 1984), drug residues which are covalently bound to tissues should present little risk to the consumer of meat containing these residues. Thus, all these toxicological studies with benzimidazoles suggest that the extractable metabolites or parent drugs are responsible for the observed toxicity. Therefore, the extractable residue fraction in edible tissues is of interest toxicologically. The toxic effects of benzimidazoles, which are important in the determination of an acceptable daily intake (ADI) of residues by humans, are teratogenicity and mutagenicity.

The study of the relationship between metabolism and teratogenicity of benzimidazoles allows the differentiation between metabolites which are teratogenic and metabolites which are not teratogenic, for structural and/or kinetic reasons. It is therefore possible to determine which metabolites are the ultimate teratogens. For the consumer, it is important to know which metabolites may be teratogenic, and also the inactive metabolites which may be metabolically converted to teratogenic metabolites. Using the concept of "Sum of Potentially Teratogenic Metabolites" (SPTM) it is possible

to determine the ADI in food. With each drug, the number of metabolites which may contribute to the SPTM varies. For example, carbendazim is teratogenic in the rat (NOAEL = 9.5 mg/kg). However, its 2-amino-benzimidazole metabolite is not, because it does not have a carbamate group which is thought to be necessary for both mutagenic (Seiler, 1976a) and teratogenic (Delatour and Richard, 1976b) activity. In addition, the 5-hydroxy metabolite of carbendazim is neither teratogenic in the rat nor cytotoxic *in vitro* (Styles and Garner, 1974). It is not believed that carbendazim can be regenerated from any of its metabolites. Therefore, teratogenicity is due to carbendazim alone (i.e. SPTM = 1).

A study of the teratogenicity of febantel and its metabolites in the rat (Delatour *et al.*, 1982b) demonstrated that the SPTM = 4 (febantel plus three metabolites, including fenbendazole and oxfendazole). In the case of a pro-drug of oxfendazole (Hennessy *et al.*, 1983) and the compound R34803 (Michiels *et al.*, 1982), a similar SPTM was observed.

The ADI of drug residues found in edible tissues is calculated from the NOAEL in the most sensitive species, using metabolites included in the SPTM. The uncertainty (safety) factor chosen for biological effects such as teratogenicity, is 1,000 (U.S. EPA, 1980). Therefore, for carbendazim, a NOAEL of 9.5 mg/kg in the rat corresponds to an ADI of 570 µg/day in humans (60 kg body weight), a value which is sensibly low considering results from genotoxicity studies (NOAEL = 5 mg/kg, uncertainty factor = 100).

Mutagenic activity of benzimidazoles has been demonstrated mainly in bacteria and fungi. Only carbendazim has been shown to possess mutagenic activity in mammals (using the mammalian spot test and the micronucleus test). The dose-effect in the micronucleus test indicated that carbendazim was genotoxic in the mouse at an oral dose of 100 mg/kg, but, at a dose of 50 mg/kg, carbendazim was not genotoxic (Seiler, 1975b).

WITHDRAWAL TIMES

Withdrawal times may be calculated from the depletion of total residues, depletion of extractable metabolites, or depletion of potentially toxic metabolites. More correctly, withdrawal times should be calculated on the basis that the sample contains less than the acceptable daily intake. In the past, arbitrary figures were chosen in setting withdrawal times, partly because analytical methods were insensitive, and partly because of difficulties in assessing the exact nature of residues. In recent years, methods have become much more sensitive and specific, with residues being detectable at much lower concentrations and for much longer periods of time.

CHAPTER 3

MATERIALS AND METHODS

The analysis of benzimidazole anthelmintics is difficult, due to the fact that they are poorly soluble in water (Ngomuo 1983) (Table 3.1) and have limited solubility in only a few organic solvents.

High Performance Liquid Chromatographic techniques require that the injected sample is uncontaminated (i.e. free of lipids, proteins, etc). Thus, development of a method for the determination of benzimidazoles and their metabolites in tissues posed a great problem as regards co-extracted lipids. This problem of co-extracted lipids is normally overcome in the analysis of other drugs by one or a combination of the following methods:

(1) Precipitation techniques using Sodium Tungstate, Zinc Sulphate, or other salts.

(2) Solvent/Solvent partitioning using two solvents, one of which is miscible with water.

(3) Column chromatographic clean-up.

(4) Acid/Base partitioning.

Considerable effort was made to utilise these techniques for benzimidazoles and their metabolites, but none of these was found useful since for the following reasons:

(1) In precipitation methods, contaminating lipids were precipitated out, but the supernatant, although clear, contained only about ten per cent of the total drug added.

(2) Petroleum ether/acetonitrile partition chromatography serves a dual role as both an extraction and a clean-up procedure. By using a polar and a non-polar solvent together, the fat is dissolved in the non-polar solvent, leaving the compounds of interest in the more polar solvent. The more polar solvent is then extracted using the non-polar solvent which is then evaporated to a small volume for transfer to a Florisil column.

This technique is used in many residue analyses (e.g. for organochlorine compounds), but benzimidazoles are poorly soluble in petroleum ether and acetonitrile, therefore, separation from lipids was not possible by this technique.

(3) In column chromatography using Florisil, sulphide and sulphoxide benzimidazole eluates contained large quantities of lipid, using a range of organic solvents and methods such as are commonly used for pesticide residue analysis.

(4) Normally, basic substances such as benzimidazoles and their metabolites, can be extracted from an aqueous alkaline solution into organic solvents and can then be re-extracted free from neutral lipids by extraction of the organic solvent with acid.

It was established that the parent benzimidazoles and their metabolites are only very poorly extractable by acids from

organic solvents (less than ten per cent recovery). Therefore, the possibility of clean-up of tissue residue extracts using back extraction into aqueous solvents was not possible.

Although benzimidazoles and their metabolites are poorly extracted into acid from organic solvents, it was discovered that they are stable even in strong acids (i.e. 3N HCl).

This property of the benzimidazoles was utilised in developing a suitable method which was found to give good recoveries of all the benzimidazoles studied and their toxicologically important metabolites (Tables 3.2-3.9).

METHOD FOR THE DETERMINATION OF BENZIMIDAZOLE RESIDUES IN LIVER, MUSCLE AND KIDNEY

Reagents:

All aqueous solutions were prepared using permanganate distilled water.

Diethyl ether (Analar) - washed with ferrous sulphate and stabilised with pyrogallol before use.

Methanol (Analar) - re-distilled before use.

Sodium carbonate

(Analar)

Ammonium carbonate

(Analar)

Hydrochloric acid

(Analar)

Frozen samples were thawed overnight in a refrigerator.

(1) The tissue was finely chopped with a scalpel and about 2.5g was weighed accurately and added to a 50ml ground glass stoppered tube.

(2) 5ml of 3N HCl were added and the tube stoppered and placed in a boiling water bath for 60 minutes.

(3) The tube was removed from the boiling water bath and cooled to room temperature.

(4) The tube was vortexed and, while being vortexed, 1.2g of sodium carbonate were slowly added. This brought the sample to a pH of approximately 9.

(5) 40ml of diethyl ether were added to the tube and the tube shaken on a slow rotary mixer for 15 minutes.

(6) 30ml of the diethyl ether were removed into a 50ml thin-walled tube and evaporated to dryness using a dry bath at 50°C with air.

(7) When the tube was dry 2ml of 1N HCl were added and the tube vortexed and filtered through Whatman No.1 paper pre-wetted with 1N HCl. The tube was vortexed with a further 4 x 2ml of 1N HCl, again filtering each 2ml fraction.

(8) After filtering, the filtrate was vortexed and 0.8g of sodium carbonate slowly added. This brought the sample to a pH of approximately 9.

(9) 40ml of diethyl ether were added to the filtrate and the tube was shaken on a slow rotary mixer for 15 minutes.

(10) 30ml of the diethyl ether were removed into a 50ml thin-walled tube and evaporated to approximately 4ml using a dry bath at 50°C with air.

(11) The remaining 4ml were transferred to a 10ml conical tube.

(12) The 50ml tube was washed with 2 x 2ml of diethyl ether and the washings were added to the 10ml conical tube.

(13) The washings were evaporated to dryness, then the side of the 10ml conical tube was washed with approximately 1ml of diethyl ether and again evaporated to dryness.

(14) The sample was resuspended in 75 microliters (ul) of methanol, placed in a sonic bath for 2 minutes and 5ul were injected onto the H.P.L.C. column.

Recoveries were estimated using blank tissue samples spiked with working standards which were prepared in methanol.

Spiking was carried out immediately after addition of the tissue to the tube, i.e. before the 3N HCl was added.

Parent compounds were measured along with their respective sulphoxide and sulphone metabolites.

The limit of detection for these compounds varied according to column performance, although a limit of 0.05 micrograms (ug) per gram could consistently be achieved.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Unfortunately, benzimidazoles and their metabolites cannot be measured using a single chromatographic system (Marriner and Bogan, 1980).

(1) With a mobile phase of methanol:ammonium carbonate the following were easily measured:

albendazole, fenbendazole, thiabendazole and 5- hydroxy

-thiabendazole. The sulphoxide and sulphone metabolites have identical retention times.

(2) With a mobile phase of methanol:perchloric acid the following were measured:

albendazole sulphoxide, albendazole sulphone, oxfendazole and oxfendazole sulphone.

Therefore, two chromatographic systems have to be used to measure the parent benzimidazoles and their important metabolites.

Pumps : Gilson Model 302

Detectors : Cecil CE 2012 variable wavelength U.V. spectrophotometer

Columns : 100mm x 5mm i.d. (Shandon Southern)

Packing : ODS-Hypersil. 5 micron

Wavelengths : 292nm

Absorbances : 0.05 a.u.f.s.

Solvents and Flow Rates:

(1) Methanol: Ammonium Carbonate (0.05M) 70:30 (1 ml/min)

(2) Methanol: Water 65:35 + 1µl of perchloric acid (0.7 ml/min)
(1.1% w/v) per ml of solvent

(3) Methanol: Ammonium Carbonate (0.05M) 40:60 (1 ml/min)

Retention Times : varied slightly and are therefore only approximate.

	Retention Time	Solvent
Albendazole	4 min	1
Albendazole Sulphoxide	4 min	2
Albendazole Sulphone	2.5 min	2

	Retention Time	Solvent
Fenbendazole	5 min	1
Oxfendazole	4.5 min	2
Oxfendazole Sulphone	2.5 min	2

	Retention Time	Solvent
Thiabendazole	8 min	3
5-hydroxythiabendazole	3 min	3

Although thiabendazole and 5-hydroxythiabendazole were measured using a mobile phase of methanol:ammonium carbonate; under the conditions used to measure albendazole and fenbendazole, their retention times were too short and therefore the proportions of methanol and ammonium carbonate had to be altered to allow their detection. Hence, the requirement for a third mobile phase.

It was found that a shortened version of the tissue method could be applied to milk samples giving good recoveries for all benzimidazoles used and their principal metabolites (Tables 3.10-3.12).

METHOD FOR THE DETERMINATION OF BENZIMIDAZOLE RESIDUES IN MILK

Reagents:

All aqueous solutions were prepared using permanganate distilled water.

Diethyl ether (Analar) - washed with ferrous sulphate and stabilised with pyrogallol before use.

Methanol (Analar) - re-distilled before use.

Sodium Carbonate
(Analar)

Ammonium Carbonate
(Analar)

Hydrochloric Acid
(Analar)

(1) 5ml of milk were added to a 50ml thin-walled tube.

(2) 5ml of 1N HCl were added and the tube vortexed.

(3) The sample was filtered through Whatman No.1 paper pre-wetted with 1N HCl into a 50ml ground glass stoppered tube. The tube was vortexed with a further 5ml of 1N HCl (2+2+1ml) and the washings filtered.

(4) The filtrate was vortexed and 0.8g of sodium carbonate added slowly. This brought the sample to a pH of approximately 9.

(5) 40ml of diethyl ether were added and the tube was shaken on a slow rotary mixer for 15 minutes.

(6) 30ml of diethyl ether were then removed into a 50ml thin-walled tube and evaporated to approximately 4ml.

(7) The remaining 4ml were transferred to a 10ml conical tube.

(8) The 50ml tube was washed with 2 x 2ml of diethyl ether and the washings were added to the 10ml conical tube.

(9) The diethyl ether was evaporated to dryness and the side of the tube was washed with 1ml of diethyl ether and again evaporated to dryness.

(10) The sample was resupsended in 75ul of methanol, placed in a sonic bath for 2 minutes and 5ul were injected onto the H.P.L.C. column.

Recoveries were estimated by the same method as that used for tissues.

This method was also applied to fat-free milk samples which were prepared in the following way:-

(1) To 10ml of milk were added 200µl of a commercial preparation of rennin (Rennet).

(2) The milk was incubated in a water bath at 40°C for one hour.

(3) The milk was centrifuged at 3000 rpm for 15 minutes and the supernatant decanted off into a tube for extraction by the milk method previously described.

The limit of detection for benzimidazoles and their metabolites in milk and fat-free milk varied, again due to column performance, although a limit of 0.05µg per ml could consistently be achieved.

The analysis of benzimidazoles and their metabolites in plasma and ruminal fluid was straightforward since a method was already available (Bogan and Marriner, 1980) and is as follows:-

METHOD FOR THE DETERMINATION OF BENZIMIDAZOLES AND THEIR METABOLITES IN PLASMA AND RUMINAL FLUID

(1) To 4ml of plasma or ruminal fluid in a 50ml stoppered tube were added 0.4g of sodium chloride, 0.4ml of ammonium hydroxide (0.1N) and 20ml of diethyl ether.

(2) The tube was shaken for 15 minutes on a slow rotary mixer.

(3) 15ml of the diethyl ether were removed into a 50ml thin-walled tube and a further 20ml of diethyl ether was added to the first tube, which was shaken for a further 15 minutes.

(4) 20ml of diethyl ether was removed and combined with the initial 15ml of diethyl ether.

(5) The diethyl ether was evaporated to approximately 4ml using a dry bath at 50°C with air.

(6) The remaining 4ml was transferred to a 10ml conical tube.

(7) The 50ml tube was washed with 2 x 2ml of diethyl ether and the washings added to the 10ml conical tube.

(8) The diethyl ether was then evaporated to dryness and the side of the 10ml conical tube washed with approximately 1ml of diethyl ether and again evaporated to dryness.

(9) The sample was resuspended in 100µl of methanol, placed on a sonic bath for 2 minutes and 5µl were injected onto the H.P.L.C. column.

TABLE 3.1 The solubilities of albendazole, fenbendazole, oxfendazole and thiabendazole in phosphate buffer pH 7.4 at 18°C

DRUG	MAXIMUM SOLUBILITY (µg/ml)
ALBENDAZOLE	0.26
FENBENDAZOLE	0.02
OXFENDAZOLE	1.90
THIABENDAZOLE	15.37

TABLE 3.2 Estimation of albendazole (ABZ) following spiking of liver, muscle and kidney

LIVER

ABZ ADDED (µg)	ABZ MEASURED (µg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.62	1.64 ± 0.02	1.2	0.66
2.5	1.65			
1.25	0.82	0.81 ± 0.01	1.2	0.65
1.25	0.80			
0.5	0.31	0.32 ± 0.01	3.1	0.64
0.5	0.33			

MUSCLE

ABZ ADDED (µg)	ABZ MEASURED (µg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.55	1.56 ± 0.01	0.6	0.62
2.5	1.57			
1.25	0.77	0.76 ± 0.01	0.6	0.61
1.25	0.76			
0.5	0.28	0.28 ± 0.01	1.8	0.56
0.5	0.29			

KIDNEY

ABZ ADDED (µg)	ABZ MEASURED (µg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.63	1.62 ± 0.01	0.6	0.65
2.5	1.61			
1.25	0.81	0.80 ± 0.01	1.2	0.64
1.25	0.79			
0.5	0.32	0.31 ± 0.01	3.2	0.62
0.5	0.30			

TABLE 3.3 Estimation of albendazole sulphoxide (ABSX) following spiking of liver, muscle and kidney

LIVER

ABSX ADDED (µg)	ABSX MEASURED (µg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.47	1.46 ± 0.01	0.7	0.58
2.5	1.45			
1.25	0.70	0.70 ± 0.01	0.7	0.56
1.25	0.71			
0.5	0.28	0.27 ± 0.01	3.7	0.54
0.5	0.26			

MUSCLE

ABSX ADDED (µg)	ABSX MEASURED (µg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.35	1.34 ± 0.02	1.1	0.54
2.5	1.32			
1.25	0.67	0.66 ± 0.02	2.3	0.53
1.25	0.64			
0.5	0.28	0.28 ± 0.01	1.8	0.56
0.5	0.29			

KIDNEY

ABSX ADDED (µg)	ABSX MEASURED (µg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.44	1.43 ± 0.01	0.7	0.57
2.5	1.42			
1.25	0.68	0.66 ± 0.02	2.3	0.53
1.25	0.65			
0.5	0.26	0.26 ± 0.01	1.9	0.52
0.5	0.27			

TABLE 3.4 Estimation of albendazole sulphone (ABSO) following spiking of liver, muscle and kidney

LIVER

ABSO ADDED (μg)	ABSO MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.81	1.80 ± 0.01	0.6	0.72
2.5	1.79			
1.25	0.89	0.90 ± 0.01	1.1	0.72
1.25	0.91			
0.5	0.36	0.36 ± 0.01	1.4	0.72
0.5	0.37			

MUSCLE

ABSO ADDED (μg)	ABSO MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.75	1.76 ± 0.01	0.6	0.70
2.5	1.77			
1.25	0.86	0.85 ± 0.01	1.2	0.68
1.25	0.84			
0.5	0.34	0.34 ± 0.01	1.5	0.68
0.5	0.35			

KIDNEY

ABSO ADDED (μg)	ABSO MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.80	1.78 ± 0.02	1.1	0.71
2.5	1.76			
1.25	0.85	0.86 ± 0.01	0.6	0.69
1.25	0.86			
0.5	0.34	0.33 ± 0.01	3.0	0.66
0.5	0.32			

TABLE 3.5 Estimation of fenbendazole (FBZ) following spiking of liver, muscle and kidney

LIVER

FBZ ADDED (μg)	FBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.71	1.69 ± 0.02	1.2	0.68
2.5	1.67			
1.25	0.87	0.88 ± 0.01	0.6	0.70
1.25	0.88			
0.5	0.33	0.34 ± 0.02	4.4	0.68
0.5	0.36			

MUSCLE

FBZ ADDED (μg)	FBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.65	1.65 ± 0.02	0.9	0.66
2.5	1.62			
1.25	0.83	0.84 ± 0.01	1.2	0.67
1.25	0.85			
0.5	0.29	0.30 ± 0.02	5.0	0.60
0.5	0.32			

KIDNEY

FBZ ADDED (μg)	FBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.69	1.66 ± 0.02	1.5	0.66
2.5	1.64			
1.25	0.85	0.86 ± 0.01	1.2	0.69
1.25	0.87			
0.5	0.31	0.30 ± 0.01	3.3	0.60
0.5	0.29			

TABLE 3.6 Estimation of oxfendazole (OFZ) following spiking of liver, muscle and kidney

LIVER

OFZ ADDED (μg)	OFZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.61	1.63 ± 0.02	1.2	0.65
2.5	1.65			
1.25	0.77	0.76 ± 0.01	1.3	0.61
1.25	0.75			
0.5	0.30	0.32 ± 0.02	4.7	0.64
0.5	0.33			

MUSCLE

OFZ ADDED (μg)	OFZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.53	1.54 ± 0.02	1.0	0.62
2.5	1.56			
1.25	0.73	0.72 ± 0.01	1.4	0.58
1.25	0.71			
0.5	0.26	0.28 ± 0.02	5.4	0.56
0.5	0.29			

KIDNEY

OFZ ADDED (μg)	OFZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.63	1.62 ± 0.01	0.6	0.65
2.5	1.61			
1.25	0.79	0.80 ± 0.01	1.2	0.64
1.25	0.81			
0.5	0.32	0.33 ± 0.01	3.0	0.66
0.5	0.34			

TABLE 3.7 Estimation of oxfendazole (OFZ.SO) following spiking of liver, muscle and kidney

LIVER

OFZ.SO. ADDED (μg)	OFZ.SO. MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.91	1.89 ± 0.02	1.1	0.76
2.5	1.87			
1.25	0.96	0.94 ± 0.02	1.6	0.75
1.25	0.93			
0.5	0.36	0.38 ± 0.02	3.9	0.76
0.5	0.39			

MUSCLE

OFZ.SO. ADDED (μg)	OFZ.SO. MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.87	1.86 ± 0.02	0.8	0.74
2.5	1.84			
1.25	0.90	0.91 ± 0.01	1.1	0.73
1.25	0.92			
0.5	0.33	0.35 ± 0.02	5.7	0.70
0.5	0.37			

KIDNEY

OFZ.SO. ADDED (μg)	OFZ.SO. MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.89	1.86 ± 0.02	1.3	0.74
2.5	1.84			
1.25	0.92	0.91 ± 0.01	1.1	0.73
1.25	0.90			
0.5	0.37	0.36 ± 0.01	1.4	0.72
0.5	0.36			

TABLE 3.8 Estimation of thiabendazole (TBZ) following spiking of liver, muscle and kidney

LIVER

TBZ ADDED (μg)	TBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.45	1.47 ± 0.02	1.4	0.59
2.5	1.49			
1.25	0.70	0.72 ± 0.02	2.8	0.58
1.25	0.74			
0.5	0.31	0.30 ± 0.01	3.3	0.60
0.5	0.29			

MUSCLE

TBZ ADDED (μg)	TBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.41	1.42 ± 0.01	0.7	0.57
2.5	1.43			
1.25	0.65	0.67 ± 0.02	3.0	0.54
1.25	0.69			
0.5	0.28	0.29 ± 0.01	3.4	0.58
0.5	0.30			

KIDNEY

TBZ ADDED (μg)	TBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.48	1.46 ± 0.02	1.4	0.58
2.5	1.44			
1.25	0.71	0.70 ± 0.01	1.4	0.56
1.25	0.69			
0.5	0.33	0.32 ± 0.01	3.1	0.64
0.5	0.31			

TABLE 3.9 Estimation of 5-hydroxythiabendazole (5-OH TBZ) following spiking of liver, muscle and kidney

LIVER

5-OH TBZ ADDED (μg)	5-OH TBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.55	1.52 ± 0.02	1.6	0.61
2.5	1.50			
1.25	0.76	0.75 ± 0.01	1.3	0.60
1.25	0.74			
0.5	0.31	0.29 ± 0.02	6.9	0.58
0.5	0.27			

MUSCLE

5-OH TBZ ADDED (μg)	5-OH TBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.49	1.50 ± 0.01	0.7	0.60
2.5	1.51			
1.25	0.74	0.75 ± 0.01	1.3	0.60
1.25	0.76			
0.5	0.28	0.26 ± 0.02	5.8	0.52
0.5	0.25			

KIDNEY

5-OH TBZ ADDED (μg)	5-OH TBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
2.5	1.53	1.52 ± 0.01	0.7	0.61
2.5	1.51			
1.25	0.77	0.76 ± 0.01	1.3	0.61
1.25	0.75			
0.5	0.33	0.31 ± 0.02	6.4	0.62
0.5	0.29			

TABLE 3.10 Estimations of albendazole (ABZ), albendazole sulphoxide (ABSX) and albendazole sulphone (ABSO) following spiking of cows' milk

ABZ ADDED (μg)	ABZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
5.0	3.96	3.92 ± 0.04	0.9	0.78
5.0	3.89			
2.5	1.87	1.89 ± 0.02	1.1	0.76
2.5	1.91			
0.5	0.37	0.36 ± 0.01	2.8	0.72
0.5	0.35			

ABSX ADDED (μg)	ABSX MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
5.0	3.26	3.30 ± 0.04	1.1	0.66
5.0	3.33			
2.5	1.59	1.62 ± 0.02	1.5	0.65
2.5	1.64			
0.5	0.33	0.31 ± 0.02	6.4	0.62
0.5	0.29			

ABSO ADDED (μg)	ABSO MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
5.0	4.01	3.96 ± 0.04	1.1	0.79
5.0	3.92			
2.5	1.97	2.00 ± 0.02	1.2	0.80
2.5	2.02			
0.5	0.39	0.38 ± 0.02	3.9	0.76
0.5	0.36			

TABLE 3.11 Estimations of fenbendazole (FBZ), oxfendazole (OFZ) and oxfendazole sulphone (OFZ.SO) following spiking of cows' milk

FBZ ADDED (μg)	FBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
5.0	3.81	3.79 ± 0.02	0.5	0.76
5.0	3.77			
2.5	1.86	1.90 ± 0.04	1.8	0.76
2.5	1.93			
0.5	0.37	0.38 ± 0.01	1.3	0.76
0.5	0.38			

OFZ ADDED (μg)	OFZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
5.0	2.69	2.67 ± 0.02	0.7	0.53
5.0	2.65			
2.5	1.32	1.34 ± 0.02	1.5	0.54
2.5	1.36			
0.5	0.28	0.27 ± 0.01	3.7	0.54
0.5	0.26			

OFZ.SO. ADDED (μg)	OFZ.SO. MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
5.0	3.93	3.95 ± 0.02	0.5	0.79
5.0	3.97			
2.5	1.91	1.89 ± 0.02	1.1	0.76
2.5	1.87			
0.5	0.37	0.38 ± 0.02	3.9	0.76
0.5	0.40			

TABLE 3.12 Estimations of thiabendazole (TBZ) and 5-hydroxythiabendazole (5-OH TBZ) following spiking of cows' milk

TBZ ADDED (μg)	TBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
5.0	3.54	3.58 ± 0.04	1.3	0.72
5.0	3.63			
2.5	1.80	1.76 ± 0.04	2.0	0.70
2.5	1.73			
0.5	0.37	0.35 ± 0.02	5.7	0.70
0.5	0.33			

5-OH TBZ ADDED (μg)	5-OH TBZ MEASURED (μg)	MEAN ± S.E.M.	S.E.M. %	RATIO OF ASSAYED TO ADDED
5.0	3.88	3.90 ± 0.02	0.6	0.78
5.0	3.93			
2.5	1.87	1.88 ± 0.01	0.5	0.75
2.5	1.89			
0.5	0.36	0.37 ± 0.01	2.7	0.74
0.5	0.38			

The methods described have been shown to be very suitable for the determination of benzimidazoles and their toxicologically important metabolites. Recoveries of all the compounds were generally very similar at all concentrations measured and for all tissues. As for the limits of detection, they were considered to be when the peak heights of the analytes were twice the base-line variation.

At the outset of this study it was not our intention to determine kidney residues of benzimidazoles and their metabolites, however, we were provided with kidney samples by Robert Young and Company and found that our methods were applicable to this tissue. For this reason, kidney samples were not included in all of the studies.

ANIMALS

All sheep and cattle used in the residue studies were of mixed breed and sex. The animals were of different ages, but all were adult.

FEEDING

All animals were maintained on a diet of hay with a small amount of concentrates and water ad libitum, throughout the duration of the residue trials.

DOSING

All drugs were given orally by drench.

SAMPLING

Blood samples were taken by jugular venepuncture into 10ml heparinised syringes (Monovettes). The blood was centrifuged immediately at 3000 x g for 15 minutes, the plasma removed and stored at -20^{o}C until assayed.

Rumen fluid samples were obtained via rumen cannulae which had been inserted at least one month before the study had begun. The fluid was centrifuged immediately at 3000 x g for 10 minutes, the supernatant removed and stored at -20^{o}C until assayed.

TISSUE SAMPLES

Liver, muscle and kidney samples were taken at slaughter and stored immediately at -20°C until assayed. In all of the studies carried out, liver and kidney samples were aliquots taken from large portions of chopped tissue, to give a representative sample of the whole organ. Muscle tissue was consistently taken from the thigh of the animal.

CHAPTER 4

STUDIES WITH ALBENDAZOLE AND ALBENDAZOLE SULPHOXIDE IN SHEEP

INTRODUCTION

Albendazole (5-(propylthio)-1H-benzimidazol-2-yl-carbamate) is a potent member of the benzimidazole group of anthelmintics with broad spectrum activity against gastro-intestinal roundworms including inhibited larval stages, tapeworms, liver flukes and lungworms in many species (Theodorides et al., 1976). It is the first broad spectrum anthelmintic to have useful efficacy at low dose levels against all major economically important helminth parasites infecting domestic animals. In sheep and cattle, it is widely used in the control of gastro-intestinal nematodes and tapeworms, lungworms and liver flukes (Knight and Colglazier, 1977).

The metabolism of albendazole has been extensively studied (Gyurik et al., 1981), with nine metabolites being identified in the urine of cattle, sheep, rats and mice which had been given ^{14}C-labelled albendazole orally (Figure 4.1). Albendazole is metabolised by oxidation at the sulphur atom, by aliphatic and aromatic hydroxylation, methylation at both nitrogen and sulphur, and carbamate hydrolysis. Unchanged albendazole was present in only minor amounts in urine. The percentage of each metabolite was measured after samples of urine from each species were extracted with ethyl acetate and the metabolites separated by thin-layer chromatography (Table 4.1).

The most important metabolic reactions leading to the observed urine metabolites were oxidation at alkyl and sulphur, and carbamate hydrolysis. All the observed urine metabolites were sulphoxides or sulphones. The oxidation of alkyl and aryl sulphides to more polar

sulphoxides and sulphones is commonly observed and facilitates urinary excretion (Duggan et al., 1977).

In all species studied, metabolite C (Figure 4.1) was found in higher concentrations than any of the other metabolites isolated, with the exception of mouse urine in which metabolite G was most abundant. In cattle and sheep, metabolite I was found to be a major urinary metabolite. Metabolite E was found in sheep urine in high concentrations, but in cattle urine, was found in lower concentrations than in sheep urine. Metabolite A has been identified in the plasma of cattle and sheep (Marriner and Bogan, 1980) but is not a major urinary metabolite in these species. Metabolite A is chemically identical to albendazole sulphone and metabolite C is chemically identical to albendazole sulphoxide. Albendazole 2-amino sulphone (Metabolite I), although a major urinary metabolite in cattle and sheep, is not a major metabolite in the plasma of these species. Albendazole sulphoxide and albendazole sulphone are the major metabolites found in the plasma of cattle and sheep after the administration of albendazole (Marriner and Bogan, 1980).

The facile metabolic interconversion of alkyl and aryl sulphides and sulphoxides has been reported (Duggan et al., 1980). This has been confirmed for albendazole and albendazole sulphoxide (Douch and Buchanan, 1979). When rats were dosed with albendazole sulphone, no sulphoxide metabolites were detected in urine. The metabolic oxidation of sulphoxide to sulphone appears to be irreversible (Kexel and Schmidt, 1972).

When administered orally, albendazole and albendazole sulphoxide have similar anthelmintic and embryotoxic activities. However, all of the other identified metabolites of albendazole have little anthelmintic activity and do not appear to be teratogenic in the rat (Delatour et al., 1981).

FIGURE 4.1 The proposed metabolic pathway of albendazole (ABZ) (Gyurik et al., 1981).

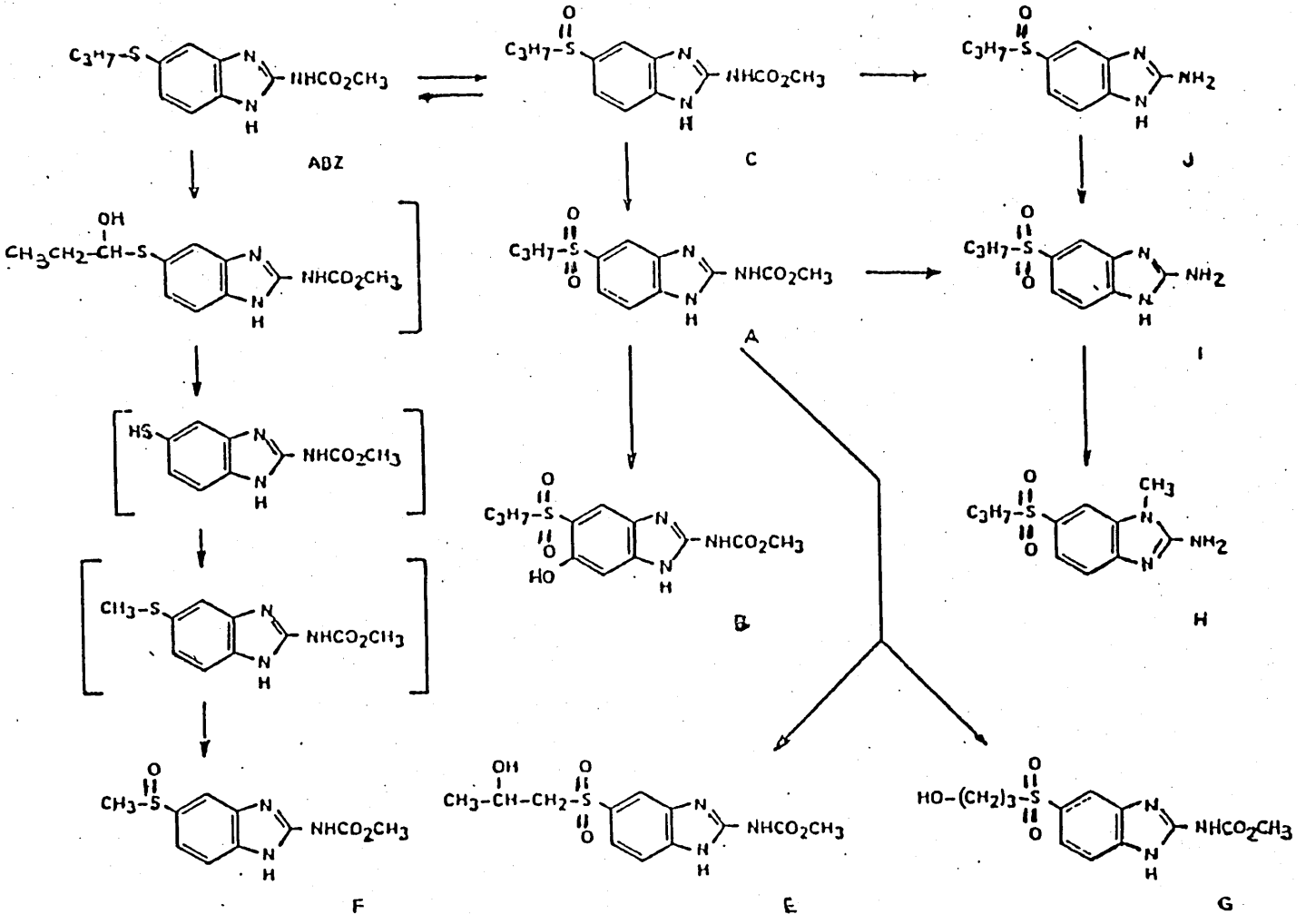


TABLE 4.1 Percentage distribution of radioactivity in the thin-layer chromatographic zones corresponding to various metabolites in extracts of urine from animals treated orally with ^{14}C-albendazole. (Gyurik et al., 1981)

METABOLITE	CATTLE	SHEEP	RAT	MOUSE
			%	
ALBENDAZOLE	0.1	1.0	1.9	0.5
A (Albendazole sulphone)	5.8	3.4	5.5	1.3
B	6.3	8.9	0.0	0.0
C (Albendazole sulphoxide)	22.9	24.3	26.6	24.2
E	7.5	22.5	14.0	21.6
F	2.8	4.5	0.0	5.7
G	9.2	4.3	24.2	29.9
H	6.6	5.1	0.0	0.0
I (Albendazole 2-aminosulphone)	20.9	20.2	14.8	3.7
J	4.4	1.6	0.5	4.7
TOTAL RECOVERED	86.5	95.6	87.6	91.6

TOXICITY OF ALBENDAZOLE

The toxicity of albendazole and its metabolites has been extensively studied (Martin, 1980; Grannec, 1980; Delatour et al., 1981; Delhoste, 1983).

Delatour et al., (1981) compared the relay embryotoxicity of albendazole with the embryotoxicity of its individual metabolites. In these studies, cattle were given albendazole at an experimental dosage of 20 mg/kg body weight. The animals were slaughtered at 24, 48 and 96 hours after dosing, their livers removed immediately after slaughter and frozen. The frozen livers were freeze-dried and the freeze-dried livers fed to gestating rats. It was found that rats fed liver from albendazole treated cattle did not differ significantly from rats fed liver from untreated control cattle with respect to observed embryotoxicity (embryolethality, foetotoxicity, terato-genicity). Scott and Dicuollo (1980) have shown that in rats treated with liver obtained from calves which had been dosed with albendazole, the bioavailability of total residues was very low (i.e. residues were very poorly absorbed from the rat gastro-intestinal tract). These results suggest that relay toxicity does not occur with albendazole.

Pregnant rats were also treated with albendazole (Delatour et al., 1981) or one of its various metabolites (Figure 4.1) from the eighth to the fifteenth day of gestation, each day at the same time. Albendazole was found to be embryotoxic in the dose range of

6.62 mg/kg to 13.25 mg/kg but, of all the known metabolites of albendazole, including all the major urinary metabolites, only metabolite C (albendazole sulphoxide) was found to be embryotoxic. In fact, albendazole sulphoxide reproduces exactly the toxic effects of the parent compound (Delatour et al., 1981).

When SKF 525-A (an inhibitor of microsomal oxidation) and albendazole are administered concurrently, the embryotoxic effects of albendazole are eliminated (Delatour et al., 1984). This is probably due to an inhibitory effect of SKF 525-A upon gastro-intestinal absorption of albendazole, rather than an inhibition of enzymatic S-oxidation (Delatour et al., 1984).

From a toxicological point of view, it is clear from these experiments that in any residue studies involving albendazole, it is most important that the parent albendazole and albendazole sulphoxide should be measured, since none of the other metabolites of albendazole have been found to be toxic.

In the following experiments with albendazole and albendazole sulphoxide, only the parent sulphide, and the sulphoxide and sulphone metabolites were measured (Figure 4.2). The reason for measuring albendazole sulphone was that, although it is not toxicologically important, it is a major plasma metabolite of albendazole (Marriner and Bogan, 1980) and persists longer in plasma than albendazole sulphoxide. Also, under the chromatographic conditions used to measure albendazole sulphoxide, albendazole sulphone is easily measured.

FIGURE 4.2 Albendazole and its important metabolites.

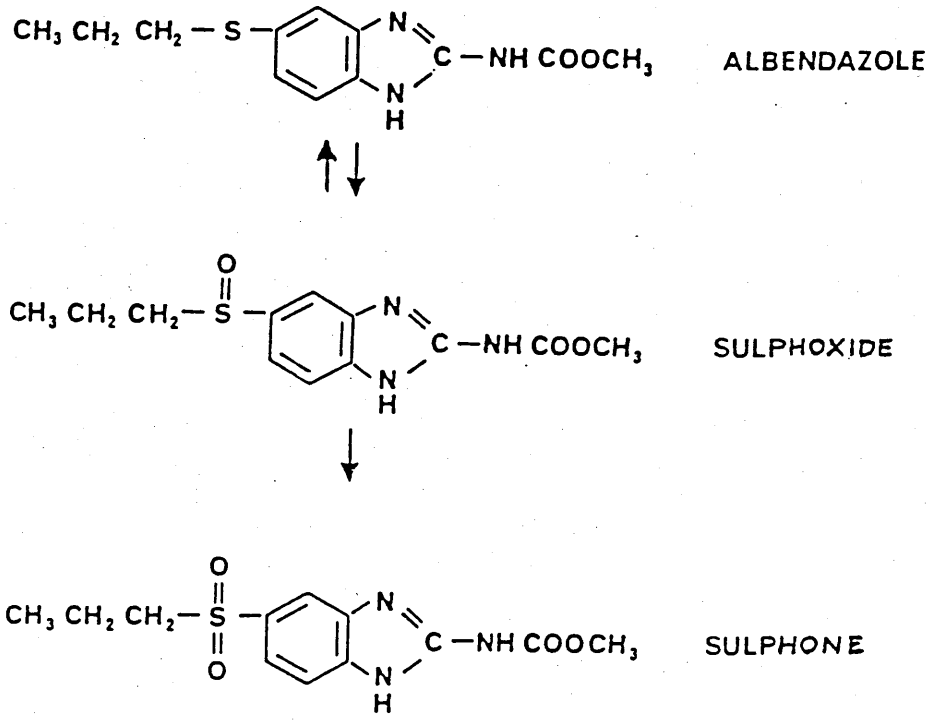


TABLE 4.2 The meat and milk withholding times for albendazole in sheep and cattle in the United Kingdom.

SPECIES	WITHHOLDING TIME (days)	
	MEAT	MILK
CATTLE	14	3
SHEEP	10	-

MATERIALS AND METHODS

These studies involved the administration of albendazole or albendazole sulphoxide to sheep, both at a dose rate of 5 mg/kg. All treatments were by oral drench, and the animals were slaughtered at 1, 3, 5 and 8 days post-treatment. For the sheep treated with albendazole, samples of liver and muscle were taken at slaughter and stored immediately at -20°C until assayed (as described in Chapter 3). They were assayed in duplicate (Table 4.3). Blood samples were also taken at intervals throughout the course of the experiment to assess tissue/plasma ratios; plasma was removed after centrifugation and stored at -20°C until assayed (Table 4.3). For the sheep treated with albandazole sulphoxide, samples of liver, muscle and kidney were taken at slaughter and assayed as previously described (Table 4.4).

A third experiment was carried out in which two sheep with permanent rumen cannulae were given albendazole sulphoxide as an oral drench, at a dose rate of 5 mg/kg. Samples of ruminal fluid (10 ml) were taken before and at 2, 4, 8, 12, 24, 36, 48, 72 and 96 hours after treatment. The samples were centrifuged, the supernatant removed and stored immediately at -20°C until assayed (as described in Chapter 3). All ruminal fluid samples were assayed for albendazole sulphoxide and albendazole (Table 4.5).

After albendazole treatment (Table 4.3), albendazole itself was found in liver in high concentrations at one day post-treatment (at a concentration of 11.80 μg/g). This was surprising since albendazole itself was detected in plasma only at very low concentrations (0.02 μg/ml) after albendazole treatment at a dose rate of 10 mg/kg (Marriner and Bogan, 1980).

At three days post-treatment, albendazole was still detectable in liver (although only a trace amount was present), but, at five days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphoxide was detectable in liver at one day post-treatment (at a concentration of 1.65 μg/g), but, highest concentrations were found at three days post-treatment (3.28 μg/g). At five days post-treatment, albendazole sulphoxide was no longer detectable in liver.

Albendazole sulphone was also detectable in liver at one day post-treatment (at a concentration of 1.94 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

In muscle, albendazole was detectable at one day post-treatment (at a concentration of 0.68 μg/g), although in a much lower concentration than was found in liver at the same time. At three days post-treatment, albendazole was no longer detectable in muscle.

Albendazole sulphoxide was detectable in muscle at one day

post-treatment (at a concentration of 0.68 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in muscle at one day post-treatment (at a concentration of 0.28 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

In plasma, albendazole itself was not detectable at any time after treatment (Table 4.6).

Albendazole sulphoxide was detectable in plasma, highest concentrations being found at one day post-treatment (1.57 μg/ml). At three days post-treatment, albendazole sulphoxide was still detectable (at a concentration of 0.04 μg/ml), but, at five days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in plasma, highest concentrations being found at one day post-treatment (0.69 μg/ml). At three days post-treatment, albendazole sulphone was still detectable (at a concentration of 0.05 μg/ml), but, at five days post-treatment, concentrations had fallen below a detectable level (< 0.02 μg/ml).

TABLE 4.3 Liver, muscle and plasma residues of albendazole and its metabolites in sheep.

LIVER

Animal No	Dose	Days after Treatment	Residues (µg/g wet wt) ABZ *	ABSX *	ABSO *
110	5mg/kg	1	11.90	1.65	1.94
65	5mg/kg	3	TRACE	3.28	N.D.
97	5mg/kg	5	N.D.	N.D.	N.D.
4	5mg/kg	8	N.D.	N.D.	N.D.

MUSCLE

110	5mg/kg	1	0.68	0.68	0.28
65	5mg/kg	3	N.D.	N.D.	N.D.
97	5mg/kg	5	N.D.	N.D.	N.D.
4	5mg/kg	8	N.D.	N.D.	N.D.

PLASMA

			Residues (µg/l)		
110	5mg/kg	1	N.D.	1.57	0.69
65	5mg/kg	3	N.D.	0.04	0.05
97	5mg/kg	5	N.D.	N.D.	N.D.
4	5mg/kg	8	N.D.	N.D.	N.D.

ABZ = albendazole
ABSX = albandazole sulphoxide
ABSO = albendazole sulphone

* = Mean values of two determinations

N.D. = Not detected (< 0.05µg/g or µg/ml)

After albendazole sulphoxide treatment (Table 4.4), albendazole sulphoxide was detectable in liver at one day post-treatment (at a mean concentration of 3.36 μg/g). At three days post-treatment, it was still detectable (at a mean concentration of 0.74 μg/g), but, at five days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in liver at one day post-treatment (at a mean concentration of 2.94 μg/g). It was still detectable at three days post-treatment (although only a trace amount was present), but, at five days post-treatment, concentrations had fallen below a detectable level.

Surprisingly, albendazole was detectable in liver in high concentrations at one day post-treatment (a mean concentration of 14.21 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

In muscle, albendazole sulphoxide was detectble at one day post-treatment (at a mean concentration of 1.87 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in muscle at one day post-treatment (at a mean concentration of 1.07 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable

level.

Albendazole was not detectable in muscle at any time after treatment.

In kidney, albendazole sulphoxide was detectable at one day post-treatment (at a mean concentration of 6.84 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in kidney at one day post-treatment (at a mean concentration of 2.14 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

Again, surprisingly, albendazole was detectable in kidney at one day post-treatment (at a mean concentration of 4.20 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

TABLE 4.4 Liver, muscle and kidney residues of albendazole sulphoxide and its metabolites in sheep.

LIVER

Animal No	Dose	Days after Treatment	Residues (µg/g wet wt) ABZ	ABSX	ABSO
583	5mg/kg	1	18.61	1.92	2.80
586	5mg/kg	1	9.82	4.81	3.09
MG	5mg/kg	3	N.D.	0.60	TRACE
MG	5mg/kg	3	N.D.	0.89	TRACE
693	5mg/kg	5	N.D.	N.D.	N.D.
CHE	5mg/kg	5	N.D.	N.D.	N.D.
692	5mg/kg	8	N.D.	N.D.	N.D.
SUF	5mg/kg	8	N.D.	N.D.	N.D.

MUSCLE

583	5mg/kg	1	N.D.	2.45	1.05
586	5mg/kg	1	N.D.	1.29	1.09
MG	5mg/kg	3	N.D.	N.D.	N.D.
MG	5mg/kg	3	N.D.	N.D.	N.D.
693	5mg/kg	5	N.D.	N.D.	N.D.
CHE	5mg/kg	5	N.D.	N.D.	N.D.
692	5mg/kg	8	N.D.	N.D.	N.D.
SUF	5mg/kg	8	N.D.	N.D.	N.D.

KIDNEY

583	5mg/kg	1	2.50	11.57	2.28
586	5mg/kg	1	5.90	2.12	2.00
MG	5mg/kg	3	N.D.	N.D.	N.D.
MG	5mg/kg	3	N.D.	N.D.	N.D.
693	5mg/kg	5	N.D.	N.D.	N.D.
CHE	5mg/kg	5	N.D.	N.D.	N.D.
692	5mg/kg	8	N.D.	N.D.	N.D.
SUF	5mg/kg	8	N.D.	N.D.	N.D.

ABZ = albendazole
ABSX = albendazole sulphoxide
ABSO = albendazole sulphone

N.D. = not detected (< 0.05 µg/g)

Marriner and Bogan (1980) reported that after the administration of albendazole to sheep, the parent sulphide was detectable only in very low concentrations in plasma. Only its metabolites albendazole sulphoxide and albendazole sulphone were detectable in plasma in high concentrations.

The surprise finding was that albendazole was detectable in high concentrations in liver after its administration to sheep. It was also detectable in liver after its administration to cattle, but to a lesser extent.

Even more surprising was the finding that albendazole was detectable in liver and kidney after the administration of albendazole sulphoxide to sheep. It was also detectable in liver and kidney after the administration of albendazole sulphoxide to cattle, but to a lesser extent.

This experiment using ruminal fluid was devised to determine if the conversion of albendazole sulphoxide to albendazole (i.e. reduction of albendazole sulphoxide) occurred in the rumen. If this was so, these unexpected results would not be so difficult to explain.

Albendazole was detectable in ruminal fluid (Table 4.5). Maximum concentrations were reached at four hours post-treatment in both sheep (a mean concentration of 1.11 μg/ml). At forty-eight hours

post-treatment, albendazole was still detectable in one of the sheep (at a concentration of 0.05 μg/ml), but, at seventy-two hours post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphoxide was also detectable in ruminal fluid. Maximum concentrations were reached at four hours post-treatment in one of the sheep (a concentration of 1.71 μg/ml) and at eight hours post-treatment in the other sheep (a concentration of 0.34 μg/ml). At thirty-six hours post-treatment, albendazole sulphoxide was still detectable in both sheep (a mean concentration of 0.20 μg/ml), but, at forty-eight hours post-treatment, concentrations had fallen below a detectable level.

TABLE 4.5 Rumen fluid concentrations of albendazole sulphoxide and its metabolites in sheep

TIME(h)	CONCENTRATION (ug/ml) ALBENDAZOLE	ALBENDAZOLE SULPHOXIDE		RATIO OF ABZ./ABSX.
0	0.00	0.00	A	0
	0.00	0.00	B	0
2	1.04	0.88	A	1.18
	0.36	0.22	B	1.64
4	1.75	1.71	A	1.02
	0.48	0.31	B	1.55
8	1.25	0.66	A	1.89
	0.36	0.34	B	1.06
12	0.73	0.44	A	1.66
	0.30	0.12	B	2.50
24	0.45	0.22	A	2.04
	0.18	0.14	B	1.28
36	0.21	0.32	A	0.66
	0.06	0.09	B	0.67
48	0.05	N.D.	A	-
	N.D.	N.D.	B	-
72	N.D.	N.D.	A	-
	N.D.	N.D.	B	-
96	N.D.	N.D.	A	-
	N.D.	N.D.	B	-

N.D. = not detected (< 0.05 µg/ml) A = sheep No.1 B = sheep No.2

ABZ = albendazole
ABSX = albendazole sulphoxide

DISCUSSION

After the administration of albendazole or albendazole sulphoxide to sheep, residues were similar qualitatively and quantitatively.

With both treatments, the concentrations of liver residues were approximately five to ten times greater than the concentrations of muscle and plasma residues.

Tissue residues appear to follow the same pattern as plasma residues in that for both tissues and plasma, highest concentrations were reached at one day post-treatment and at five days post-treatment, concentrations had fallen below detectable levels.

High concentrations of the sulphide albendazole were found in liver after both treatments. This was indeed surprising since, after the administration of albendazole to sheep at a dose rate of 10 mg/kg (twice the dose used in these studies), albendazole was detectable only in very low concentrations in the plasma of two sheep (a mean maximum concentration of 0.02 µg/ml) and was not detected in the plasma of a third sheep (limit of detection 0.02 µg/ml) (Marriner and Bogan, 1980). With albendazole treatment, it was possible that unchanged albendazole was absorbed from the gut and via the hepatic portal system, travelled to the liver where it became bound. Delatour et al., (1983) found that albendazole binds to liver protein, although the extent and mechanism of binding are unknown. This may explain, in part, the presence of high concentrations of parent albendazole in the

liver. However, it does not explain the presence of high concentrations of albendazole in the liver after the administration of albendazole sulphoxide to sheep. For albendazole to be found in liver after albendazole sulphoxide treatment, reduction at sulphur must have occurred. It has been reported (Duggan et al., 1980) that the anti-inflammatory drug sulindac (a sulphoxide) undergoes irreversible oxidation to a sulphone. In addition, it undergoes reduction to a sulphide, a reaction which is readily reversible. The facile metabolic interconversion of fenbendazole (a sulphide) and oxfendazole (its corresponding sulphoxide) has also been reported (Marriner and Bogan, 1981a and 1981b). It is extremely unusual for any drug metabolic reaction to be freely reversible, as it appears to contradict the purpose of drug metabolism, which is to make more polar metabolites.

In vitro experiments using bovine liver microsomes indicated that after microsomes were incubated with albendazole sulphoxide (for six hours), only 1.5% of the total drug was present as albendazole, the sulphoxide accounting for 95.4% of the total drug (Marriner, 1980). Therefore, it is unlikely that liver residues of albendazole after the administration of albendazole sulphoxide to sheep were due to the reduction of albendazole sulphoxide in the liver. There is no evidence to suggest that sulphoxides are reduced to sulphides in the liver, either microsomally or non-microsomally (Zeigler, 1982).

The possibility existed that albendazole sulphoxide was converted to albendazole by reduction in the rumen. The rumen is considered a purely reductive medium (Prins, 1985). No examples of

oxidation of drugs in the rumen are known. There has been one report recently that netobimin (a pro-drug of albendazole) is extensively oxidised to albendazole sulphoxide in the rumen, as much as 90% of the labelled material being present as albendazole sulphoxide twenty-four hours after the administratin of netobimin (McDougall et al., 1985). However, this result is in doubt, since albendazole sulphoxide was detected only in small amounts in ruminal fluid in this laboratory after the administration of netobimin (Bogan-Personal Communication). It is also apparent that if albendazole sulphoxide did account for 90% of the labelled material in the rumen, it would not be present in such a high percentage, as it has been demonstrated, in this laboratory, that albendazole sulphoxide is extensively reduced to albendazole in the rumen. When ruminal fluid was incubated in vitro with albendazole sulphoxide, conversion to the sulphide, albendazole, occurred (after twenty-four hours, 11.1% of the total drug present was albendazole) (Marriner, 1980). Albendazole was found in ruminal fluid (at a mean maximum concentration of 1.11 ug/ml) after the administration of albendazole sulphoxide to sheep. This may explain, in part, the high concentrations of albendazole found in liver after the administration of albendazole sulphoxide. Also, since the solubility of albendazole is lower than the solubility of albendazole sulphoxide, there may have been more conversion to albendazole than was measured in filtered ruminal fluid (i.e. some of the albendazole may have precipitated once formed).

Albendazole sulphoxide and albendazole sulphone were also detectable in tissues after both treatments to sheep. These residues

were not unexpected since, albendazole sulphoxide and albendazole sulphone are the major metabolites of albendazole found in the plasma (Marriner and Bogan, 1980) and urine (Gyurik et al., 1981) of sheep. Plasma concentrations of albendazole sulphoxide and albendazole sulphone were similar to those found in muscle after albendazole treatment.

Kidney samples were taken after the administration of albendazole sulphoxide to sheep (unfortunately this was not done after the administration of albendazole to sheep) and it was found that the sulphide albendazole was detectable in kidney, although residues were lower than those found in liver. This was surprising, but it is known that after albendazole treatment to sheep, some of the parent sulphide (1.0%) is excreted unchanged in the urine (Gyurik et al., 1981). Therefore, if albendazole was formed in the rumen after albendazole sulphoxide treatment, and was absorbed, it is reasonable to suggest that ruminal reduction is the source of sulphide in the kidney.

Albendazole sulphoxide and albendazole sulphone were also detectable in kidney. This was not unexpected since they are major urinary metabolites of albendazole in sheep (24.3% of an administered dose of albendazole was excreted as albendazole sulphoxide, and 3.4% was excreted as albendazole sulphone) (Gyurik et al., 1981).

From all the residue data for albendazole and albendazole sulphoxide in sheep, it was clear that if residues were present at five days post-treatment, they would be very low (less than 0.05 μg/g)

and therefore the meat witholding time of ten days for sheep treated with albendazole would seem to be adequate.

It is interesting that in an experiment in which ^{14}C-labelled albendazole was administered to sheep (DiCuollo et al., 1977), extractable tissue residues did not fall below a concentration of 0.1 µg/g until ten days post-treatment, although in this experiment the dose of albendazole was 16 mg/kg (more than three times the recommended dose). Unfortunately, tissue residue studies using radiolabelled drugs do not distinguish between parent drugs and their metabolites. Therefore, it is impossible to determine whether or not residues contain metabolites which may be toxic or metabolites which are non-toxic. It is important, in any drug residue study, that the exact nature of the residues be determined.

According to Parish et al., (1977), the longest residing metabolite of albendazole in tissue is albendazole 2-aminosulphone. This metabolite is not anthelmintically active and it is not important toxicologically. In addition, in no tissue samples analysed in this laboratory did it constitute a quantitatively important metabolite. When measuring residues of albendazole, it was more important to monitor concentrations of the parent sulphide and the sulphoxide metabolite, since these two compounds could be the possible cause of toxicity to humans, especially now that albendazole has been made available for use in man in some countries, at a dose rate of 400 mg per person (Morris et al., 1983).

CHAPTER 5

STUDIES WITH ALBENDAZOLE AND ALBENDAZOLE SULPHOXIDE IN CATTLE

INTRODUCTION

Albendazole has a broad spectrum of activity against gastro-intestinal roundworms including inhibited larval stages, tapeworms, liver flukes and lungworms in cattle (Theodorides et al., 1976). It is the first broad spectrum anthelmintic to have useful efficacy at low dose levels against all major economically important helminth parasites infecting domestic animals. In cattle, it is widely used in the control of gastro-intestinal nematodes and tapeworms, lungworms and liver flukes (Knight and Colglazier, 1977).

(For a more detailed introduction describing the metabolism and toxicity of albendazole and albendazole sulphoxide, please refer to Chapter 4).

MATERIALS AND METHODS

These studies involved the administration of albendazole and albendazole sulphoxide to cattle, both at a dose rate of 7.5 mg/kg. All treatments were by oral drench, and the animals were slaughtered at 1, 3, 5 and 8 days after albendazole treatment and 1, 3 and 5 days after albendazole sulphoxide treatment. For both treatments, samples of liver, muscle nd kidney were taken at slaughter and stored immediately at -20°C until assayed (as described in Chapter 3). They were assayed in duplicate (Tables 5.1 and 5.2).

In addition, milking cows were treated with albendazole and albendazole sulphoxide by oral drench, again both at a dose rate of 7.5 mg/kg. For the cows treated with albendazole, milk samples were taken before and at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hours post-treatment. Both whole milk (Table 5.3) and the aqueous fraction of milk (Table 5.4) were analysed for residues. For the cows treated with albendazole sulphoxide, milk samples were taken before and at 12, 24 and 36 hours after treatment. Only whole milk (Table 5.5) was analysed for residues. The samples provided to us did not allow a large enough volume to determine residues in the aqueous fraction of milk also.

RESULTS

After albendazole treatment (Table 5.1), albendazole itself was detectable in liver at one day post-treatment (at a concentration of 1.14 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphoxide was also detectable in liver at one day post-treatment (at a concentration of 0.43 μg/g), but again, at three days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was detectable in liver at one day post-treatment (at a concentration of 0.93 μg/g), but, like albendazole and albendazole sulphoxide, at three days post-treatment, concentrations had fallen below a detectable level.

In muscle, albendazole was not detectable at any time after treatment.

Albendazole suphoxide, like albendazole, was not detectable in muscle at any time after treatment.

Albendazole sulphone was detectable in muscle at one day post-treatment (although only a trace amount was present), but, at three days post-treatment, concentrations had fallen below a detectable level.

In kidney, albendazole was not detectable at any time after treatment.

Albendazole sulphoxide, like albendazole, was not detectable in kidney at any time after treatment.

Albendazole sulphone was detectable in kidney at one day post-treatment (at a concentration of 0.21 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

TABLE 5.1 Liver, muscle and kidney residues of albendazole and its metabolites in cattle.

Animal No	Dose	Days after Treatment	Residues (µg/g wet wt) ABZ	ABSX	ABSO
			*	*	*
LIVER					
B65	7.5mg/kg	1	1.14	0.43	0.93
B85	7.5mg/kg	3	N.D.	N.D.	N.D.
B98	7.5mg/kg	5	N.D.	N.D.	N.D.
G81	7.5mg/kg	8	N.D.	N.D.	N.D.
MUSCLE					
B65	7.5mg/kg	1	N.D.	N.D.	TRACE
B85	7.5mg/kg	3	N.D.	N.D.	N.D.
B98	7.5mg/kg	5	N.D.	N.D.	N.D.
G81	7.5mg/kg	8	N.D.	N.D.	N.D.
KIDNEY					
B65	7.5mg/kg	1	N.D.	N.D.	0.21
B85	7.5mg/kg	3	N.D.	N.D.	N.D.
B98	7.5mg/kg	5	N.D.	N.D.	N.D.
G81	7.5mg/kg	8	N.D.	N.D.	N.D.

ABZ = albendazole
ABSX = albendazole sulphoxide
ABSO = albendazole sulphone

* = Mean values of two determinations.

N.D. = Not detected (< 0.05 µg/g).

After albendazole sulphoxide treatment (Table 5.2), albendazole sulphoxide was itself detectable in liver at one day post-treatment (at a concentration of 0.44 μg/g in one calf, although only a trace amount was present in the other calf), but, at three days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in liver at one day post-treatment (at a mean concentration of 3.58 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

Surprisingly, albendazole was detectable in liver at one day post-treatment (at a mean concentration of 3.98 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

In muscle, albendazole sulphoxide was not detectable at any time after treatment.

Albendazole sulphone was detectable in muscle at one day post-treatment (although only in one calf at a concentration of 1.15 μg/g; it was not detectable in the other calf), but, at three days post-treatment, concentrations had fallen below a detectable level.

Albendazole was not detectable in muscle at any time after

treatment.

In kidney, albendazole sulphoxide was detectable at one day post-treatment (although only in one calf at a concentration of 0.54 μg/g; it was not detectable in the other calf), but, at three days post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in kidney at one day post-treatment (at a mean concentration of 2.64 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

Again, surprisingly, albendazole was detectable in kidney at one day post-treatment (at a mean concentration of 1.52 μg/g), but, at three days post-treatment, concentrations had fallen below a detectable level.

TABLE 5.2 Liver, muscle and kidney residues of albendazole sulphoxide and its metabolites in cattle.

LIVER

Animal No	Dose	Days after Treatment	Residues (µg/g wet wt) ABZ	ABSX	ABSO
78	7.5mg/kg	1	3.20	TRACE	1.30
B42	7.5mg/kg	1	4.76	0.44	5.86
A771	7.5mg/kg	3	N.D.	N.D.	N.D.
7243	7.5mg/kg	3	N.D.	N.D.	N.D.
534	7.5mg/kg	5	N.D.	N.D.	N.D
657	7.5mg/kg	5	N.D.	N.D.	N.D

MUSCLE

78	7.5mg/kg	1	N.D.	N.D.	N.D.
B42	7.5mg/kg	1	N.D.	N.D.	1.15
A771	7.5mg/kg	3	N.D.	N.D.	N.D.
7243	7.5mg/kg	3	N.D.	N.D.	N.D.
534	7.5mg/kg	5	N.D.	N.D.	N.D
657	7.5mg/kg	5	N.D.	N.D.	N.D

KIDNEY

78	7.5mg/kg	1	1.68	N.D.	0.87
B42	7.5mg/kg	1	1.36	0.54	4.42
A771	7.5mg/kg	3	N.D.	N.D.	N.D.
7243	7.5mg/kg	3	N.D.	N.D.	N.D.
534	7.5mg/kg	5	N.D.	N.D.	N.D
657	7.5mg/kg	5	N.D.	N.D.	N.D

ABZ = albendazole
ABSX = albendazole sulphoxide
ABSO = albendazole sulphone

N.D. = Not detected (< 0.05 µg/g)

After albendazole administration to cows (Table 5.3), albendazole itself was not detectable in whole milk at any time after treatment.

Albendazole sulphoxide was detectable in whole milk. Maximum concentrations were reached at twleve hours post-treatment in both animals (0.24 μg/ml and 0.52 μg/ml).

In one of the cows, albendazole sulphoxide was still detectable at twenty-four hours post-treatment (at a concentration of 0.04 μg/ml), but, at thirty-six hours post-treatment, concentrations had fallen below a detectable level.

In the other cow, albendazole sulphoxide was still detectable at thirty-six hours post-treatment (at a concentration of 0.07 μg/ml), but, at forty-eight hours post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in whole milk. Maximum concentrations were reached at twelve hours post-treatment in both animals (1.44 μg/ml and 2.18 μg/ml).

In both cows, albendazole sulphone was still detectable at twenty-four hours post-treatment (0.04 μg/ml and 0.03 μg/ml), but, at thirty-six hours post-treatment, concentrations had fallen below detectable levels.

In the aqueous fraction of milk (Table 5.4), albendazole itself was not detectable at any time after treatment.

Albendazole sulphoxide was detectable in the aqueous fraction of milk. Maximum concentrations were reached at twelve hours post-treatment in both animals (0.18 µg/ml and 0.14 µg/ml).

In one of the cows, albendazole sulphoxide was still detectable at twenty-four hours post-treatment (at a concentration of 0.04 µg/ml), but, at thirty-six hours post-treatment, concentrations had fallen below detectable levels.

In the other cow,albendazole sulphoxide was still detectable at thirty-six hours post-treatment (at a concentration of 0.02 µg/ml), but, at forty-eight hours post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in the aqueous fraction of milk. Maximum concentrations were reached at twelve hours post-treatment (0.01 µg/ml for both cows), but, at thirty-six hours post-treatment, concentrations had fallen below detectable levels.

TABLE 5.3 Milk residues (whole milk) of albendazole and its metabolites in two cows.

TIME(h)	CONCENTRATION (μg/ml) ALBENDAZOLE	ALBENDAZOLE SULPHOXIDE	ALBENDAZOLE SULPHONE	
0	0.00	0.00	0.00	A
	0.00	0.00	0.00	B
12	N.D.	0.24	1.44	A
	N.D.	0.52	2.18	B
24	N.D.	0.07	0.04	A
	N.D.	0.04	0.03	B
36	N.D.	0.07	N.D.	A
	N.D.	N.D.	N.D.	B
48	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
60	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
72	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
84	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
96	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
108	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
120	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
132	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
144	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B

N.D. = not detected (< 0.05 μg/ml) A = cow No.205 B = cow No.206

TABLE 5.4 Milk residues (aqueous fraction) of albendazole and its metabolites in two cows.

	CONCENTRATION (μg/ml)			
TIME(h)	ALBENDAZOLE	ALBENDAZOLE SULPHOXIDE	ALBENDAZOLE SULPHONE	
0	0.00	0.00	0.00	A
	0.00	0.00	0.00	B
12	N.D.	0.18	0.52	A
	N.D.	0.14	0.37	B
24	N.D.	0.05	0.01	A
	N.D.	0.04	0.01	B
36	N.D.	0.02	N.D.	A
	N.D.	N.D.	N.D.	B
48	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
60	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
72	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
84	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
96	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
108	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
120	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
132	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
144	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B

N.D. = not detected A = cow No.205 B = cow No.206

After albendazole sulphoxide treatment of cows (Table 5.5), albendazole sulphoxide itself was detectable in whole milk. Maximum concentrations were reached at twelve hours post-treatment in four of the cows (a mean concentration of 0.12 μg/ml), but, at twenty-four hours post-treatment, concentrations had fallen below a detectable level.

In the other cow, albendazole sulphoxide was detectable in whole milk at twelve hours post-treatment (at a concentration of 0.11 μg/ml), but, maximum concentrations were reached at twenty-four hours post-treatment (a concentration of 0.18 μg/ml). At thirty-six hours post-treatment, concentrations had fallen below a detectable level.

Albendazole sulphone was also detectable in whole milk. Maximum concentrations were reached at twelve hours post-treatment in all five cows (a mean concentration of 0.46 μg/ml). At twenty-four hours post-treatment, concentrations had fallen below a detectable level in four of the cows.

In the other cow, albendazole sulphone was still detectable at twenty-four hours post-treatment (although only a trace amount was present), but, at thirty-six hours post-treatment, concentrations had fallen below a detectable level.

Albendazole was not detectable in whole milk at any time after treatment.

TABLE 5.5 The concentration (μg/ml) of albendazole sulphoxide (ABSX) and albendazole sulphone (ABSO) in milk at intervals after oral administration of albendazole sulphoxide at a dose rate of 7.5 mg/kg.

		TIME POST-TREATMENT (hours)			
COW NO		0	12	24	36
1	ABSX	N.D.	0.26	N.D.	N.D.
	ABSO	N.D.	0.73	N.D.	N.D.
6	ABSX	N.D.	0.11	N.D.	N.D.
	ABSO	N.D.	0.53	N.D.	N.D.
19	ABSX	N.D.	0.06	N.D.	N.D.
	ABSO	N.D.	0.28	N.D.	N.D.
33	ABSX	N.D.	0.06	N.D.	N.D.
	ABSO	N.D.	0.32	N.D.	N.D.
45	ABSX	N.D.	0.11	0.18	N.D.
	ABSO	N.D.	0.45	TRACE	N.D.

N.D. = Not detected

DISCUSSION

After the administration of albendazole or albendazole sulphoxide to cattle, residues were similar qualitatively and quantitatively. With both treatments, concentrations of albendazole, albendazole sulphoxide and albendazole sulphone in tissues were much lower than those found in sheep tissues. In addition, residues did not persist as long as they did in sheep. In general, cattle have a greater capacity for the oxidation of benzimidazoles than sheep do, and in the case of albendazole, the conversion of the sulphoxide to the sulphone metabolite is more rapid and complete in cattle than in sheep. Therefore, since this conversion is irreversible (Gyurik et al., 1981), it was not surprising that no detectable residues of albendazole sulphoxide were found in muscle after both treatments. This increased oxidative capacity of cattle for benzimidazoles may also explain the finding that liver residues of albendazole and albendazole sulphoxide were much lower in cattle than in sheep. The finding that residues of the sulphide were detectable in liver after both treatments was surprising, because albendazole itself does not circulate in the plasma of cattle after these treatments. In the case of albendazole treatment, it was probable that unchanged albendazole was absorbed and bound in the liver (as has already been discussed for albendazole residues in sheep liver), but this does not explain residues of the sulphide after albendazole sulphoxide treatment. In this case, it was probable that albendazole sulphoxide was reduced to albendazole to some extent in the rumen (as has been shown to occur in sheep) and the newly-formed albendazole absorbed and bound in the liver.

With both treatments, it would appear that liver residue concentrations were higher than muscle and kidney residue concentrations (although kidney residues were higher than those found in muscle). It would seem that the concentrations of liver residues were around twice the amount of those found in kidney, which were around four times greater than those found in muscle.

Albendazole was detectable in kidney after the administration of albendazole sulphoxide. Albendazole residues were not unexpected in kidney, as it is known that after albendazole treatment of cattle, some of the parent sulphide (0.1%) is excreted unchanged in the urine (Gyurik et al., 1981).

From all the residue data for albendazole and albendazole sulphoxide in cattle, it was clear that if residues were present at three days post-treatment, they would be very low (less than 0.05 μg/g) and therefore the meat witholding time of fourteen days for cattle treated with albendazole would seem to be adequate.

Delatour et al., (1984) have pointed out that in recent years, an increasing number of drugs have been reported for which metabolism includes some covalent binding of metabolites to macromolecules, which gives rise to non-extractable tissue-bound residues. These residues are often the result of highly reactive intermediates which have only been isolated in the last few years. Isolation is difficult because of their highly reactive nature. These tissue-bound residues are of considerable interest because of their possible participation in

target species toxicity, as well as their potential toxicological significance in terms of food hygiene for humans.

In these studies with albendazole and albendazole sulphoxide, the extraction procedure used involved boiling the tissues in strong acid (see Chapter 3). Therefore, any drug-related bound residues would have been extractable and hence, detectable. It has been shown (Delatour et al., 1984) that bound residues of albendazole do not appear to have any toxicological significance because they are strongly covalently bound and therefore they are not absorbed. Toxicity of residues is related to the pharmacokinetics of the parent sulphide and its free metabolites.

Milk was free of residues of the sulphide albendazole after the administration of albendazole or albendazole sulphoxide to cows.

Albendazole sulphoxide and albendazole sulphone were detectable in milk after both treatments. These results were not surprising since, albendazole sulphoxide and albendazole sulphone were the major metabolites found in cattle plasma after both treatments.

The interesting finding was that after the administration of albendazole to cows, residues in the aqueous fraction of milk were very similar to those found in whole milk. Maximum concentrations were found at twelve hours post-treatment in both cases and the residue profiles were identical. From the results it seems that after albendazole treatment, residues of albendazole sulphoxide and

albendazole sulphone were almost exclusively associated with the aqueous fraction of milk. These results are important in that in the manufacture of cream, cheese and other milk products, the lipid fraction of milk is used. Therefore, if residues are associated with the aqueous fraction of milk, they would pose less of a problem to the consumer of these products.

From all the residue data for albendazole and albendazole sulphoxide in cows' milk, it was clear that residues did not persist beyond thirty-six hours (if residues were present beyond this time, they would be very low: less than 0.05 μg/ml). Therefore, the milk witholding time of seventy-two hours for cattle treated with albendazole would appear to be adequate.

CHAPTER 6

STUDIES WITH FEBANTEL

Febantel (N-2-(2,3-bis-methoxy-carbonyl-guanidino) -5-(phenyl-thio)- phenyl-2-methoxy-acetamide) is a broad spectrum anthelmintic which is active against gastro-intestinal roundworms and lungworms in sheep and cattle, and many other species (Behrens, 1978; Burger, 1978; Corba, 1981; Ciordia et al., 1982; Dimitrov et al., 1982). It is a member of the pro-benzimidazole group of anthelmintics.

The metabolism of febantel has been extensively studied (Delatour et al., 1982b), with nine metabolites being identified in the urine of sheep and rats which had been given febantel orally (Figure 6.1). Febantel is metabolised by cyclization, oxidation at sulphur, aromatic hydroxylation and carbamate hydrolysis. The mechanism of cyclization is poorly understood. It has been suggested that it is mono-oxygenase dependent (Douch et al., 1974), but it seems more likely that cyclization occurs to a great extent in the gut (i.e. before absorption has taken place) (Bogan, Personal Communication). It is known that febantel undergoes hydrolytic removal of a methoxyacetyl group prior to cyclization to a benzimidazole (in this case fenbendazole).

After cyclization, the most important metabolic reactions leading to the observed urine metabolites were oxidation at sulphur and carbamate hydrolysis. Apart from metabolite A (which is a sulphide), the important urinary metabolites were all sulphoxides or sulphones. The oxidation of alkyl and aryl sulphides to more polar sulphoxides and sulphones is commonly observed and facilitates urinary excretion (Duggan et al., 1977).

In sheep and rats, the most abundant urinary metabolites were: metabolite A (fenbendazole), metabolite A' (the sulphoxide of febantel, which cyclizes to oxfendazole), metabolite C (oxfendazole) and metabolite B (oxfendazole sulphone).

When sheep were given febantel orally at a dose rate of 7.5 mg/kg, fenbendazole, oxfendazole and oxfendazole sulphone were all detectable in plasma. Febantel itself was also detectable in plasma, although only in very low concentrations (less than 0.05 µg/ml) (Delatour et al., 1985a).

When cattle were given febantel orally at a dose rate of 7.5 mg/kg, fenbendazole, oxfendazole and oxfendazole sulphone were also detectable in plasma. In addition, febantel and its sulphoxide metabolite were also detectable in plasma (Delatour et al., 1985a).

Fenbendazole and oxfendazole have also been identified in the milk of cows treated with febantel at a dose rate of 7.5 mg/kg (Delatour et al., 1983b).

In sheep, fenbendazole, oxfendazole and oxfendazole sulphone are also found in plasma after the administration of oxfendazole (Marriner and Bogan, 1981a) and fenbendazole (Marriner and Bogan, 1981b). Similarly, in cattle, fenbendazole, oxfendazole and oxfendazole sulphone are found in plasma after the administration of oxfendazole and fenbendazole (Ngomuo et al., 1984). From these results, it is clear that fenbendazole, oxfendazole and oxfendazole sulphone are the major

metabolites of each of these three anthelmintics (febantel, fenbendazole and oxfendazole).

The facile metabolic interconversion of alkyl aryl sulphides has been reported (Duggan et al., 1980) and this is known to occur with fenbendazole and oxfendazole (Marriner and Bogan, 1981a and 1981b), although the metabolic oxidation of sulphoxides (i.e. oxfendazole) to sulphones (i.e. oxfendazole sulphone) appears to be irreversible (Kexel and Schmidt, 1972).

Regarding toxicity, febantel is well tolerated in all domestic animals. It has been extensively studied with regard to its teratogenic potential, since one of its metabolites (oxfendazole) which is itself marketed, has been shown to be teratogenic.

From the teratogenic point of view, febantel is said to be safe in the ewe at an oral dosage of 10 mg/kg (Terblanche, 1979), while at 45 mg/kg administered as a single dose on day seventeen of pregnancy, renal and skeletal abnormalities occurred in more than ten per cent of the lambs (Chement, 1982). Metabolite C (oxfendazole) was not teratogenic in sheep at doses of 7.5 mg/kg, 10 mg/kg and 15 mg/kg, but, at doses of 22.5 mg/kg and 25 mg/kg on day seventeen of pregnancy, it induced embryolethality and malformations (Delatour et al., 1977; Piercy et al., 1979). The study of the embryotoxic effects of individual metabolites of oxfendazole has indicated that the parent compound seems to be responsible for the observed teratogenicity (Delatour et al., 1982a). Oxfendazole (metabolite C) is also the ultimate teratogen of febantel (Delatour et al., 1982b).

In the rat, febantel, metabolite A' (the sulphoxide of febantel) and metabolite C (oxfendazole) have been found to be teratogenic (Delatour et al., 1982b).

From all this toxicological data, it is clear that in any residue studies involving febantel, it is most important that the parent compound should be measured, as should metabolite A' (febantel sulphoxide) and metabolite C (oxfendazole). In addition, metabolite A (fenbendazole) should be measured as it is readily interconvertible with metabolite C (oxfendazole).

In the following experiment with febantel, metabolite A, metabolite B (oxfendazole sulphone) and metabolite C were measured (Figure 6.2). The reason for measuring metabolite B was that, although it is not toxicologically important, it is a major metabolite of febantel in the plasma of sheep (Delatour et al., 1985a). Also, under the chromatographic conditions used to measure metabolite C, metabolite B is easily measured. Unfortunately, the method used did not allow the determination of febantel itself, but, since it is detectable in plasma only in very low concentrations after its administration to sheep at a dose rate of 7.5 mg/kg (Delatour et al., 1985a), it is unlikely that it would be detectable in plasma in this experiment (in which the sheep were dosed at 5 mg/kg). Furthermore, the chromatographic conditions used for the determination of metabolites A, B and C, did not allow for the determination of metabolite A'.

In this experiment, the animals were slaughtered at various times

after the administration of febantel. Liver, muscle and plasma were analysed for residues until they could no longer be detected (limits of detection 0.05 µg/g for tissues and 0.05 µg/ml for plasma). Table 6.1 lists the meat and milk witholding times for febantel in sheep and cattle.

FIGURE 6.1 The proposed metabolic pathway of febantel (Delatour et al., 1982 b).

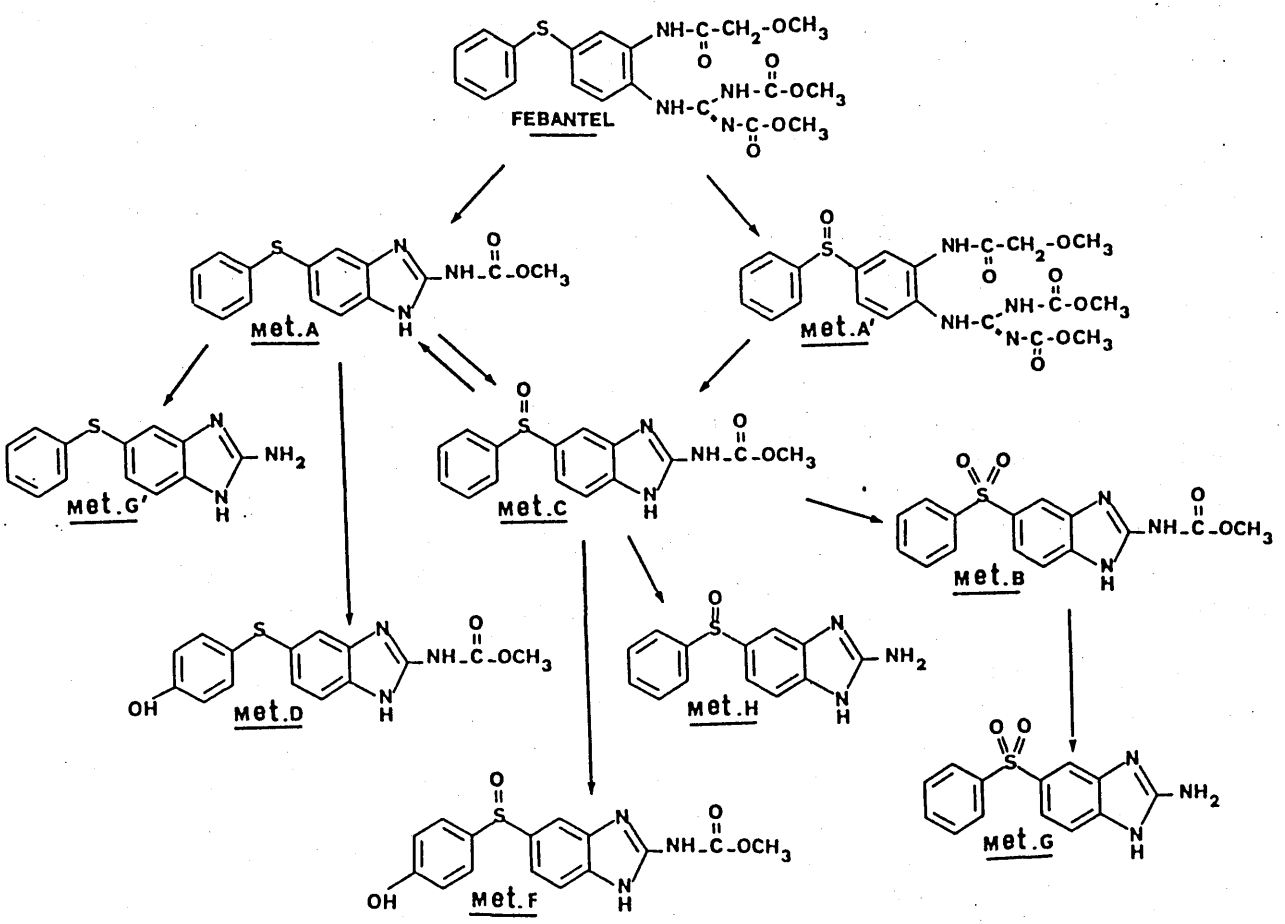


FIGURE 6.2 Febantel and its important metabolites.

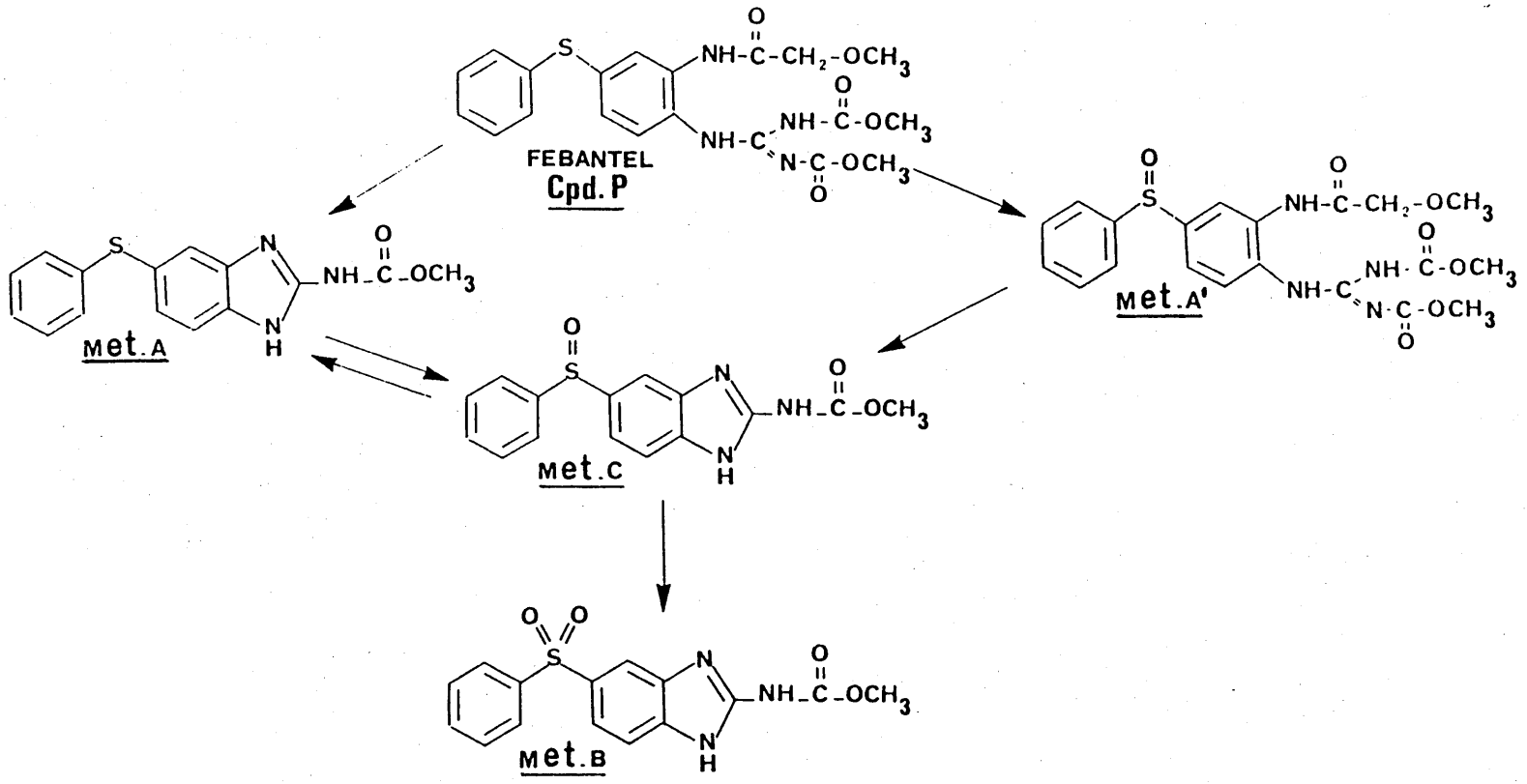


TABLE 6.1 The meat and milk witholding times for febantel in sheep and cattle

SPECIES	WITHHOLDING TIME (days)	
	MEAT	MILK
CATTLE	8	2
SHEEP	8	-

RESIDUES OF THE MAJOR METABOLITES OF FEBANTEL IN THE TISSUES OF SHEEP

In this experiment, five sheep were given febantel orally, at a dose rate of 5 mg/kg (recommended dosage) (Table 6.2). They were slaughtered at 1, 7, 14, 21 and 28 days post-treatment.

Samples of liver and muscle were taken at slaughter and stored immediately at -20°C until assayed (as described in Chapter 3).

Blood samples were also taken at intervals throughout the course of the experiment to assess the tissue/plasma ratio of residues; plasma was removed after centrifugation and stored at -20°C until assayed.

All liver and muscle samples were assayed in duplicate (Tables 6.3 and 6.4).

TABLE 6.2 Weights, dose volumes and dosages for the five sheep used in the febantel residue study

SHEEP NO.	WT.(kg)	VOL. SUSPENSION 2.5% w/v (ml)	TOTAL DOSE (mg)	DOSE RATE (mg/kg)
123	72	14.4	360	5
88	75	15.0	375	5
89	68	13.6	340	5
90	84	16.8	420	5
102	77	15.4	385	5

TABLE 6.3 Liver residues of febantel metabolites in sheep

Animal No	Dose	Days after Treatment	Residues (µg/g wet wt) Fenbendazole	Oxfendazole	Oxfendazole Sulphone
123	5mg/kg	1	* (4.89) 5.02 (5.16)	(4.05) 4.05 (4.05)	(1.42) 1.40 (1.38)
88	5mg/kg	7	(1.30) 1.09 (0.88)	(2.86) 2.66 (2.33)	(0.25) 0.20 (0.15)
89	5mg/kg	14	(0.30) 0.28 (0.26)	(0.57) 0.50 (0.42)	N.D.
90	5mg/kg	21	N.D.	(0.15) 0.12 (0.10)	N.D.
102	5mg/kg	28	N.D.	N.D.	N.D.

* = Mean value of two determinations

N.D. = Not detected

FIGURE 6.3 Semi-logarithmic plot of liver residues of fenbendazole and oxfendazole after the administration of fenbendazole to sheep.

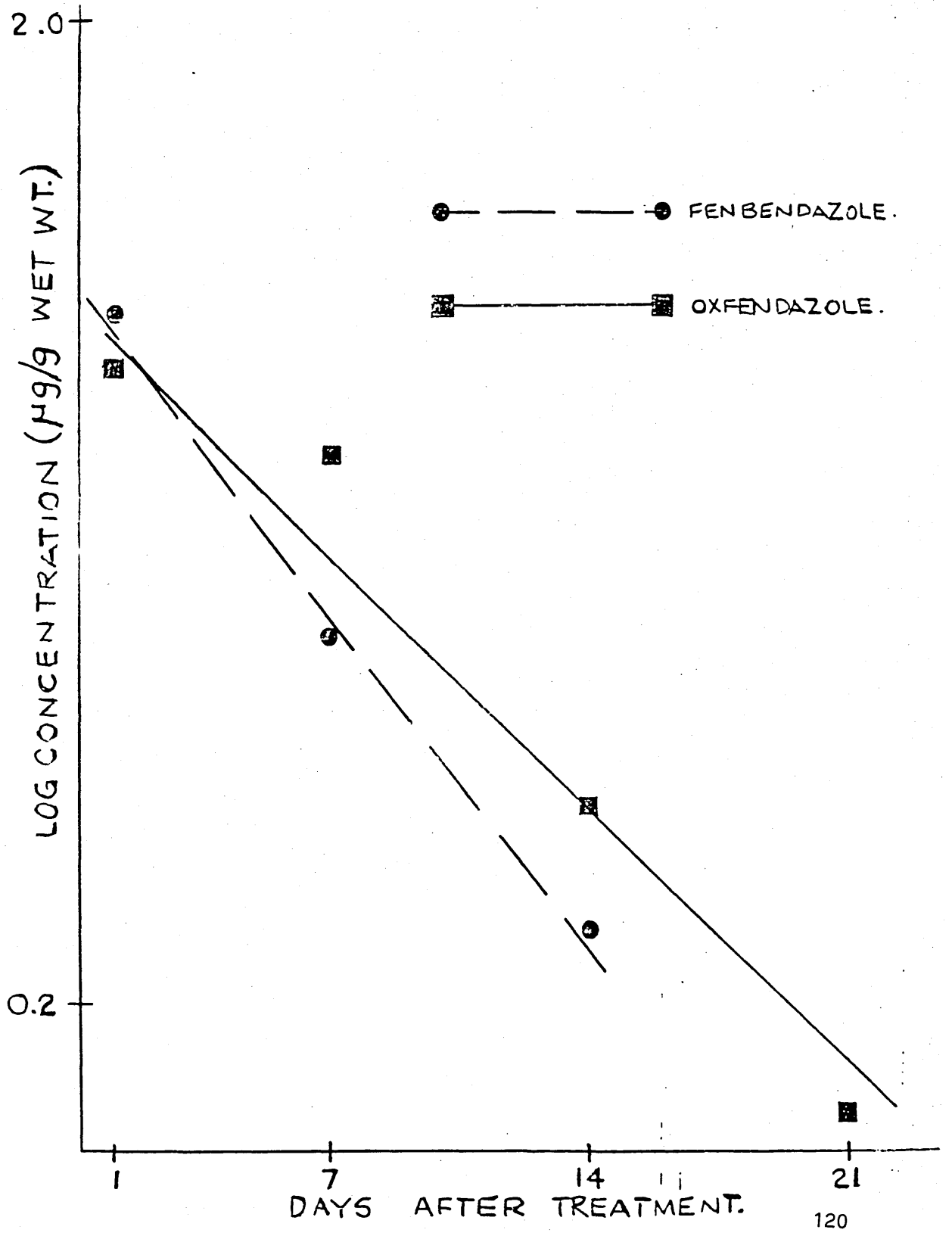


TABLE 6.4 Muscle residues of febantel metabolites in sheep

Animal No	Dose	Days after Treatment	Residues (µg/g wet wt) Fenbendazole	Oxfendazole	Oxfendazole Sulphone
123	5mg/kg	1	* (0.35) 0.41 (0.47)	(0.16) 0.16 (0.17)	(0.14) 0.14 (0.14)
88	5mg/kg	7	N.D.	Trace	N.D.
89	5mg/kg	14	N.D.	N.D.	N.D.
90	5mg/kg	21	N.D.	N.D.	N.D.
102	5mg/kg	28	N.D.	N.D.	N.D.

* = Mean value of two determinations

N.D. = Not detected

TABLE 6.5 Plasma concentrations of febantel metabolites in sheep

Animal No	Dose	Days after Treatment	Concentration (µg/ml) Febantel	Fenbendazole	Oxfendazole	Oxfendazole Sulphone
123	5mg/kg	1	N.D.	0.07	0.29	0.32
88	5mg/kg	7	N.D.	N.D.	N.D.	N.D.
89	5mg/kg	14	N.D.	N.D.	N.D.	N.D.
90	5mg/kg	21	N.D.	N.D.	N.D.	N.D.
102	5mg/kg	28	N.D.	N.D.	N.D.	N.D.

N.D. = Not detected

After febantel treatment, fenbendazole was detectable in liver in high concentrations at one day post-treatment (a concentration of 5.02 μg/g). Residues persisted and at fourteen days post-treatment fenbendazole was still detectable (at a concentration of 0.28 μg/g), but, at twenty-one days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole was also detectable in liver in high concentrations at one day post-treatment (a concentration of 4.05 μg/g). Again, residues persisted and at twenty-one days post-treatment oxfendazole was still detectable (at a concentration of 0.12 μg.g), but, at twenty-eight days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was detectable in liver at one day post-treatment (at a concentration of 1.40 μg/g). It was still detectable at seven days post-treatment (at a concentration of 0.20 μg/g), but, at fourteen days post-treatment, concentrations had fallen below a detectable level.

A semi-logarithmic representation of the concetrations of fenbendazole and oxfendazole in liver versus time, after the administration of febantel, was plotted (Figure 6.3). Linear regression analysis revealed straight lines for both compounds (R = 0.997 for fenbendazole and 0.984 for oxfendazole) indicating first-order elimination.

In muscle, fenbendazole was detectable at one day post-treatment (at a concentration of 0.41 ug/g), although in a much lower concentration than was found in liver at the same time. Residues did not persist and at seven days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole was also detectable in muscle at one day post-tretment (at a concentration of 0.16 µg/g). At seven days post-tretment it was still detectable (although only a trace amount was present), but, at fourteen days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was detectable in muscle at one day post-treatment (at a concentration of 0.14 µg/g). Residues did not persist and at seven days post-treatment, concentrations had fallen below a detectable level.

In plasma (Table 6.5), fenbendazole was detectable at one day post-treatment (at a concentration of 0.07 µg/ml). Residues did not persist and at seven days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole was also detectable in plasma at one day post-treatment (at a concentration of 0.29 µg/ml). Again, residues did not persist and at seven days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was detectable in plasma at one day post-treatment (at a concentration of 0.32 µg/ml). As with fenbendazole and oxfendazole, residues did not persist and at seven days post-treatment, concentrations had fallen below a detectable level.

Febantel itself was not detectable in plasma at any time after treatment.

DISCUSSION

After the administration of febantel to sheep, its major metabolites (fenbendazole, oxfendazole and oxfendazole sulphone) were detectable in liver in high concentrations. Fenbendazole and oxfendazole persisted in liver. At fourteen days post-treatment, fenbendazole was still detectable, but oxfendazole persisted longer, and was still detectable at twenty-one days post-treatment. Febantel is considered a pro-drug of fenbendazole and oxfendazole. Therefore, one might expect the residue profile of febantel to be similar to the profiles of fenbendazole and oxfendazole. This is in fact the case (see Chapters 7 and 8), residues of oxfendazole being detectable in liver at twenty-one days after the administration of both fenbendazole (Chapter 7) and oxfendazole (Chapter 8).

In muscle, concentrations of these metabolites were very low compared to those found in liver, and they did not persist as long (at seven days post-treatment only a trace amount of oxfendazole was detectable in muscle).

In plasma, concentrations of these metabolites were also very low (similar to those found in muscle). At seven days post-tretment, concentrations of fenbendazole, oxfendazole and oxfendazole sulphone had fallen below detectable levels. Febantel itself was not detectable in plasma at any time after treatment. This was not surprising since, after the administration of febantel to sheep at a dose rate of 7.5 mg/kg, the parent compound was detectable only in very low concentrations (a maximum of 0.02 µg/ml) in plasma (Delatour et al.,

1985a). Therefore, it is very unlikely that it would be detectable in plasma after a dose of 5 mg/kg.

From these results, it seems clear that residues of the metabolites of febantel are high and persist well beyond the recommended meat withholding time of eight days for sheep treated with febantel. Therefore, an eight day withholding time may be inadequate, especially considering that the metabolite which is still detectable in liver at twenty-one days post-treatment (oxfendazole) is a known teratogen. However, since the teratogenic and embryotoxic effects of oxfendazole are dose-related, a determination of a NEL, followed by a calculation of an ADI for a certain safety factor would be required to determine the minimum withholding period. Also involved is a consideration of the relationship between residue concentration and time. The elimination of oxfendazole from the liver of sheep treated with febantel is first-order (Figure 6.3), and the extrapolation of these results indicates that perhaps a twenty-one day withholding time would ensure a greater degree of safety. Furthermore, any complete determination of withholding periods for these compounds must include relay-toxicity studies involving absorption, elimination and toxic effects of residues in monogastric animals. Such studies would determine whether they are absorbed from contaminated food and whether there is any potential for teratogenesis or embryotoxicity.

CHAPTER 7

STUDIES WITH FENBENDAZOLE

INTRODUCTION

Fenbendazole (methyl-5-(phenylthio)-2-benzimidazole carbamate) is a potent member of the benzimidazole group of anthelmintics. It was the first anthelmintic which proved to be effective against inhibited larval stages of the important parasite of cattle Ostertagia ostertagi (Duncan et al., 1976). It has also been shown to be extremely effective against lungworms in cattle (Duwel, 1974) and sheep (Ross, 1975), and has also been shown to have some activity against tapeworms and liver flukes (Corba et al., 1979).

The metabolism and pharmacokinetics of fenbendazole have been studied in various species. Fenbendazole has been measured in the plasma of cattle, sheep and other species (Duwel et al., 1975) using a fluorimetric method which unfortunately did not distinguish the parent drug from its metabolites. Similarly, plasma and abomasal fluid concentrations of ^{14}C-labelled fenbendazole have been reported in cattle after intra-ruminal administration (Prichard et al., 1978), but again, the method did not distinguish between the parent drug and its metabolites. Duwel (1977) has also reported on the terminal metabolites of ^{14}C-labelled fenbendazole in sheep and cattle, but plasma concentrations of its metabolites were not reported. The principal metabolite in sheep and cattle urine results from hydroxylation of the phenyl ring, although carbamate hydrolysis also occurs to some extent. In pig urine, the quantity of metabolites is higher and two other metabolites are present (which are not present in sheep and cattle urine), one of which is a hydroxyl derivative. Metabolites of fenbendazole are also excreted in the faeces (Duwel, 1977).

After the administration of fenbendazole to sheep (Marriner and Bogan, 1981b), the parent sulphide was detected in plasma along with the sulphoxide metabolite (oxfendazole) and the sulphone metabolite (oxfendazole sulphone). Similarly, after the administration of fenbendazole to cattle (Ngomuo et al., 1984), oxfendazole and oxfendazole sulphone were found to be the major plasma metabolites.

In the horse, oxfendazole and oxfendazole sulphone were also found to be the major plasma metabolites after fenbendazole administration (Marriner and Bogan, 1985).

Oxfendazole is also detectable in pig plasma after fenbendazole administration (Anonymous, 1984).

It has been reported (Anonymous, 1983) that at one day after a cow was given ^{14}C-labelled fenbendazole at a dose rate of 10 mg/kg, total liver residues were 17.6 µg/g, of which approximately 80% was fenbendazole and 6% was oxfendazole. Duwel (1977) has reported on total tissue and milk residues of fenbendazole. Residues in cows' milk were low after the administration of fenbendazole at a dose rate of 7.5 mg/kg. Maximum concentrations were 0.30 µg/ml at twenty-eight hours post-treatment, and at fifty-four hours post-treatment, residues could no longer be detected (limit of detection of 0.05 µg/ml).

In sheep, cattle and pigs treated with fenbendazole at recommended dose rates, residues were low in most tissues after two days, and approached the limit of detection (0.05 µg/g) at five days.

However, residues were somewhat higher in the liver and persisted for up to fourteen days.

These residue studies did not distinguish fenbendazole itself from its important metabolites oxfendazole and oxfendazole sulphone. Fenbendazole and oxfendazole are metabolically interconvertible. Therefore, when fenbendazole is administered, oxfendazole should be measured as a matter of course.

Toxicity studies have revealed that the sulphoxide metabolite of fenbendazole (oxfendazole) may be important as regards teratogenicity. Investigations have shown that fenbendazole is well tolerated in the host animal. In toxicity tests involving a single administration to small rodents, it was not possible to determine the minimum lethal dose. The oral LD_{50} is certainly greater than 10,000 mg/kg in rats and mice (Duwel, 1977).

Apart from transient and dose-dependent increases of individual transaminases (cytosolic liver enzymes which indicate hepatotoxicity when found in plasma) in a few members of each group of animals treated, no side-effects were observed. In sheep, a single oral dose of 5,000 mg/kg was well tolerated, and in cattle, a single oral dose of 2,000 mg/kg was well tolerated (Duwel, 1977).

In sheep, fenbendazole is not teratogenic (Becker, 1975), but its sulphoxide metabolite (oxfendazole) has been shown to be teratogenic (Delatour et al., 1977).

Neither fenbendazole (Becker, 1975) nor oxfendazole (Piercy et al., 1979) have been shown to be teratogenic in cattle.

Pigs have been reported to be insensitive with respect to the teratogenicity of fenbendazole (Anonymous, 1984) and oxfendazole (Morgan, 1982) during the first month of pregnancy.

No teratogenic effects have been observed for fenbendazole (Becker, 1975) or oxfendazole (Anonymous, 1978) in the horse.

Fenbendazole appears to be non-teratogenic in the rat (Baeder et al., 1974). However, embryolethal and teratogenic effects have been observed for oxfendazole in the rat (Delatour et al., 1977).

It has also been reported that fenbendazole is embryotoxic but not teratogenic in rabbits at a dose of 63 mg/kg (Anonymous, 1984).

The reason that fenbendazole is not teratogenic is probably that because it is very insoluble, insufficient can dissolve (no matter how high the dose) to result in blood concentrations sufficiently high to produce toxic effects (Marriner and Bogan, 1981b). It has also been demonstrated that the sulphone metabolite of fenbendazole is not teratogenic (Delatour et al., 1982a).

From this toxicological data, it is clear that in any residue studies involving fenbendazole, it is most important that the parent sulphide (fenbendazole) should be measured, as should the sulphoxide

metabolite (oxfendazole), since none of the other metabolites of fenbendazole have been found to be toxic. Although fenbendazole itself is not teratogenic, it is readily interconvertible with oxfendazole (which is teratogenic), hence, it is important that both compounds are measured.

In the following experiments with fenbendazole, the parent sulphide, and the sulphoxide and sulphone metabolites were measured (Figure 7.1). The reason for measuring oxfendazole sulphone was that, although it is not toxicologically important, it is a major metabolite of fenbendazole in the plasma of sheep (Marriner and Bogan, 1981b) and cattle (Ngomuo et al., 1984). Also, under the chromatographic conditions used to measure oxfendazole, oxfendazole sulphone is easily measured.

In all experiments, the animals were slaughtered at various times after the administration of fenbendazole. Liver, muscle, milk and plasma were analysed for residues of fenbendazole and its metabolites until they could no longer be detected (limits of detection 0.05 μg/g for tissues and 0.05 μg/ml for mlik and plasma). It was also assessed whether or not residues persisted beyond recommended withdrawal periods (Table 7.1).

FIGURE 7.1 Fenbendazole and its important metabolites.

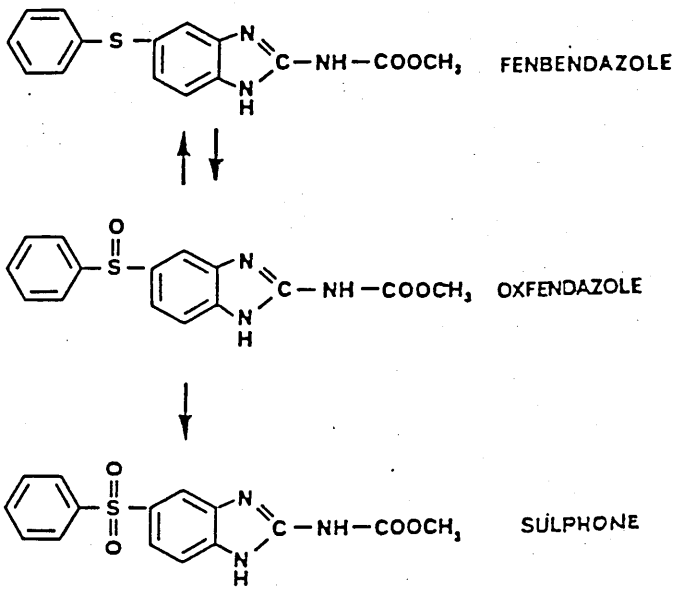


TABLE 7.1 The meat and milk withholding times for fenbendazole in sheep and cattle

SPECIES	WITHHOLDING TIME (days)	
	MEAT	MILK
CATTLE	14	3
SHEEP	14	-

MATERIALS AND METHODS

These studies involved the administration of fenbendazole to sheep at a dose rate of 5 mg/kg as an oral drench. The animals were slaughtered at 24 hours, 4, 8, 14, 21 and 28 days post-treatment. Samples of liver and muscle were taken at slaughter and stored immediately at -20°C until assayed (as described in Chapter 3). They were assayed in duplicate (Table 7.2). Blood samples were also taken at intervals throughout the course of the experiment to assess tissue/plasma ratios; plasma was removed after centrifugation and stored at -20°C until assayed (Table 7.2)..

In addition, milking cows were treated with fenbendazole by oral drench, at a dose rate of 7.5 mg/kg. Milk samples were taken before and at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hours after treatment. Both whole milk (Table 7.3) and the aqueous fraction of milk (Table 7.4) were analysed for residues.

RESULTS

After fenbendazole treatment of sheep (Table 7.2), fenbendazole itself was detectable in liver in high concentrations at one day post-treatment (a concentration of 8.88 μg/g). Residues persisted, and at fourteen days post-treatment fenbendazole was still detectable (at a concentration of 0.38 μg/g), but, at twenty-one days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole was also detectable in liver in high concentrations at one day post-treatment (a concentration of 5.54 μg/g), although highest concentrations were detectable at four days post-treatment (a concentration of 12.15 μg/g). Again, residues persisted and at twenty-one days post-treatment oxfendazole was still detectable (at a concentration of 0.33 μg/g), but, at twenty-eight days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was detectable in liver at one day post-treatment (at a concentration of 0.78 μg/g). It was still detectable at fourteen days post-treatment (althougn only a trace amount was present), but, at twenty-one days post-treatment, concentrations had fallen below a detectable level.

A semi-logarithmic representation of the concentrations of fenbendazole and oxfendazole in liver versus time, after the administration of fenbendazole, was plotted (Figure 7.2). Linear regression analysis revealed straight lines for both compounds (R=0.974 for fenbendazole and 0.999 for oxfendazole), indicating

first-order elimination.

In muscle, fenbendazole was detectable at one day post-treatment (at a concentration of 0.84 μg/g), although in a much lower concentration than was found in liver at the same time. At four days post-treatment it was still detectable (although only a trace amount was present), but, at eight days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole was also detectable in muscle at one day post-treatment (although only a trace amount was present). At fouur days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was detectable in muscle at one day post-treatment (at a concentration of 0.15 μg/g). It was still detectable at four days post-treatment (at a concentration of 0.08 μg/g), but, at eight days post-treatment, concentrations had fallen below a detectable level.

In plasma (Table 6.5), fenbendazole was detectable at one day post-treatment (at a concentration of 0.16 μg/ml). It was still detectable at four days post-treatment (at a concentration of 0.03 μg/ml), but, at eight days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole was also detectable in plasma at one day

post-treatment (at a concentration of 0.29 μg/ml), but, at four days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was detectable in plasma at one day post-treatment (at a concentration of 0.07 μg/ml), but again, at four days post-treatment, concentrations had fallen below a detectable level.

TABLE 7.2 Liver, muscle and plasma residues of fenbendazole and its metabolites in sheep

LIVER

Animal No	Dose	Days After Treatment	Residues (μg/g wet wg) FBZ *	OFZ *	OFZ.SO *
100	5mg/kg	1	8.88	5.54	0.78
101	5mg/kg	4	5.60	12.14	0.84
99	5mg/kg	8	0.93	3.49	0.23
32	5mg/kg	14	0.38	0.52	TRACE
47	5mg/kg	21	N.D.	0.33	N.D.
58	5mg/kg	28	N.D.	N.D.	N.D.

MUSCLE

100	5mg/kg	1	0.84	TRACE	0.15
101	5mg/kg	4	TRACE	N.D.	0.08
99	5mg/kg	8	N.D.	N.D.	N.D.
32	5mg/kg	14	N.D.	N.D.	N.D.
47	5mg/kg	21	N.D.	N.D.	N.D.
58	5mg/kg	28	N.D.	N.D.	N.D.

PLASMA

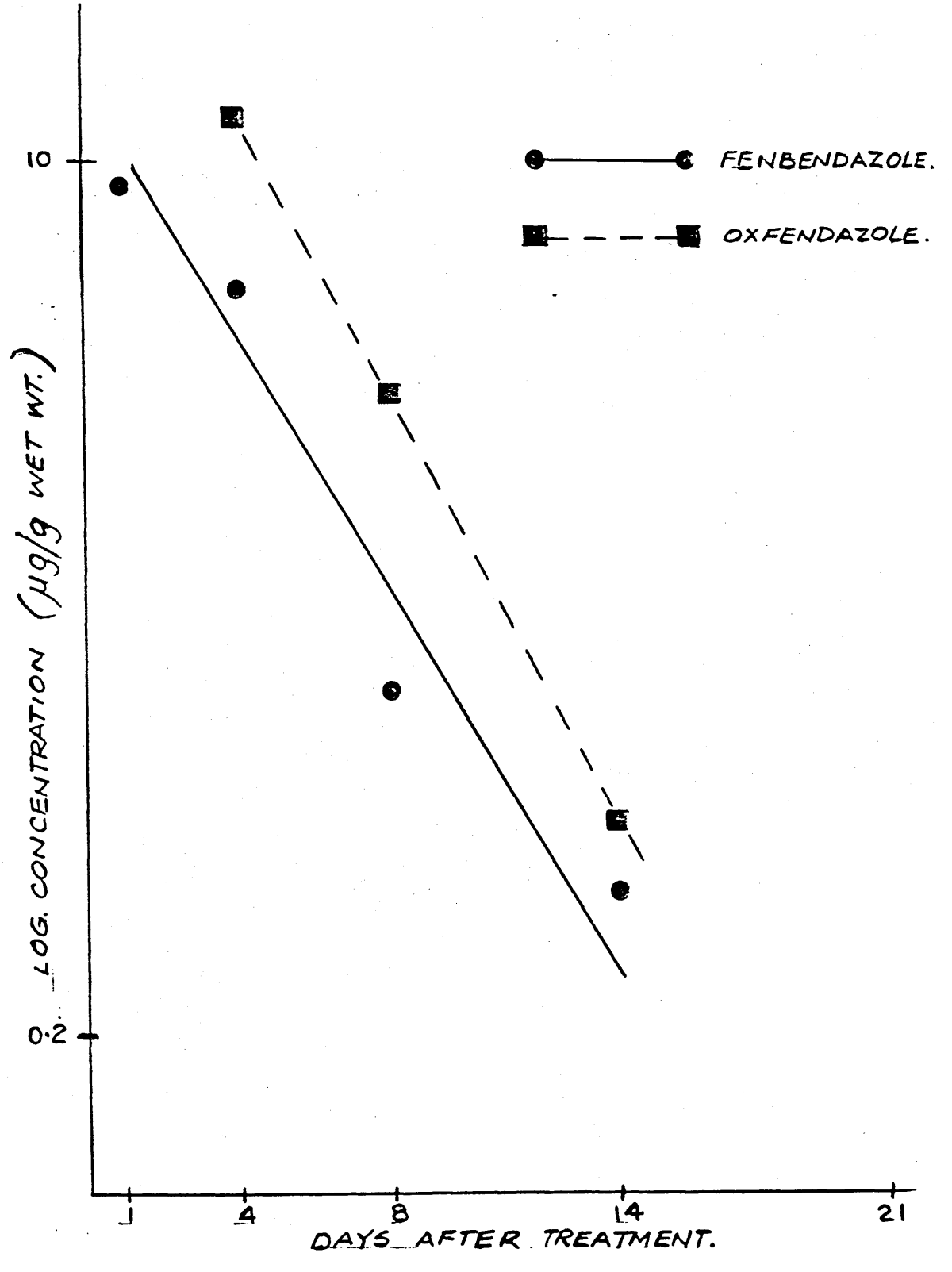
100	5mg/kg	1	0.16	0.29	0.07
101	5mg/kg	4	0.03	N.D.	N.D.
99	5mg/kg	8	N.D.	N.D.	N.D.
32	5mg/kg	14	N.D.	N.D.	N.D.
47	5mg/kg	21	N.D.	N.D.	N.D.
58	5mg/kg	28	N.D.	N.D.	N.D.

FBZ = Fenbendazole
OFZ = Oxfendazole
OFZ.SO = Oxfendazole sulphone

* = Mean values of two determinations

N.D. = Not detected (< 0.05 μg/g or μg/ml)

FIGURE 7.2 Semi-logarithmic plot of liver residues of fenbendazole and oxfendazole after the administration of fenbendazole to sheep.



Fenbendazole itself was not detectable in whole milk at any time after treatment (Table 7.3).

Oxfendazole was detectable in whole milk. Maximum concentrations were reached at twelve hours post-treatment in both animals (0.19 μg/ml and 0.32 μg/ml).

In one of the cows, oxfendazole was still detectable at thirty-six hours post-treatment (at a concentration of 0.07 μg/ml), but, at forty-eight hours post-treatment, concentrations had fallen below a detectable level.

In the other cow, oxfendazole was still detectable at sixty hours post-treatment (at a concentration of 0.03 μg/ml), but, at seventy-two hours post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was also detectable in whole milk. Maximum concentrations were reached at thirty-six hours post-treatment (0.09 μg/ml and 0.12 μg/ml).

In both cows, oxfendazole sulphone was still detectable at sixty hours post-treatment (only a trace amount was present in one of the cows, and a concentration of 0.02 μg/ml in the other cow), but, at seventy-two hours post-treatment, concentrations had fallen below detectable levels.

In the aqueous fraction of milk, fenbendazole itself was not detectable at any time after treatment (Table 7.4).

Oxfendazole was detectable in the aqueous fraction of milk. Maximum concentrations were reached at twelve hours post-treatment in both animals (0.12 μg/ml and 0.08 μg/ml).

In one of the cows, oxfendazole was still detectable at twenty-four hours post-treatment (at a concentration of 0.08 μg/ml), but, at thirty-six hours post-treatment, concentrations had fallen below a detectable level.

In the other cow, oxfendazole was still detectable at thirty-six hours post-treatment (at a concentration of 0.04 μg/ml), but, at forty-eight hours post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was also detectable in the aqueous fraction of milk. Maximum concentrations were reached at twelve hours post-treatment in both animals (0.01 μg/ml in both animals).

In both cows, oxfendazole sulphone was still detectable at thirty-six hours post-treatment (again at a concentration of 0.01 μg/ml in both animals), but, at forty-eight hours post-treatment, concentrations had fallen below detectable levels.

TABLE 7.3 Milk residues (whole milk) of fenbendazole and its metabolites in two cows

	CONCENTRATION (μg/ml)			
TIME(h)	FENBENDAZOLE	OXFENDAZOLE	OXFENDAZOLE SULPHONE	
0	0.00	0.00	0.00	A
	0.00	0.00	0.00	B
12	N.D.	0.19	0.05	A
	N.D.	0.32	0.05	B
24	N.D.	0.12	0.07	A
	N.D.	0.25	0.08	B
36	N.D.	0.07	0.09	A
	N.D.	0.15	0.12	B
48	N.D.	N.D.	0.06	A
	N.D.	0.06	0.10	B
60	N.D.	N.D.	TRACE	A
	N.D.	0.03	0.02	B
72	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
84	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
96	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
108	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
120	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
132	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
144	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B

N.D. = not detected (< 0.05 μg/ml) A = cow No.203 B = cow No.204

TABLE 7.4 Milk residues (aqueous fraction) of fenbendazole and its metabolites in two cows

	CONCENTRATION (μg/ml)			
TIME(h)	FENBENDAZOLE	OXFENDAZOLE	OXFENDAZOLE SULPHONE	
0	0.00	0.00	0.00	A
	0.00	0.00	0.00	B
12	N.D.	0.08	0.01	A
	N.D.	0.12	0.01	B
24	N.D.	0.06	0.01	A
	N.D.	0.08	0.01	B
36	N.D.	0.04	0.01	A
	N.D.	N.D.	0.01	B
48	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
60	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
72	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
84	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
96	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
108	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
120	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
132	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B
144	N.D.	N.D.	N.D.	A
	N.D.	N.D.	N.D.	B

N.D. = not detected (< 0.5 μg/ml) A = cow No.203 B = cow No.204

DISCUSSION

After the administration of fenbendazole to sheep, residues were high and persisted for twenty-one days. The parent sulphide and its sulphoxide metabolite (oxfendazole) were found in liver in high concentrations. This was not unexpected since, after the administration of fenbendazole to sheep at a dose rate of 10 mg/kg, the parent drug and oxfendazole, along with oxfendazole sulphone, were found to be the major metabolites in plasma (Marriner and Bogan, 1981b). Therefore, one would expect to find all three in tissues, as they circulate freely in plasma. However, muscle residues were very low. Total liver concentrations were thirty to forty times greater than total muscle and plasma concentrations. It is interesting to note that fenbendazole and its metabolites were undetectable in plasma at eight days post-treatment whereas in liver, residues were still detectable at twenty-one days post-treatment. It is a feature of all benzimidazoles studied, that they accumulate in the liver of sheep (i.e. albendazole, oxfendazole and thiabendazole) and are bound by some unknown mechanism.

One may have expected only one of the metabolites of fenbendazole to persist in tissues. However, fenbendazole and oxfendazole undergo a facile metabolic interconversion. Therefore, this may explain, in part, the persistence of both fenbendazole and oxfendazole in the liver of sheep. Furthermore, Marriner and Bogan (1981a,b) reported that plasma concentrations of oxfendazole were 2.5 times higher after the administration of oxfendazole to sheep than they were after the administration of fenbendazole to sheep.

Interestingly, liver residues of fenbendazole were very similar after both treatments to sheep. In addition, the insolubility of fenbendazole does not prevent high concentrations of oxfendazole occurring in the liver after the administration of fenbendazole to sheep, alghough higher concentrations of oxfendazole occur in the liver of sheep after the administration of oxfendazole (see Chapter 8). These data suggest that fenbendazole is absorbed after oral treatment, to an extent great enough that toxicity (e.g. teratogenicity) could occur at higher dose levels.

From all the residue data for fenbendazole in sheep, it was clear that if residues were present at twenty-eight days post-treatment, they would be very low (less than 0.05 μg/g). However, residues were detectable at twenty-one days post-treatment. Therefore, the meat witholding time of fourteen days for sheep treated with fenbendazole may be inadequate, although an appraisal of toxicity, taking into account no-effect levels, acceptable daily intakes and safety factors would have to be undertaken to establish a realistic withdrawal time.

Residues of fenbendazole in sheep, cattle and pig tissues have been reported previously (Duwel, 1977). A fluorimetric method was used which unfortunately did not distinguish between the parent drug and its metabolites. However, Duwel (1977) found that after the administration of fenbendazole to sheep at a dose rate of 5 mg/kg (recommended dosage), residues persisted in liver. At fourteen days post-treatment, residues were detectable at a concentration of

0.20 μg/g, but at twenty-one days post-treatment, residues were no longer detectable (less than 0.10 μg/g). In this experiment, residues were probably falsely low, because it has been found (see Chapter 9) that oxfendazole (the most persistent metabolite in liver after the administration of fenbendazole to sheep) is much less fluorescent than fenbendazole.

After the administration of fenbendazole to milking cows, the parent sulphide was not detectable in milk at any time. Oxfendazole and oxfendazole sulphone were detectable in milk after fenbendazole treatment. This was not surprising because oxfendazole and oxfendazole sulphone have been shown to be major metabolites in the plasma of cattle after fenbendazole treatment (Ngomuo et al., 1984). Residues in whole milk were higher than residues in the aqueous fraction of milk, which suggests that they are associated with both the lipid and the aqueous fractions of milk. The absence of the parent sulphide from milk may be due to the fact that after its administration to cattle, its concentration in plasma never exceeded 0.10 μg/ml (when fenbendazole was given at a dose rate of 7.5 mg/kg). Therefore, if it was present in milk, its concentration would be very low (probably below the limit of detection of of 0.05 μg/ml).

Residues of fenbendazole in cows' milk have been reported previously (Duwel, 1977), although again, the method did not distinguish between the parent drug and its metabolites. Maximum concentrations of 0.30 μg/ml were found at twenty-eight hours post-treatment, and at fifty-four hours post-treatment, concentrations had fallen below detectable levels (less than 0.10 μg/ml).

From all the residue data for fenbendazole in cows' milk, it was clear that residues did not persist beyond sixty hours (if residues were present beyond this time, they would be very low: less than 0.05 µg/ml). Therefore, the milk witholding time of seventy-two hours for cattle treated with fenbendazole would seem to be adequate.

CHAPTER 8

STUDIES WITH OXFENDAZOLE

INTRODUCTION

Oxfendazole (methyl-5(6)-(phenylsulphinyl)- 2-benzimidazole carbamate) is a potent member of the benzimidazole group of anthelmintics. It has broad spectrum activity against gastro-intestinal roundworms (including inhibited larval stages), tapeworms and lungworms in many species (Averkin et al., 1975; Downey, 1976; Chalmers, 1977; Lyons et al., 1977).

The metabolism and pharmacokinetics of oxfendazole have been studied in various species (Prichard et al., 1978; Marriner and Bogan, 1981a; Delatour et al., 1982a; Ngomuo et al., 1984). Prichard et al (1978) measured oxfendazole in sheep plasma using a previously developed radioimmunoassay procedure (Nerenberg et al., 1978). The drawback to this method was that any oxfendazole measured may have been falsely high, as some cross-reactivity may have occurred with its metabolites.

After the administration of oxfendazole to sheep (Marriner and Bogan, 1981a), the parent oxfendazole was detected in plasma along with the sulphide metabolite (fenbendazole) and the sulphone metabolite (oxfendazole sulphone). Similarly, after the administration of oxfendazole to cattle (Ngomuo et al., 1984), the parent oxfendazole was detected in plasma along with fenbendazole and oxfendazole sulphone.

In the horse, oxfendazole, fenbendazole and oxfendazole sulphone were all detected in plasma after oxfendazole administration (Marriner and Bogan, 1985).

A High Performance Liquid Chromatographic method has been reported for the determination of ^{14}C-labelled oxfendazole in cows' milk (Tsina and Matin, 1981), but again, the method did not distinguish between parent drug and metabolites.

It is important to distinguish between parent drug and metabolites, especially in this case, where the parent compound is known to be teratogenic.

Oxfendazole has a wide margin of safety in all species of domesticated animal. Its oral LD_{50} is greater than 6,400 mg/kg in rats and greater than 1,600 mg/kg in dogs (Averkin et al., 1975).

Oxfendazole has been extensively studied in the rat with regard to its potential teratogenicity. Delatour et al., (1984) conducted a study in which pregnant rats were treated from day eight to day fifteen of pregnancy, either with oxfendazole alone or oxfendazole combined with SKF-525A (an inhibitor of microsomal oxidation). They found that oxfendazole was teratogenic in the rat, but, oxfendazole combined with SKF-525A increased the embryotoxicity of oxfendazole. This was surprising since SKF-525A had an inhibitory effect upon gastro-intestinal absorption of oxfendazole (Delatour et al., 1984). Therefore, one would not expect increased embryotoxicity when blood concentrations of oxfendazole were decreased when it was administered in combination with SKF-525A.

From all this toxicological data, it is clear that in any

residue studies involving oxfendazole, it is most important that the parent sulphoxide (oxfendazole) should be measured, as should the sulphide metabolite (fenbendazole), since none of the other metabolites of oxfendazole have been found to be toxic. Although the sulphide metabolite (fenbendazole) is not teratogenic, it is readily interconvertible with oxfendazole (which is teratogenic), hence, it is important that both compounds are measured.

In all experiments, the animals were slaughtered at various times after the administration of oxfendazole. Liver, muscle, milk and plasma were analysed for residues of oxfendazole and its metabolites until they could be no longer detected (limits of detection 0.05 µg/g for tissues and 0.05 µg/ml for milk and plasma). It was also assessed whether or not residues persisted beyond recommended withdrawal periods (Table 8.1).

TABLE 8.1 The meat and milk withholding times for oxfendazole in sheep and cattle

SPECIES	WITHHOLDING TIME (days)	
	MEAT	MILK
CATTLE	14	5
SHEEP	14	-

MATERIALS AND METHODS

These studies involved the administration of oxfendazole to sheep by oral drench, at a dose rate of 5 mg/kg. They were slaughtered at 24 hours, 4, 8, 14, 21 and 28 days post-treatment. Samples of liver and muscle were taken at slaughter and stored immediately at -20°C until assayed (as described in Chapter 3). They were assayed in duplicate (Table 8.2). Blood samples were also taken at intervals throughout the experiment to assess the tissue/plasma ratio of residues; plasma was removed after centrifugation and stored at -20°C until assayed (Table 8.2).

Cattle were also treated with oxfendazole, either orally by drench or intra-ruminally, both groups being given oxfendazole at a dose rate of 4.5 mg/kg. This experiment was part of a study conducted by Syntex (Bairden et al, 1983), to compare plasma concentrations reached after both treatments, and to correlate bioavailability with efficacy between the two routes of administration. The cattle treated orally were slaughtered at eight days post-treatment. The cattle treated intra-ruminally (by means of a Synanthic Intra-Ruminal Injector; Syntex Agribusiness, Palo Alto, California) were slaughtered at ten days post-treatment. Samples of liver and muscle were taken at each slaughter and stored immediately at -20°C until assayed (as previously described). They were assayed in duplicate (Table 8.3).

A third experiment was carried out in which milking cows were treatment with oxfendazole by oral drench, again at a dose rate of 4.5 mg/kg. Milk samples were taken before and at 12, 24, 36, 48, 60,

72, 84, 96, 108, 120, 132 and 144 hours after treatment. Both whole milk (Table 8.4) and the aqueous fraction of milk (Table 8.5) were analysed for residues.

RESULTS

After oxfendazole treatment of sheep (Table 8.2), oxfendazole itself was detectable in liver in high concentrations at one day post-treatment (a concentration of 4.96 μg/g), although highest concentrations were detectable at four days post-treatment (a concentration of 5.56 μg/g). Residues persisted, and at twenty-one days post-treatment oxfendazole was still detectable (at a concentration of 0.12 μg/g), but, at twenty-eight days post-treatment, concentrations had fallen below a detectable level.

Fenbendazole was also detectable in liver in high concentrations at one day post-treatment (a concentration of 8.20 μg/g). Residues persisted, and at fourteen days post-treatment fenbendazole was still detectable (at a concentration of 0.86 μg/g), but, at twenty-one days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was detectable in liver at one day post-treatment (at a concentration of 0.70 μg/g), although highest concentrations were detectable at four days post-treatment (a concentration of 0.87 μg/g). At eight days post-treatment oxfendazole sulphone was still detectable (at a concentration of 0.05 μg/g), but, at fourteen days post-treatment, concentrations had fallen below a detectable level.

A semi-logarithmic representation of the concentrations of oxfendazole and fenbendazole in liver versus time, after the administration of oxfendazole, was plotted (Figure 8.1). Linear regression analysis revealed straight lines for both compounds

(R=0.986 for oxfendazole and 0.940 for fenbendazole), indicating first-order elimination.

In muscle, oxfendazole itself was not detectable at any time after treatment.

Fenbendazole was detectable in muscle at one day post-treatment (at a concentration of 0.94 μg/g), although in a much lower concentration than was found in liver at the same time. It was still detectable at four days post-treatment (at a concentration of 0.33 μg/g), but, at eight days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was also detectable in muscle at one day post-treatment (at a concentration of 0.07 μg/g), although highest concentrations were detectable at four days post-treatment (a concentration of 0.12 μg/g). At eight days post-treatment, concentrations had fallen below a detectable level.

In plasma (Table 7.5), oxfendazole was detectable at one day post-treatment (at a concentration of 0.43 μg/ml). It was still detectable at four days post-treatment (at a concentration of 0.04 μg/ml), but, at eight days post-treatment, concentrations had fallen below a detectable level.

Fenbendazole was also detectable in plasma at one day post-treatment (at a concentration of 0.11 μg/ml). Again, it was still

detectable at four days post-treament (at a concentration of 0.02 µg/ml), but, at eight days post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was detectable in plasma at one day post-treatment (at a concentration of 0.08 µg/ml). It was still detectable at four days post-treatment (at a concentration of 0.02 µg/ml), but, at eight days post-treatment, concentrations had fallen below a detectable level.

FIGURE 8.1 Semi-logarithmic plot of consentrations of oxfendazole and fenbendazole in liver after the administration of oxfendazole in sheep.

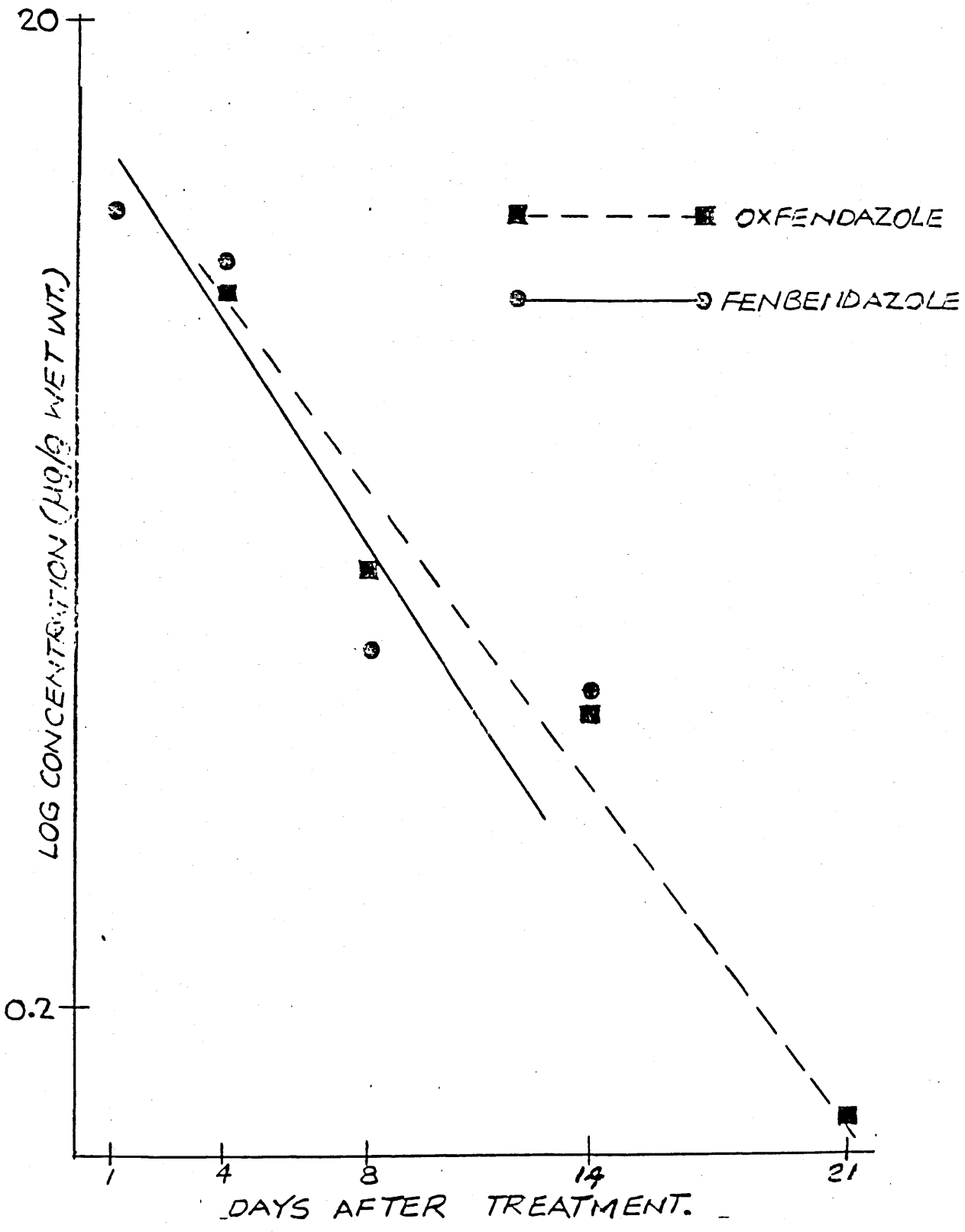


TABLE 8.2 Liver, muscle and plasma residues of oxfendazole and its metabolites in sheep

LIVER

Animal No	Dose	Days After Treatment	Residues (µg/g wet wt) FBZ *	OFZ *	OFZ.SO *
102	5mg/kg	1	8.20	4.96	0.70
99	5mg/kg	4	6.54	5.56	0.87
89	5mg/kg	8	1.04	1.54	0.05
83	5mg/kg	14	0.86	0.80	N.D.
116	5mg/kg	21	N.D.	0.12	N.D.
107	5mg/kg	28	N.D.	N.D.	N.D.

MUSCLE

102	5mg/kg	1	0.94	N.D.	0.07
99	5mg/kg	4	0.33	N.D.	0.12
89	5mg/kg	8	N.D.	N.D.	N.D.
83	5mg/kg	14	N.D.	N.D.	N.D.
116	5mg/kg	21	N.D.	N.D.	N.D.
107	5mg/kg	28	N.D.	N.D.	N.D.

PLASMA

			Residues (µg/ml)		
102	5mg/kg	1	0.11	0.43	0.08
99	5mg/kg	4	0.02	0.04	0.02
89	5mg/kg	8	N.D.	N.D.	N.D.
83	5mg/kg	14	N.D.	N.D.	N.D.
116	5mg/kg	21	N.D.	N.D.	N.D.
107	5mg/kg	28	N.D.	N.D.	N.D.

FBZ = Fenbendazole
OFZ = Oxfendazole
OFZ.SO = Oxfendazole sulphone

* = Mean values of two determinations
N.D. = Not detected (<0.05 µg/g or µg/ml)

After oxfendazole treatment of cattle (Table 8.3), oxfendazole itself was detectable in liver at eight days post-treatment (at a mean concentration of 0.56 μg/g) in all four calves. It was still detectable at ten days post-treatment (at a mean concentration of 0.20 μg/g), again in all four calves.

Fenbendazole was also detectable in liver at eight days post-treatment (at a mean concentration of 0.89 μg/g) in all four calves. Again, it was still detectable at ten days post-treatment (at a mean concentration of 0.37 μg/g), again in all four calves.

Oxfendazole sulphone was detectable in liver at eight days post-treatment, although only in three of the four calves (in which only a trace amount was detectable). It was still detectable at ten days post-treatment, although only in one of the four calves (in which only a trace amount was detectable).

In muscle, oxfendazole, fenbendazole and oxfendazole sulphone were not detectable at either eight or ten days post-treatment.

TABLE 8.3 Liver residues of oxfendazole and its metabolites in cattle

ORAL ADMINISTRATION

Animal No	Dose	Days After Treatment	Residues (μg/g wet wt) FBZ	OFZ	OFZ.SO
			*	*	*
17	4.5mg/kg	8	1.04	0.26	TRACE
20	4.5mg/kg	8	0.72	0.67	TRACE
33	4.5mg/kg	8	0.70	0.16	TRACE
56	4.5mg/kg	8	1.09	1.14	N.D.
	Mean + S.E.M.	0.89±0.10	0.56±0.22		

INTRA-RUMINAL ADMINISTRATION

17	4.5mg/kg	10	0.50	0.20	N.D.
20	4.5mg/kg	10	0.32	0.16	N.D.
33	4.5mg/kg	10	0.32	0.27	N.D.
56	4.5mg/kg	10	0.33	0.19	TRACE
	Mean + S.E.M.	0.37±0.04	0.20±0.02		

FBZ = Fenbendazole
OFZ = Oxfendazole
OFZ.SO = Oxfendazole sulphone

* = Mean values of two determinations
N.D. = Not detected (<0.05 μg/g)

Muscle samples from each of these animals were taken at equivalent times to the liver samples. None of the samples had residues of FBZ, OFZ or OFZ.SO at the limit of detection (0.05 μg/g).

After oxfendazole treatment, oxfendazole itself was detectable in whole milk (Table 8.4). In one of the cows, maximum concentrations were reached at twelve hours post-treatment (0.53 μg/ml). At thirty-six hours post-treament it was still detectable (at a concentration of 0.14 μg/ml), but, at forty-eight hours post-treatment, concentrations had fallen below a detectable level. In the other cow, maximum concentrations were reached at twenty-four hours post-treatment (0.36 μg/ml). At seventy-two hours post-treatment it was still detectable (at a concentration of 0.05 μg/ml), but, at eighty-four hours post-treatment, concentrations had fallen below a detectable level.

Oxfendazole sulphone was also detectable in whole milk. Maximum concentrations were reached at thirty-six hours post-treatment in both animals (0.15 μg/ml and 0.16 μg/ml). In one of the cows, it was still detectable at sixty hours post-treatment (at a concentration of 0.02 μg/ml), but, at seventy-two hours post-treatment, concentrations had fallen below a detectable level. In the other cow, it was still detectable at eighty-four hours post-treatment (although only a trace amount was present), but, at ninety-six hours post-treatment, concentrations had fallen below a detectable level.

Fenbendazole was not detectable in whole milk at any time after oxfendazole treatment.

Oxfendazole was also detectable in the aqueous fraction of milk (Table 8.5). Maximum concentrations were reached at twelve hours

post-treatment in both animals (0.12 μg/ml and 0.14 μg/ml). In both cows, it was still detectable at twenty-fours post-treatment (in both cases at a concentration of 0.10 μg/ml), but, at thirty-six hours post-treatment, concentrations had fallen below detectable levels.

Oxfendazole sulphone was also detectable in the aqueous fracton of milk. In one of the cows, maximum concentrations were reached at twelve hours post-treatment (0.03 μg/ml). At twenty-four hours post-treatment it was still detectable (at a concentration of 0.01 μg/ml), but, at thirty-six hours post-treatment, concentrations had fallen below a detectable level. In the other cow, maximum concentrations were reached at twenty-four hours post-treatment (0.07 μg/ml), but, at thirty-six hours post-treatment, concentrations had again fallen below a detectable level.

Fenbendazole was not detectable in the aqueous fraction of milk at any time after oxfendazole treatment.

TABLE 8.4 Milk residues (whole milk) of oxfendazole and its metabolites in two cows

	CONCENTRATION (µg/ml)		
TIME(h)	FENBENDAZOLE	OXFENDAZOLE	OXFENDAZOLE SULPHONE
0	0.00	0.00	0.00 A
	0.00	0.00	0.00 B
12	N.D.	0.53	0.10 A
	N.D.	0.34	0.08 B
24	N.D.	0.35	0.13 A
	N.D.	0.36	0.11 B
36	N.D.	0.14	0.16 A
	N.D.	0.19	0.15 B
48	N.D.	N.D.	0.06 A
	N.D.	0.10	0.15 B
60	N.D.	N.D.	0.02 A
	N.D.	0.02	0.10 B
72	N.D.	N.D.	N.D. A
	N.D.	0.05	0.11 B
84	N.D.	N.D.	N.D. A
	N.D.	N.D.	TRACE B
96	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
108	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
120	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
132	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
144	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B

N.D. = not detected A = cow No.201 B = cow No.202

TABLE 8.5 Milk residues (aqueous fraction) of oxfendazole and its metabolites in two cows

	CONCENTRATION (µg/ml)		
TIME(h)	FENBENDAZOLE	OXFENDAZOLE	OXFENDAZOLE SULPHONE
0	0.00	0.00	0.00 A
	0.00	0.00	0.00 B
12	N.D.	0.14	0.03 A
	N.D.	0.12	0.03 B
24	N.D.	0.10	0.01 A
	N.D.	0.10	0.07 B
36	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
48	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
60	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
72	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
84	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
96	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
108	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
120	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
132	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B
144	N.D.	N.D.	N.D. A
	N.D.	N.D.	N.D. B

N.D. = not detected A = cow No.201 B = cow No.202

DISCUSSION

After the administration of oxfendazole to sheep, residues were high and persisted for twenty-one days. The parent drug and its sulphide metabolite (fenbendazole) were found in liver in high concentrations. This was not unexpected since, after the administration of oxfendazole to sheep at a dose rate of 10 mg/kg, the parent sulphoxide and fenbendazole, along with oxfendazole sulphone, were found to be the major metabolites in plasma (Marriner and Bogan, 1981a). Therefore, one would expect to find all three in tissues, as they circulate freely in plasma. However, muscle residues were very low. Total liver residues were around twenty times greater than total muscle and plasma residues. It is interesting to note that oxfendazole and its metabolites were undetectable in plasma at eight days post-treatment whereas in liver, residues were still detectable at twenty-one days post-treatment. As has been observed for albendazole, fenbendazole and thiabendazole, residues accumulate in the liver of sheep.

Residues of the parent drug and its sulphide metabolite both persisted. As has been explained previously (see Chapter 7), oxfendazole and fenbendazole undergo a facile metabolic interconversion. Therefore, this may explain, in part, the persistence of both oxfendazole and fenbendazole in the liver of sheep.

An interesting finding was that after the administration of oxfendazole to sheep, residues of the parent compound, fenbendazole and oxfendazole sulphone were very similar at both one and four days post-treatment. This is not very surprising if one considers the

residence time of oxfendazole and its metabolites in the gastro-intestinal tract of sheep and the plasma concentration versus time profiles for these compounds, as reported by Marriner and Bogan (1981a,b). After the oral administration of oxfendazole or fenbendazole to sheep, all three compounds were detectable in plasma for up to seven days, although the period from zero to four days accounted for the majority of the area under the plasma concentration/time curves for these compounds. From this data one may assume that absorption of these compounds from the gastro-intestinal tract is still occurring after four days, resulting in liver concentrations which are similar after both one and four days. This is strengthened by abomasal fluid concentrations of these compounds (Marriner and Bogan, 1981a,b). For example, after the administration of fenbendazole to sheep, the mean concentration of oxfendazole in abomasal fluid was 0.64 μg/ml after twenty-four hours and it was very similar after four days (0.56 μg/ml).

From all the residue data for oxfendazole in sheep, it was clear that if residues were present at twenty-eight days post-treatment, they would be very low (less than 0.05 μg/g). However, residues were detectable at twenty-one days post-treatment. Therefore, the meat witholding time of fourteen days for sheep treated with oxfendazole may be inadequate, although an evaluation of toxicity, taking into account no-effect levels, acceptable daily intakes and safety factors should be carried out before making this judgement. For example, if one assumes that the "average" meal consists of 500g muscle, 100g fat, 100g liver, 100g kidney and 500ml of milk, then one could estimate

maximum daily intakes at certain withdrawal times. At fourteen days after the administration of oxfendazole to sheep, residues in liver only were detectable, and totalled 1.66 μg/g (equivalent to 166 μg which would be consumed). Assuming the average weight of a human to be 70 kg, this would result in a maximum daily intake of approximately 2 μg/kg/day for a fourteen day withdrawal period. This amount would be very unlikely to pose any problem to humans as regards toxicity, although the assumption must be made that the susceptability of humans to the toxic effects of these compounds is similar to that of other animals which have been shown to be susceptible. Furthermore, comprehensive toxicity studies, including relay-bioavailability studies, would have to be carried out to determine ADIs, NELs and withdrawal periods accurately.

After the administration of oxfendazole to cattle, residues of oxfendazole, fenbendazole and oxfendazole sulphone were detectable in liver. This was not unexpected since, after the administration of oxfendazole to cattle at a dose rate of 4.5 mg/kg, oxfendazole, fenbendazole and oxfendazole sulphone were all detectable in plasma (Ngomuo et al., 1984). Unfortunately, this residue study was incomplete, as the tissues were obtained from cattle which had been used in an efficacy trial. However, from the amount of residues of oxfendazole (a mean concentration of 0.19 μg/g) and fenbendazole (a mean concentration of 0.37 μg/g) still detectable in liver at ten days post-treatment, it is likely that residues would still be detectable in liver beyond the fourteen day meat witholding time for cattle treated with oxfendazole.

After the administration of oxfendazole to milking cows, the parent drug and oxfendazole sulphone were detectable in milk. This was not surprising because oxfendazole and oxfendazole sulphone are found in the plasma of cattle after oxfendazole treatment (Ngomuo et al., 1984). Fenbendazole was not detectable in milk at any time after oxfendazole treatment. The absence of fenbendazole from milk may be due to the fact that after the administration of oxfendazole to cattle at a dose rate of 4.5 mg/kg, the concentration of fenbendazole in plasma was sufficiently low that if it were present in milk, its concentration would be very low (probably below the limit of detection of 0.05 μg/ml).

Residues of oxfendazole and oxfendazole sulphone in whole milk were higher than residues in the aqueous fraction of milk, which suggests that they are associated with both the lipid and the aqueous fractions of milk (which was the case with milk residues after fenbendazole treatment). Residues in whole milk were of the order of four times greater than those in the aqueous fraction, therefore, one could assume that the majority of residues were associted with the fat in milk. One may speculate that fat residues (although not determined) would be very similar to those in the fat of milk, assuming uniform distribution of these compounds into all types of fat.

From all the residue data for oxfendazole in cows' milk, it was clear that residues did not persist beyond eighty-four hours (if residues were present beyond this time, they would be very low: less than 0.05 μg/ml). Therefore, the milk witholding time of one hundred

and twenty hours for cattle treated with oxfendazole would seem to be adequate.

Overall, a complete evaluation of residues should include kidney and fat, which were not determined in these studies. If these tissues were included, a more complete picture of the residue profiles could be drawn, allowing more accurate estimations of acceptable daily intakes and withdrawal times.

CHAPTER 9

STUDIES WITH THIABENDAZOLE

Thiabendazole (2-(4'-thiazolyl)-benzimidazole) is a member of the benzimidazole group of anthelmintics with broad spectrum activity against gastro-intestinal roundworms and tapeworms in sheep, cattle and many other species (Brown et al., 1961; Cuckler, 1961). It is also effective against trichinosis in rats and mice (Campbell, 1961) and in swine (Campbell and Cuckler, 1962). It has been shown to be effective against inhibited Ostertagia larvae in cattle (Prichard et al., 1978), but only if drug concentrations were maintained by continuous infusion or frequently repeated administration. Thiabendazole had previously been shown to be ineffective (at a single dose) against inhibited larvae (Armour, 1969; Armour et al., 1975; Anderson, 1977).

The metabolism and pharmacokinetics of thiabendazole have been studied in sheep (Tocco et al., 1964) and in cattle, goats and pigs (Tocco et al., 1965) using ^{14}C-labelled thiabendazole. Both thiabendazole and 5-hydroxythiabendazole were detected in urine, but plasma concentrations were not reported. It is known, however, that 5-hydroxythiabendazole is conjugated in the liver, both glucuronide and sulphate conjugates being found (Tocco et al., 1964). In cattle, thiabendazole was administered orally, at dose rates of 50 mg/kg and 200 mg/kg (Tocco et al., 1965). Unchanged thiabendazole was not detectable in plasma at any time after treatment (even at a dose rate of 200 mg/kg). Maximum plasma concentrations of 5-hydroxythiabendazole occurred between four and seven hours after treatment. Thereafter, concentrations of 5-hydroxythiabendazole fell rapidly and were virtually undetectable after twenty-four hours (50 mg/kg) and

forty-eight hours (200 mg/kg). Also, unchanged thiabendazole was not detected in urine at any time after treatment.

Although 5-hydroxythiabendazole is the major metabolite of thiabendazole in sheep urine and plasma (see later) and in cattle plasma, it is considered to be anthelmintically inactive (Stone et al., 1965). Hence, anthelmintic activity may be greater in sheep than in cattle (since thiabendazole is more rapidly converted to 5-hydroxythiabendazole in cattle).

Several High Performance Liquid Chromatographic methods for the determination of thiabendazole have been reported (Maeda and Tsuji, 1976; Farrow et al., 1977; Isshiki et al., 1980; Tafuri et al., 1981). However, for two reasons, none of these methods are suitable for measuring thiabendazole in plasma. Firstly, they are not designed for the determination of thiabendazole in plasma. Secondly, they do not distinguish between thiabendazole and its major metabolite (5-hydroxythiabendazole). Measurement of 5-hydroxythiabendazole is important as it has been observed that the accumulation of 5-hydroxythiabendazole was linked with hepatotoxicity in a human patient who was unable to eliminate it because of impaired renal function (Schumaker et al., 1978).

Acute toxicity studies with thiabendazole have demonstrated (like all the other benzimidazoles) that it is well tolerated in the host animal, although the literature is lacking in detailed information regarding the toxicity of thiabendazole. However, in the rat,

thiabendazole appears to be non-teratogenic (Tanaka et al., 1982), but minor abnormalities (retardation of ossification of the sternum) were suggested at a dose rate of 500 mg/kg (Khera et al., 1979). In the mouse, various abnormalities have been described for thiabendazole at extremely high dose rates of 700 mg/kg to 2400 mg/kg (Kubo et al., 1982; Ogata et al., 1982 and 1984).

In the following experiments with thiabendazole, both the parent compound and 5-hydroxythiabendazole were measured.

In all experiments, the animals were slaughtered at various times after the administration of thiabendazole. Liver, muscle, milk and plasma were analysed for residues of thiabendazole and 5-hydroxythiabendazole until they could be no longer detected (limits of detection 0.05 µg/g for tissues and 0.05 µg/ml for milk and plasma).

RESIDUES OF THIABENDAZOLE AND 5-HYDROXYTHIABENDAZOLE IN THE TISSUES OF SHEEP

In this experiment, four sheep were given thiabendazole orally, at a dose rate of 44 mg/kg (recommended dosage) (Table 9.1). They were slaughtered at 1, 2, 3 and 4 days post-treatment.

Samples of liver and muscle were taken at slaughter and stored immediately at -20°C until assayed (as described in Chapter 3).

All liver and muscle samples were assayed in duplicate (Tables 9.2 and 9.3).

TABLE 9.1 Weights, dose volumes and dosages for the four sheep used in the thiabendazole residue study

SHEEP NO.	WT. (kg)	VOL. SUSPENSION 17.6% w/v (ml)	TOTAL DOSE (mg)	DOSE RATE (mg/kg)
91	74	18.5	3256	44
92	61	15.2	2684	44
93	64	16.0	2816	44
94	69	17.2	3036	44

TABLE 9.2 Liver residues of thiabendazole and 5-hydroxythiabendazole in four sheep after treatment with thiabendazole

Animal No	Dose	Days after Treatment	Residues (µg/g wet wt) Thiabendazole	5-Hydroxy-thiabendazole
91	44 mg/kg	1	* (2.28) 2.24 (2.20)	(2.47) 2.44 (2.41)
92	44 mg/kg	2	(0.33) 0.31 (0.29)	N.D.
93	44 mg/kg	3	(0.82) 0.80 (0.79)	N.D.
94	44 mg/kg	4	N.D.	N.D.

* = Mean value of two determinations

N.D. = not detected

TABLE 9.3 Muscle residues of thiabendazole and 5-hydroxythiabendazole in four sheep after treatment with thiabendazole

Animal No	Dose	Days after Treatment	Residues (µg/g wet wt) Thiabendazole	5-Hydroxy-thiabendazole
91	44 mg/kg	1	* (0.31) 0.34 (0.38)	(0.19) 0.24 (0.28)
92	44 mg/kg	2	N.D.	N.D.
93	44 mg/kg	3	N.D.	N.D.
94	44 mg/kg	4	N.D.	N.D.

* Mean value of two determinations

N.D. = not detected

After thiabendazole treatment, thiabendazole itself was detectable in liver at one day post-treatment (at a concentration of 2.24 μg/g). It was still detectable at three days post-treatment (at a concentration of 0.80 μg/g), but, at four days post-treatment, concentrations had fallen below a detectable level.

5-Hydroxythiabendazole was also detectable in liver at one day post-treatment (at a concentration of 2.44 μg/g), but, at two days post-treatment, concentrations had fallen below a detectable level.

In muscle, thiabendazole was detectable at one day post-treatment (at a concentration of 0.34 μg/g), but, at two days post-treatment, concentrations had fallen below a detectable level.

5-Hydroxythiabendazole was also detectable in muscle at one day post-treatment (at a concentration of 0.24 μg/g), but, at two days post-treatment, concentrations had fallen below a detectable level.

THIABENDAZOLE AND 5-HYDROXYTHIABENDAZOLE IN THE PLASMA OF SHEEP

In the course of the investigation into the residues of benzimidazole anthelmintics in the tissues of sheep, it was found that the fate of thiabendazole in the blood of sheep is different from that of cattle and goats, the two species in which blood concentrations of thiabendazole and its principal metabolite 5-hydroxythiabendazole have been measured separately (Tocco et al., 1965). Tocco et al., (1964) have reported on total thiabendazole and metabolite concentrations using ^{14}C and ^{35}S-labelled thiabendazole in sheep.

In this investigation, six Finn-Dorset X adult lambs on a hay and concentrates diet were given thiabendazole orally, at a dose rate of 44 mg/kg (recommended dosage). Blood samples were collected by venepuncture before and at 2, 4, 6, 8, 12, 24, 36, 48 and 72 hours post-treatment; plasma was removed by centrifugation and stored at $-20^{o}C$ until assayed (as described in Chapter 3). All samples were assayed for thiabendazole and 5-hydroxythiabendazole (a gift from Merck, Rahway). (Figure 9.1 and Table 9.4).

FIGURE 9.1 The mean concentrations (± S.E.M.) of thiabendazole and 5-hydroxythiabendazole in the plasma of six sheep given thiabendazole (44mg/kg).

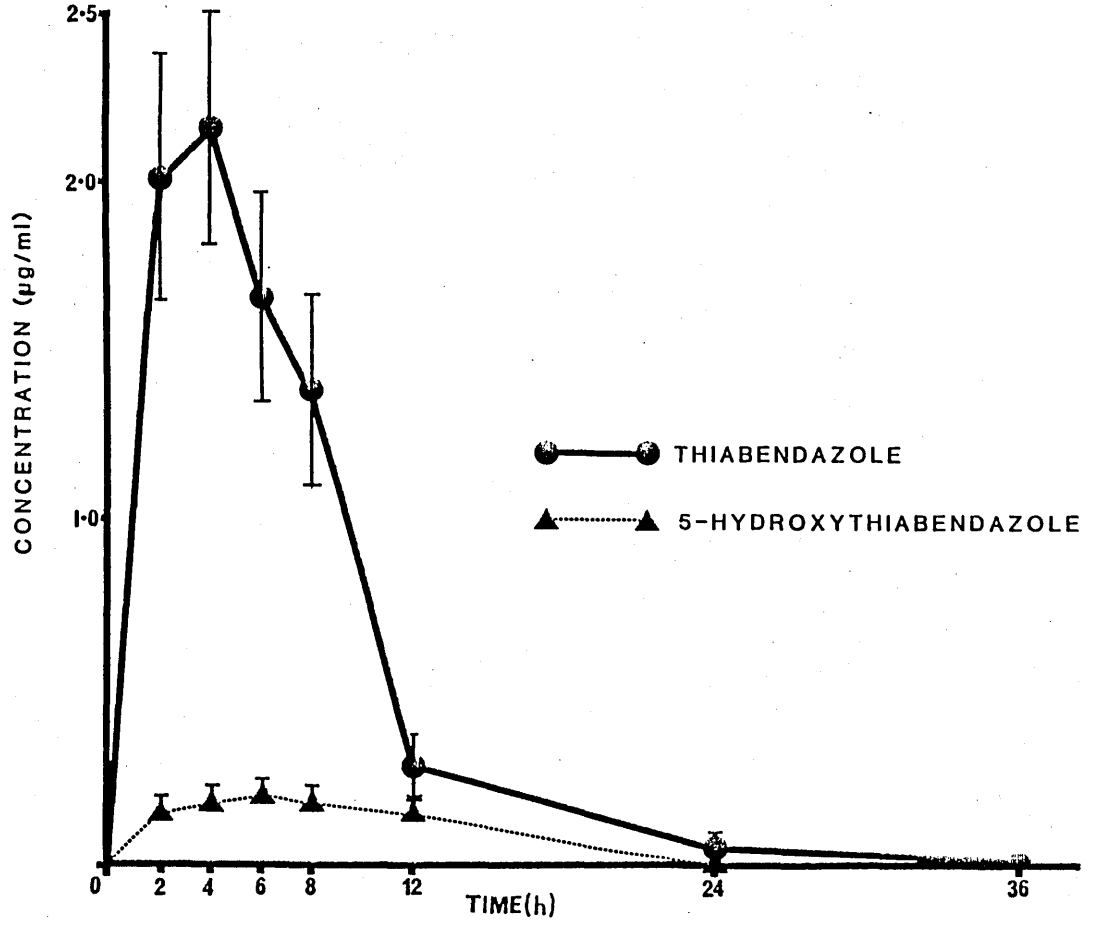


TABLE 9.4 Plasma concentrations of thiabendazole and 5-hydroxythiabendazole in six sheep given thiabendazole (44mg/kg).
TBZ = Thiabendazole
5-OH TBZ = 5-hydroxythiabendazole

PLASMA CONCENTRATION (ug/ml)

	SHEEP 1		SHEEP 2		SHEEP 3		SHEEP 4		SHEEP 5		SHEEP 6	
TIME(h)	TBZ.	5-OH TBZ.	TBZ.	5-OH TBZ.	TBZ.	5-OH TBZ.	TBZ.	5-OH TBZ.	TBZ.	5-OH TBZ.	TBZ.	5-OH TBZ.
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.79	0.08	1.29	0.08	2.44	0.13	2.82	0.13	1.79	0.18	2.94	0.16
4	0.83	0.09	1.71	0.11	2.24	0.16	2.75	0.19	2.04	0.18	3.40	0.20
6	0.60	0.09	1.37	0.14	1.64	0.19	2.34	0.16	1.28	0.20	2.74	0.29
8	0.81	0.14	1.13	0.13	N.S.	N.S.	1.79	0.18	0.77	0.14	2.39	0.22
12	0.29	0.15	0.19	0.07	0.03	0.09	0.57	0.27	0.05	0.06	0.50	0.14
24	0.02	N.D.	0.02	N.D.	0.01	N.D.	0.03	N.D.	N.D.	N.D.	0.04	N.D.
36	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
72	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. = not detected N.S. = no sample

After thiabendazole treatment, thiabendazole itself was detectable in plasma. Maximum concentrations were reached between two and four hours (a mean maximum concentration of 2.16 μg/ml) post-treatment. At twenty-four hours post-treatment, thiabendazole was still detectable in plasma (at a mean concentration of 0.02 μg/ml), but at thirty-six hours post-treatment, concentrations had fallen below a detectable level.

5-hydroxythiabendazole was also detectable in plasma after thiabendazole treatment. Maximum concentrations were reached at six hours post-treatment (a mean maximum concentration of 0.18 μg/ml). At twelve hours post-treatment, 5-hydroxythiabendazole was still detectable in plasma (at a mean concentration of 0.13 μg.ml), but at twenty-four hours post-treatment, concentrations had fallen below a detectable level.

RESIDUES OF THIABENDAZOLE AND 5-HYDROXYTHIABENDAZOLE IN COWS' MILK

In this experiment, two Friesian cows in mid-lactation were given thiabendazole orally, at a dose rate of 66 mg/kg (recommended dosage) (Table 9.5). Their weights were estimated to be 550 kg each.

The cows were milked twice daily (approximately every 12 hours), thiabendazole being administered immediately after the afternoon milking.

Samples were taken before and at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hours after treatment.

The samples were stored immediately at -20°C until assayed (as described in Chapter 3).

All milk samples were analysed in two ways:-

1) Whole milk was analysed.

2) Milk was separated into aqueous phase (whey) and lipid phase (curds). The aqueous phase was then analysed for residues.

TABLE 9.5 Weights, dose volumes and dosages for the two cows used in the thiabendazole milk residue study

COW NO.	WT. (kg)	VOL. SUSPENSION 17.6% w/v (ml)	TOTAL DOSE (mg)	DOSE RATE (mg/kg)
207	550	206	36300	66
208	550	206	36300	66

Neither thiabendazole nor 5-hydroxythiabendazole were detectable in whole milk at any time after treatment (limit of detection of 0.5 µg/ml for both compounds).

Similarly, neither thiabendazole nor 5-hyxroxythiabendazole were detectable in the aqueous fraction of milk at any time after treatment (limit of detection of 0.5 µg/ml for both compounds).

DISCUSSION

After the administration of thiabendazole to sheep, residues of the parent drug and its metabolite 5-hydroxythiabendazole were detectable in tissues. The most surprising finding was that thiabendazole itself was detectable in liver three days after its administration to sheep. This result was unexpected because thiabendazole was not detectable in plasma after twenty-four hours. This situation was similar to that which occurred with febantel, fenbendazole and oxfendazole. That is, tissue residues are higher and persist much longer than plasma residues.

In contrast to cattle and goats, where parent thiabendazole is not detectable in plasma, thiabendazole was present in the plasma of sheep for twenty-four hours after dosing and at higher concentrations than the 5-hydroxymetabolite.

These findings are consistent with those of Tocco et al., (1964 and 1965), who showed that metabolism and excretion of thiabendazole is more extensive in cattle than in sheep. They found that 94% of total radioactivity in lambs was accounted for as 5-hydroxythiabendazole or its conjugates, with 2% being excreted unchanged (the remaining 4% was unidentified), whereas in a calf no unchanged thiabendazole was detectable in the urine.

This result may also have implications for the efficacy of thiabendazole in sheep. It is considered that 5-hydroxythiabendazole is

not anthelmintically active (Stone et al., 1965). Hence, anthelmintic activity in the systemic compartment may be greater in sheep than in cattle. For example, thiabendazole is considered to be without effect against lungworm (Dictyocaulus viviparus) in cattle, whereas Gibbs and Pullin (1963) found useful activity against lungworm (Dictyocaulus filaria) in sheep. Thiabendazole also shows activity against arrested larval stages of gastro-intestinal nematodes in sheep, but not in cattle, although this may also be a consequence of access of anthelmintic to the larvae in sheep being easier than in cattle because of the thinner gastro-intestinal mucosa (Armour, 1983).

The anthelmintic activity of thiabendazole is probably limited by two factors. Firstly, it is the most soluble of the benzimidazoles (Ngomuo, 1983) and, secondly, it is rapidly metabolized. For both these reasons, the duration of exposure of the parasites to the drug is short compared to the other benzimidazoles. Prichard et al., (1978) have shown that extending the duration of exposure of parasites to thiabendazole can produce activity even against inhibited larvae in cattle.

The concentrations of thiabendazole found in these sheep (mean maximum concentration 2.16 μg/ml at this dose of 44 mg/kg) are low compared to those of the anthelmintically active metabolite (albendazole sulphoxide) of albendazole in sheep (mean maximum concentration 3.2 μg/ml at a dose of 10 mg/kg) (Marriner and Bogan, 1980). Since Tocco et al., (1964) found 75% of an administered dose of ^{14}C and ^{35}S-labelled thiabendazole to be excreted in the urine of lambs

in forty-eight hours, these low plasma concentrations of thiabendazole and 5-hydroxythiabendazole are a consequence of rapid excretion of thiabendazole and 5-hydroxythiabendazole, rather than poor absorption.

After the administration of thiabendazole to milking cows, it was found that neither thiabendazole nor 5-hydroxythiabendazole were detectable in milk at any time. This was not surprising because the parent drug was not detectable in plasma after its administration to cattle (Tocco et al., 1965) and also the metabolite 5-hydroxythiabendazole was rapidly excreted because of its high aqueous solubility.

From all the residue data for thiabendazole in sheep, it was clear that if residues were present at four days post-treatment, they would be very low (less than 0.05 μg/g). However, residues were detectable for three days. Therefore, the fact that in sheep treated with thiabendazole there is no meat witholding time should be investigated further. The zero milk witholding time for cattle treated with thiabendazole seems to be adequate.

CHAPTER 10

FLUORIMETRIC DETECTION OF BENZIMIDAZOLES

High performance liquid chromatography with ultra-violet detection has been used over the past few years to estimate concentrations of benzimidazoles and their metabolites in plasma and, more recently, in tissues.

Ultra-violet detection allows detection of most benzimidazoles and their metabolites to concentrations of 0.05 μg/ml (μg/g for tissues) or less.

During the course of tissue residue studies, a fluorimetric detector (Perkin-Elmer LS-4 Fluorescence Spectrometer) became available and it was decided that the possibility of increasing the sensitivity and specificity of the methods for the determination of benzimidazoles and their metabolites in plasma and tissues should be investigated.

DETECTION OF BENZIMIDAZOLE RESIDUES USING FLUORIMETRY

Without change to the extraction methods or HPLC conditions, ultra-violet detection was compared with fluorimetric detection. For some of the drugs and metabolites there was a marked increase in sensitivity up to fifty times, using fluorimetric detection. Unfortunately this increase in sensitivity did not apply to all the drugs and metabolites, but nevertheless, in no case was fluorimetric detection less sensitive than ultra-violet and this method of detection could be adopted as the routine. In some cases, the limit of detection was improved to 0.0005 μg/g tissue, such as for albendazole sulphone.

Fluorimetry offers much improved limits of detection for albendazole and thiabendazole and their metabolites and little improvement for fenbendazole, oxfendazole and febantel. However, although the limits of detection for these are not improved, the specificity of the detection methods appears to be better using fluorimetry, such that the risk of "false positives" is much reduced. A possible use of fluorimetry may be as a further confirmatory test for any residues detected by U.V. spectrometry. The limits of detection using fluorescence and the limits of detection using ultra-violet are shown in Table 10.1.

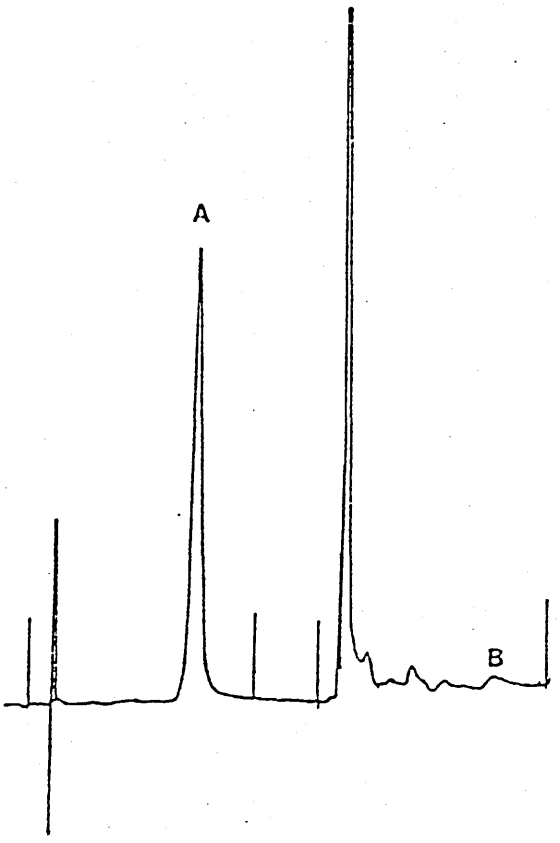
Typical chromatograms obtained using the method applied to liver samples from sheep treated with albendazole and thiabendazole using the two methods of detection are shown in Figures 10.1-10.4.

TABLE 10.1 Comparison of ultra-violet and fluorimetric methods of detection

	Limit of detection (μg/g tissue)	
	U.V.	Fluorimetry
Albendazole	0.02	0.001
Albendazole sulphoxide	0.02	0.005
Albendazole sulphone	0.02	0.0005
Fenbendazole	0.02	0.01
Oxfendazole	0.02	0.02
Oxfendazole sulphone	0.02	0.02
Thiabendazole	0.01	0.001
5-hydroxythiabendazole	0.02	0.02

Injection volumes and other conditions were the same for both detection systems. The U.V. detection system was operated at the limit for a stable baseline of 0.05 AUFS. The fluorimeter (Perkin-Elmer LS-4) could be operated at a scale of X5.

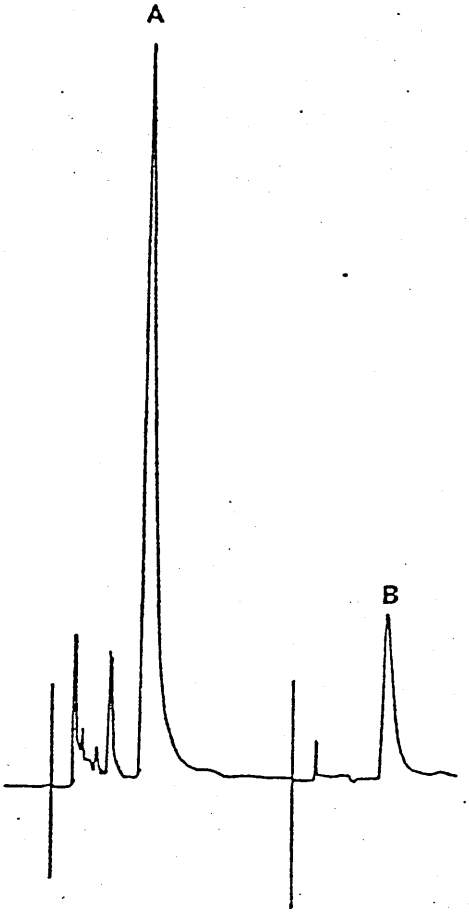
FIGURE 10.1 Ultra-violet detection of thiabendazole.



A = Thiabendazole (20µg/ml standard in methanol).

B = Liver sample from a sheep killed four days after the administration of thiabendazole (44mg/kg).

FIGURE 10.2 Fluorimetric detection of thiabendazole.

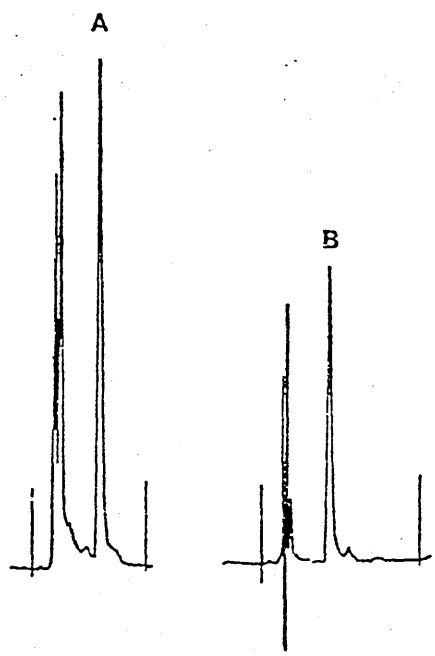


A = Thiabendazole (20µg/ml standard in methanol).

B = Liver sample from a sheep killed four days after the administration of thiabendazole (44mg/kg).

Fixed scale = 2.5.

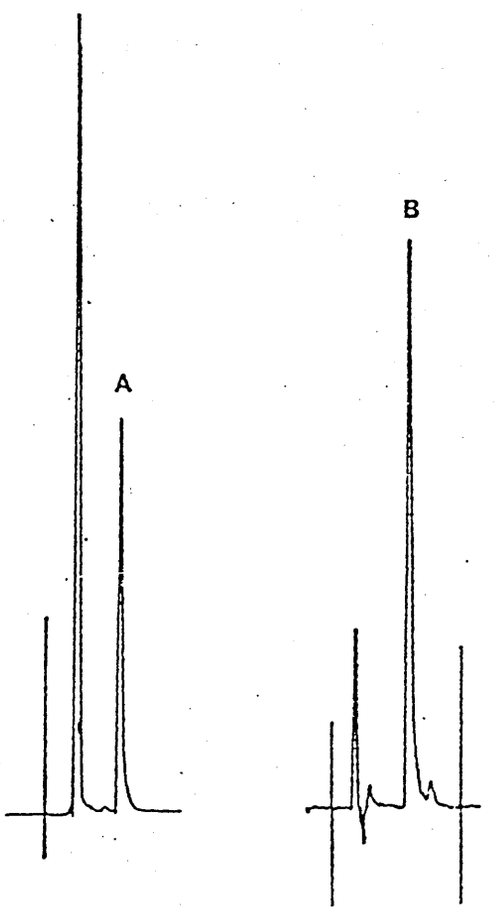
FIGURE 10.3 Ultra-violet detection of albendazole.



A = Liver sample from a sheep killed one day after the administration of albendazole (5mg/kg).

B = Albendazole (20µg/ml standard in methanol).

FIGURE 10.4 Fluorimetric detection of albendazole.



A = Liver samples (diluted x 5) from a sheep killed one day after the administration of albendazole (5mg/kg).

B = Albendazole (10µg/ml standard in methanol).

Fixed scale = 1.0.

ABATTOIR SURVEY

Samples of liver obtained from 25 calves, 25 cattle and 50 sheep post mortem from abattoirs distributed throughout the U.K. and listed in Tables 10.2-10.4 were analysed by the standard method using both U.V. and fluorimetric methods of detection. Thus, for all residues being sought the detection limit was 0.05 µg/g or better.

In no sample of liver were any benzimidazole or metabolite residues found. No spurious "peaks" or "false positives" were found in any sample.

TABLE 10.2 Details of randomly selected abattoir liver samples from cattle.

SAMPLE No.	SPECIES	SEX	ANIMAL AGE (Yrs)	ABBATOIR AREA	DATE
CA84/174	B	F	5	W. ENGLAND	30/05/84
CA84/182	B	M(1)	6	S.W. ENGLAND	7/06/84
CA84/185	B	F	-	N.W. ENGLAND	13/06/84
CA84/186	B	F	2	S.W. ENGLAND	18/05/84
CA84/191	B	F	3+	S.W. ENGLAND	21/06/84
CA84/196	B	F	2	W. SCOTLAND	14/06/84
CA84/199	B	M	2	W. SCOTLAND	14/06/84
CA84/201	B	M	1+	S.E. ENGLAND	22/06/84
CA84/202	B	F	5	N.W. ENGLAND	21/06/84
CA84/208	B	-	-	N.W. ENGLAND	27/06/84
CA84/217	B	M(1)	2	W. ENGLAND	28/06/84
CA84/220	B	F	2	E. ENGLAND	13/07/84
CA84/224	B	F	2	S. ENGLAND	10/07/84
CA84/232	B	M	1+	S.E. ENGLAND	06/07/84
CA84/233	B	M	2	S.E. ENGLAND	12/07/84
CA84/237	B	M	2	E. SCOTLAND	23/07/84
CA84/238	B	M	1	S.W. ENGLAND	17/07/84
CA84/276	B	M	1+	W. SCOTLAND	28/08/84
CA84/303	B	F	-	N.W. ENGLAND	14/09/84
CA84/309	B	M	2	S.W. ENGLAND	18/09/84
CA84/315	B	F	1+	N.E.SCOTLAND	19/09/84
CA84/318	B	F	1+	W. SCOTLAND	19/09/84
CA84/335	B	F	5	S.W. ENGLAND	27/09/84
CA84/341	B	F	-	S.W. ENGLAND	02/10/84
CA84/361	B	F	1+	S.W. ENGLAND	05/11/84

KEY B = BOVINE F = FEMALE M = MALE M(1) = BULL

TABLE 10.3 Detail of randomly selected abattoir liver samples from calves.

SAMPLE No.	SPECIES	SEX	ANIMAL AGE (Wks)	ABBATOIR AREA	DATE
CV84/100	B	M(1)	1	S. ENGLAND	01/05/84
CV84/105	B	M(1)	4	C. ENGLAND	04/05/84
CV84/110	B	M	-	S.W. ENGLAND	26/05/84
CV84/113	B	M	1	S.W. ENGLAND	23/05/84
CV84/123	B	M	13	S.W. ENGLAND	12/06/84
CV84/132	B	-	20	S. ENGLAND	13/06/84
CV84/134	B	M	1	W. SCOTLAND	28/06/84
CV84/135	B	M(1)	-	S.W. ENGLAND	29/06/84
CV84/I37	B	M	13	S.W. ENGLAND	17/07/84
CV84/149	B	M(1)	1	S. ENGLAND	18/07/84
CV84/150	B	M	3	C. ENGLAND	26/07/84
CV84/151	B	M(1)	-	S.W. ENGLAND	24/07/84
CV84/156	B	M	2	N.W. ENGLAND	30/07/84
CV84/159	B	M	12	N.E. ENGLAND	16/07/84
CV84/160	B	M	-	S.E. ENGLAND	25/07/84
CV84/168	B	M	36	W. SCOTLAND	23/08/84
CV84/171	B	F	1	WALES	25/07/84
CV84/174	B	M	1	S.W. ENGLAND	12/09/84
CV84/181	B	M	1	S.W. ENGLAND	19/09/84
CV84/184	B	M(1)	1	C. ENGLAND	03/10/84
CV84/185	B	M(1)	1	S.E. ENGLAND	03/10/84
CV84/195	B	M	3	S.W. ENGLAND	22/10/84
CV84/201	B	-	-	S.W. ENGLAND	11/10/84
CV84/202	B	M	1	W. SCOTLAND	19/10/84
CV84/206	B	M	1	N. ENGLAND	31/10/84

KEY B = BOVINE F = FEMALE M = MALE M(1) = BULL

TABLE 10.4 Details of randomly selected abattoir liver samples from sheep.

SAMPLE No.	SPECIES	SEX	AMIMAL AGE (Mth)	ABBATOIR AREA	DATE
S84/78	O	F	12	S. ENGLAND	04/04/84
S84/88	O	F	-	S.W. ENGLAND	10/04/84
S84/103	O	F	12	N.W. ENGLAND	26/03/84
S84/108	O	F	4	S. ENGLAND	15/05/84
S84/124	O	M	4	N.E.SCOTLAND	17/05/84
S84/126	O	F	12	W. ENGLAND	30/05/84
S84/144	O	F	5	S.W. ENGLAND	07/06/84
S84/152	O	M	-	N.W. ENGLAND	20/06/84
S84/160	O	M	4	C. ENGLAND	19/06/84
S84/167	O	M	-	WALES	06/07/84
S84/177	O	F	-	N. ENGLAND	20/07/84
S84/181	O	M	-	S.W. ENGLAND	17/07/84
S84/184	O	F	8	N.E. ENGLAND	17/07/84
S84/187	O	F	6	S.W. ENGLAND	30/07/84
S84/188	O	F	6	W. SCOTLAND	24/07/84
S84/192	O	M	-	C. ENGLAND	08/08/84
S84/203	O	M	-	WALES	17/08/84
S84/208	O	F	7	E. ENGLAND	29/08/84
S84/215	O	M	5	N.W. ENGLAND	11/09/84
S84/225	O	F	10	N.W. ENGLAND	11/08/84
S84/233	O	F	6	C. ENGLAND	19/09/84
S84/241	O	M	5	N.W. ENGLAND	17/09/84
S84/262	O	F	11	N.W. ENGLAND	18/10/84
S84/263	O	F	10	N.E. ENGLAND	15/10/84
S84/266	O	F	-	S.E. ENGLAND	01/11/84
S84/271	O	M	8	E. SCOTLAND	12/11/84
S84/272	O	M	12	N.E. ENGLAND	20/11/84
S84/275	O	-	8	S. ENGLAND	22/11/84
S84/278	O	M	10	S. ENGLAND	15/11/84
S84/281	O	F	8	N.E.SCOTLAND	13/11/84
S84/284	O	M	8	WALES	27/11/84
S84/285	O	F	-	WALES	26/11/84
S84/287	O	M	8	W. ENGLAND	07/11/84
S84/290	O	M	18	N.W. ENGLAND	26/10/84
S84/306	O	F	11	N.W. ENGLAND	28/11/84
S84/308	O	M	8	WALES	24/10/84
S85/34	O	F	12	C. ENGLAND	12/02/85
S85/36	O	M	12	N.W. ENGLAND	13/02/85
S85/46	O	M	11	WALES	08/03/85
S85/47	O	F	12	WALES	12/03/85
S85/49	O	M	9	W. ENGLAND	14/03/85
S85/50	O	M	12	E. ENGLAND	19/03/85
S85/55	O	F	8	S.W. ENGLAND	21/03/85
S85/63	O	M	72	E. ENGLAND	15/02/85
S85/70	O	M	-	N.E.SCOTLAND	18/04/85
S85/71	O	F	-	E. ENGLAND	15/04/85
S85/72	O	M	12	N. SCOTLAND	23/04/85
S85/75	O	F	72	W. ENGLAND	09/04/85
S85/76	O	M	15	W. SCOTLAND	18/04/85
S85/80	O	F	-	N.W. ENGLAND	18/04/85

KEY O = OVINE F = FEMALE M = MALE

CHAPTER 11

GENERAL DISCUSSION

The feature common to all the benzimidazoles studied with respect to tissue residues was that the concentrations in liver were considerably greater than those in muscle, kidney, plasma and milk. The limits of detection were similar for all tissues and plasma, and the residues in liver were detectable for longer periods than those of the other tissues and plasma.

In general, the temporal pattern of residues accorded with the known plasma pharmocokinetics of each of the compounds. That is, there was a direct correlation between the residence time of these compounds in plasma and their persistence in tissues, in that those benzimidazoles which did not persist in plasma did not persist in tissues, and those which did persist in plasma did persist in tissues. Thus, the order of diminution of residues was: thiabendazole followed by albendazole, followed by febantel, fenbendazole and oxfendazole.

Liver residues of febantel, fenbendazole and oxfendazole all followed the same pattern. After the administration of these three compounds, oxfendazole was detectable in liver in greater concentrations than any of the other metabolities. It has been demonstrated previously that oxfendazole is the most important metabolite in the plasma of sheep after the administration of febantel (Delatour et al., 1985a), fenbendazole (Marriner and Bogan, 1981b) and oxfendazole itself (Marriner and Bogen, 1981a). Therefore, it is clear that the similar residue profiles after the administration of these three anthelmintics was due to the contribution of high concentrations of oxfendazole in the liver in each case. Oxfendazole was also the

longest residing metabolite in the liver of sheep after all three treatments, which may be due to the fact that it is intermediate between fenbendazole and oxfendazole sulphone after the administration of all three compounds, such that it can be converted to either one, with a resultant decreased rate of elimination.

It appears that benzimidazoles bind to liver tissue, although the mechanism and extent of binding have not been investigated. It has been reported (Delatour et al., 1983) that albndazole binds to liver protein, although the mechanism of binding is unknown. This may explain, in part, the high residues found in liver after the administration of benzimidazoles to sheep, although it is more likely that these high residues are a result of entero-hepatic circulation of benzimidazoles. After oral administration, high benzimidazole concentrations were detectable in sheep abomasal fluid for up to six days (Marriner and bogan 1981a and 1981b). This was attributed to passive diffusion of drug from the systemic compartment to the abomasum. Therefore, entero-hepatic circulation and passive diffusion of benzimidazoles into the abomasum (which would allow drug to be re-absorbed) may explain, in part, the high residues found in liver and also the persistence of these residues long after concentrations in plasma had fallen below detectable levels. It may also explain the finding that liver residues were much higher than muscle and kidney residues after the administration of benzimidazoles to sheep. The greatest hazard to the consumer would arise, therefore, from the consumption of liver, and any screening programme for the examination of meat from animals suspected of being treated with a benzimidazole

close to slaughter, should concentrate on the analysis of liver for residues.

Compared to sheep, residues in cattle tissues were very much lower, although only albendazole, albendazole sulphoxide and oxfendazole were used to study benzimidazole residues in the tissues of cattle. It is known that the capacity for the metabolism of benzimidazoles is greater in cattle than in sheep. Pharmacokinetic studies of oxfendazole in sheep (Marriner and Bogan, 1981a) and cattle (Ngomuo et al., 1984) have shown that plasma concentrations of anthelmintically active metabolites were less in cattle than in sheep at the same dose rate. Therefore, the finding that tissue residues in cattle were very much lower than tissue residues in sheep did not come as a great surprise. It is interesting that the teratogenic effects of benzimidazoles in cattle are considerably lower than those in sheep. For example, albendazole is teratogenic in sheep at a dose rate of 11 mg/kg, whereas it is not teratogenic in cattle at a dose rate of 25 mg/kg.

It is interesting to note that in sheep, liver residues of febantel, fenbendazole, oxfendazole and thiabendazole all were detectable beyond their recommended withdrawal periods. In particular, thiabendazole (which has a nil withdrawal time in cattle and sheep) was still detectable in liver three days after its administration to sheep. There have been no reports of tissue residues of thiabendazole, and therefore, one can only assume that it was given a nil withdrawal time because, either residues have not been found or, if residues were

found, they were not considered a problem as regards toxicity to humans. More likely, however, is that residues of thiabendazole have not been determined because it has a licence of right. It is also interesting to note that febantel has only an eight day withdrawal time in cattle and sheep, despite having similar kinetics to fenbendazole and oxfendazole in sheep (both these anthelmintics have fourteen day withdrawal periods in cattle and sheep). Therefore, if all three of these anthelmintics have similar pharmacokinetics, and their residue profiles are similar (both qualitatively and quantitatively), then they should all have the same withdrawal period. Although residues of fenbendazole and oxfendazole were present beyond their recommended withdrawal times, these residues may not be considered a problem, as regards toxicity to the consumer. For example, these residues were only detectable in liver at twenty-one days, and assuming that the average human diet does not consist of large quantities of liver, it is unlikely that exposure to these residues would be very high. In addition, if large quantities of liver were consumed, it is again unlikely that these residues would be absorbed to any significant extent, although relay toxicity studies would have to be carried out in other monogastric animals before one could be certain of this.

In milk, residues of all the drugs studied were low compared to tissue residues. None of these drugs were detectable in milk beyond recommended milk withholding times. The absence of thiabendazole residues from cows' milk was not surprising, since thiabendazole has a much higher aqueous solubility than any of the other drugs studied, and as a result it is more rapidly absorbed and eliminated than any of the

others. However, uniquely among the benzimidazoles, thiabendazole has fungicidal properties, which is likely to cause problems in the production of cheese and yoghurt from sheep's milk (which is more likely to contain residues after thiabendazole treatment).

It is interesting to compare milk residues of oxfendazole after the administration of fenbendazole and after the administration of oxfendazole itself. Residues persisted longer after the administration of oxfendazole than they did after the administration of fenbendazole. This is probably due to the fact that higher concentrations of oxfendazole are achieved in the plasma of cattle after the administration of oxfendazole than are achieved after the administration of fenbendazole (Ngomuo et al., 1984). Therefore, if fenbendazole is not detectable in milk, it is obvious that residues will be higher in the milk of cattle treated with oxfendazole than those in the milk of cattle treated with fenbendazole.

The milk withholding time for febantel in cattle is forty-eight hours.If febantel has similar plasma kinetics to fenbendazole and oxfendazole, and a similar tissue residue profile, then one can assume that milk residues of febantel would be similar to those after fenbendazole and oxfendazole treatment. If this was the case, it is very likely that residues of febantel in milk would persist longer than forty-eight hours, since residues of fenbendazole and oxfendazole in milk persisted beyond forty-eight hours.

Liver residues of benzimidazoles are more likely to be a problem,

because of the persistence of metabolites which are known teratogens. Of all the drugs studied, albendazole, albendazole sulphoxide and oxfendazole are teratogenic. Albendazole sulphoxide is the major metabolite of albendazole and is more teratogenic than oxfendazole. The teratogenic dose of albendazole in man is not known, but it can be estimated from other species, the most sensitive being rabbits, sheep and rats, in which the plasma concentrations of albendazole sulphoxide which are associated with teratogenicity are in the order of 7 to 10 μg/ml (Bogan, Personal Communication). In man, 400 mg of albendazole given to ten volunteers gave rise to a maximum concentration of 0.55 μg/ml of albendazole sulphoxide in plasma, with a mean concentration of 0.20 μg/ml (Marriner et al., 1986). If it is assumed that the sensitivity of humans to the teratogenic effects of albendazole is similar to other sensitive species, a dose of 5.1g of albendazole (73 mg/kg for a 70 kg person) would be required to give a plasma concentration of 7 μg/ml of albendazole sulphoxide (i.e. a concentration which is known to be teratogenic). This concentration is considered to be the lowest plasma concentration at which teratogenicity occurs. Therefore, a dose of 73 mg/kg of albendazole in humans could be considered to be the lowest-observed-adverse-effect level (LOAEL). The uncertainty factor (safety factor) chosen for major biological effects such as teratogencity, is 1,000. Therefore, for albendazole in humans, a LOAEL of 73 mg/kg corresponds to an acceptable daily intake (ADI) of 0.073 mg/kg/day (5.11 mg/day for a 70 kg person). Assuming a human will consume a maximum of 1 kg of liver per day, the acceptable residue of albendazole should be of the order of 5 μg/g. It would appear that most regulatory authorities, since the teratogenic

dose for man is not known, err considerably on the side of caution and specify limits of, for example, 0.1 μg/g when setting withdrawal periods. However, albendazole and other benzimidazoles are probably limited by their solubility, and it is probable that increasing the dose does not give a proportional increase in concentrations in plasma and tissues. Therefore, this result is probably an underestimate of the acceptable daily intake of albendazole. In addition, teratogenicity occurs because of the existence of particular concentrations on a specific day in gestation. Variation from this timing produces much lowered teratogenicity. Therefore, the likelihood of an animal having a sufficiently high residue on a specific day of pregnancy would seem a remote possibility in general. Also, farmers are quite aware of the economics of anthelmintic treatment, and it is unlikely that an animal going to slaughter would be treated with an anthelmintic close to slaughter. Our survey of one hundred livers obtained from abattoirs would bear out the premise than an animal going to slaughter contains no residues of benzimidazole anthelmintics. Nevertheless, there is a risk and surveillance should be maintained to assess that risk. In addition, benzimidazoles are heat stable and would not be destroyed by the cooking of contaminated meat or liver. However, as discussed previously, the bioavailability of these compounds is likely to be very low in humans.

The analytical methods used to measure benzimidazoles and their metabolites were relatively simple and straightforward and appeared to work well in practice, with important biological concentrations being easily measured. With other anthelmintics, important biological

concentrations are present for long periods after treatment compared to benzimidazoles. Therefore, residues of these anthelmintics may pose a greater problem to the consumer than benzimidazoles. For example, ivermectin is known to be lipid soluble and long-lasting, producing plasma concentrations for twenty-eight days and having efficacy for twenty-eight days. Also, residues of salicylanilide flukicides are known to persist for up to three months (Mohammed-Ali, 1985). It is therefore likely that animals will occasionally arrive at slaughter containing residues of these drugs. In addition, benzimidazoles are always administered orally, so they are safer in the general respect that injected anthelmintics (e.g. ivermectin, salicylanilides) may give rise to very high local concentrations (to which the consumer may be exposed) which would not occur with orally administered anthelmintics.

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