VETERINARY ANTHELMINTICS:

THEIR EFFICACY
AND
EFFECTS ON ABOMASAL PHYSIOLOGY

A thesis presented in
partial fulfilment of the requirements
for the degree of
MASTER OF VETERINARY SCIENCE
in Veterinary Clinical Pharmacology
at Massey University

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March 1998

For Pauline, with all my love

ABSTRACT

PART 1. A Review of the Veterinary Anthelmintic Literature

A comprehensive review was undertaken of the pharmacology, efficacy, side effects and toxicity of veterinary anthelmintics used against nematode parasites. Anthelmintics reviewed for use in cattle, sheep, goats, horses, dogs and cats include copper, nicotine, arsenic, tetrachlorethylene, phenothiazine, diethylcarbamazine, piperazine, toluene, cyacethydrazide, bephenium, thenium, organophosphates, and methyridine. The review was limited to cattle for the benzimidazoles, pyrantel, morantel, tetramisole, levamisole, avermectin and milbemycins anthelmintics. Efficacy data is provided in a tabular format which classifies each anthelmintic according to method of administration and dose.

PART 2 Efficacy of two formulations of moxidectin pour-on and the effects of treatment on serum pepsinogen and gastrin levels and tissue gastrin in cattle

Three groups of eight yearling Friesian bulls were used to compare the efficacy of two 5 g/L pour-on formulations of moxidectin applied at 1ml/10kg (500 mcg moxidectin per kg bodyweight) in removing naturally acquired gastrointestinal parasites.

At slaughter, 14-16 days after treatment, the burdens of *Ostertagia* spp. and *Trichostrongylus axei* were significantly lower in both the treated groups versus the controls ($P<0.01$). Anthelmintic efficacies (based on geometric mean worm burdens of treated and control groups) were all greater than 99.8% for *Ostertagia* spp. and *Trichostrongylus axei* in both treated groups compared with the controls. A significantly lower burden of adult *Cooperia* spp. was recorded for Formulation 1 ($P<0.05$). The anthelmintic efficacy of the two treatments against *Cooperia* adults, early L4 and late L4 were 96.25% ($P<0.05$), 97.31% and 91.08% respectively in calves treated with Formulation 1, and 71.44%, 67.14% and 64.29% respectively for calves treated with Formulation 2. Low numbers of large intestinal worms, *Trichuris ovis* and *Oesophagostomum* spp. in the control cattle precluded any valid efficacy assessment of these species.

Based on these results, Formulation 1 is distinguished from Formulation 2 by its significantly greater efficacy against adult *Cooperia* spp.

Serum pepsinogen and gastrin levels were monitored in the three groups of calves after treatment. All groups showed a steady decline in levels of both pepsinogen and gastrin until termination on day 14. On only one occasion was there a significant difference in serum pepsinogen between treated and untreated calves. Following treatment there was a more rapid and significant decrease in gastrin levels in calves treated with Formulation 1 than Formulation 2. At no stage was there a significant difference between the controls and calves treated with Formulation 2. There was no correlation between pepsinogen and gastrin levels for any of the groups. Neither was there a correlation between numbers of *Ostertagia* spp. and serum pepsinogen or gastrin. It is suggested that the decreases seen in the control group were due to lack of larval challenge and normal loss of adult worms resulting insufficient numbers to sustain a hypergastrinaemia.

Gastrin concentrations were also measured in tissue samples from the pyloric antrum or the proximal duodenum. There was no significant difference in antral and proximal duodenal gastrin concentrations between the treatment and the control groups. Antral levels were between 1148-1323 pM/g which were 25-35 times those found in duodenal tissue (32.3-50.9 pM/g).

ACKNOWLEDGEMENTS

The present thesis could not have been completed without the help and assistance of numerous people. The two people who deserve a special mention are, my wife Pauline, for her continuous moral support, seemingly endless patience, as well as considerable technical assistance; and my Chief Supervisor, Associate Professor Tony Charleston of the Department of Veterinary Pathology and Public Health, Faculty of Veterinary Science, for his excellent guidance, tolerance and undying patience, especially in the closing stages of this thesis.

Special thanks are also due to Dr Alan Alexander, Associate Professor Brian Cooper and Professor Norm Williamson who in conjunction with Associate Professor Tony Charleston helped me design this course of study. I very much appreciated Dr Alan Alexander allowing me the opportunity to undertake this course of study while I was employed by the Animal Health Services Centre. Both Dr Alexander and Mr Steve Lees of the Animal Health Service Centre provided considerable assistance with the treatment and care of the animals as well as all the sampling that occurred throughout this trial.

Mrs Barbara Adlington and Ms Shirley Calder of the Department of Veterinary Pathology and Public Health provided excellent technical assistance and patience in assisting me with the worm counts. I also appreciated the assistance of Dr Bill Pomroy of the same department for technical assistance with speciation of the nematodes. I would also like to express my appreciation to Dr Heather Simpson and Ms Jane Candy for all their help with the serum and tissue gastrin assays, and I very much appreciated the assistance of Dr David Lawton in helping assay the pepsinogen samples.

The resources of the Kelvin Grove Hotel and the moral support of Mrs Barbara Adlington and Mr Bill Penman were always much appreciated. I would also like to express my appreciation to Mr Tim Knox, Chief Agricultural Compounds Officer, Ministry of Agriculture and Forestry for allowing me time to complete this thesis.

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PART 1.

A Review of the Veterinary Anthelmintic Literature

1.0 INTRODUCTION

The influence of helminth parasites on intensive agriculture production systems is considerable in terms of animal health and welfare. There is now a heavy reliance on the use of anthelmintics to maintain these farming practices, which more recently is starting to take a heavy toll on the continued efficacy of these compounds. Up until the 1960's, anthelmintics of various levels of efficacy were used to aid in the management of nematodes. With the discovery of the benzimidazoles (Brown *et al.*1961), a new era in parasite control occurred. The success of this compound stimulated considerable research and as the knowledge of anthelmintic pharmacology has developed, so has the development of more potent drug analogues, and better and more effective methods of administration (Prichard, 1986a).

In order for parasites to exist in an animal, they must maintain an appropriate feeding site and ingest and move food through their digestive tracts. Anthelmintics can be divided into two general classes as to how they affect parasites. There are those which inhibit metabolic processes, and those which inhibit neuromuscular co-ordination. The benzimidazoles inhibit metabolic processes by binding to microtubules which results in disruption of normal cell processes, such as formation of the mitotic spindle in cell division, maintenance of cell shape, cellular motility, nutrient absorption and intracellular transport. Anthelmintics which have their effects on neuromuscular co-ordination can be divided into two groups. Those which inhibit the breakdown of excitatory neurotransmitters (cholinesterase inhibitors - organophosphates) or mimic the action of excitatory neurotransmitters (imidazothiazoles - levamisole, and pyrimidines - pyrantel and morantel) and result in spastic paralysis. The second group either mimic the inhibitory action of the inhibitor neurotransmitter or cause hyperpolarisation (piperazine, avermectins/milbemycins) resulting in flaccid paralysis. Any effect on parasite neuromuscular co-ordination, results in the parasite losing its ability to maintain its position within the gastrointestinal tract or pulmonary system, and is subsequently expelled.

The "ideal" anthelmintic should have a broad spectrum of activity against mature and immature (including inhibited larvae); be easy to administer to a large number of animals; have a wide margin of safety and be compatible with other compounds; result in minimal residues; and be economical to use.

The following review identifies anthelmintics which affect nematode parasites in cattle, sheep, horses, dogs and cats. It describes the history of each of the anthelmintics, their pharmacology and their efficacy against nematodes of the gastrointestinal tract and pulmonary system, and side effects and toxicity to the treated animals. For the four main action families, the benzimidazoles, imidazothiazoles, tetrahydropyrmidines and the avermectins/milbemycins, the review has been limited to cattle.

2.0 COPPER, NICOTINE and ARSENIC

2.1 INTRODUCTION

The use of copper salts for gastrointestinal parasites was first recorded in South Africa by Hutcheon in 1891 (in the Agricultural Journal of the Cape of Good Hope. 3, 179-181) when it was used for the treatment of abomasal nematodes in sheep and goats (Hall and Foster, 1918; Gibson, 1962c). Since then, several different combinations of copper sulphate, nicotine sulphate and arsenical salts have been used to help combat gastrointestinal parasites (Prichard, 1978). It was only in the 1940's that these were superseded by phenothiazine (Gibson, 1962c).

2.2 PHARMACOLOGY

Drenches used in domestic animals have included copper sulphate solution alone, or combined with 40% nicotine sulphate, sodium arsenite or tetrachloroethylene (Hall and Foster, 1918; Gordon, 1935; Ross and Gordon, 1935; Gordon and Ross, 1936; Gordon, 1939a; 1939b; 1939c; Lapage, 1940; McEwen, 1940; Singer and Baker, 1940;Stewart and Crofton, 1941; Boddie *et al.*1941; Porter *et al.*1941; Gordon and Whitten, 1941; Willman and Baker, 1943; Martin, 1944; Eveleth and Goldsby, 1950).

The mode of action differs with each of these compounds. Both copper and lead (in lead arsenate drenches) have a general poisoning effect on enzymes. The arsenicals react with mercapto groups, and alter the tertiary structure of many proteins and the active site(s) of enzymes in both the parasite and host (Prichard, 1978; Rew, 1978). Nicotine is a general neuromuscular blocking agent and causes spastic paralysis (Prichard, 1978). It is a stimulant of nicotinic receptors in both autonomic ganglia and neuromuscular junctions in mammals (Taylor, 1992a). Subsequently when animals are dosed, there is central nervous system stimulation, irritation of the gastrointestinal tract and increased peristalsis due to stimulation of the parasympathetic autonomic nervous system. It is assumed that there is also stimulation of the parasite nervous system resulting in a spastic paralysis, which leads to detachment from its feeding site and subsequent expulsion because of the increased intestinal paralysis.

2.3 EFFICACY

These compounds are only given orally for anthelmintic efficacy. It was found in sheep that copper sulphate was ineffective unless swallowed into the abomasum. In sheep, copper salts stimulate

closure of the oesophageal groove that in up to 88% of animals results in direct passage of the drench to the abomasum. However, in 30% of animals this mechanism could fail with subsequent entry of the drench into the rumen (Gordon, 1939b). Repetition of this in some animals will subsequently lead to inefficacy and clinical parasitism (Gordon, 1939b; Gordon and Whitten, 1941).

While in the early days these anthelmintics provided some treatment against gastrointestinal parasites, they were limited in their efficacy. The number of published critical or controlled experiments is small, with many publications being either field observations or trials using small numbers of animals.

Hall and Foster, (1918) using 100-200ml of 1% copper sulphate solution in sheep found this dose to be efficacious for abomasal parasites, unlike capsules containing 0.5g powered copper sulphate. Using faecal egg count reduction and worm counts, no difference was found between starved and unstarved group animals given 30ml of 2% copper sulphate (Ross and Gordon, 1934). This trial did prove the efficacy of copper sulphate against adult *Haemonchus contortus*. Further trials were than undertaken by to try to evaluate the efficacy of copper sulphate against *H.contortus* in adult sheep (Gordon, 1939b). The criterion for efficacy was a greater then 70% reduction in faecal egg counts (FEC). One fluid ounce (28 ml) of 4% solution was ineffective, only resulting in a 29% reduction in FEC, but two fluid ounces were very effective (92% reduction in FEC) (Gordon, 1939b). Because of the larger dose volumes involved with the lower concentrations, the efficacy of 0.5, 2 and 10% solutions containing the same total dose of copper sulphate were compared. The 42 ml of a 2% solution was the most efficacious, resulting in an 81% reduction in FEC.

Because of the efficacy limitations from using copper sulphate alone, several other chemicals were combined with this anthelmintic. These included nicotine sulphate (combination was called CuNic), sodium arsenite, carbon disulphide and tetrachloroethylene (Hall and Foster, 1918; Ross and Gordon, 1935; Gordon, 1935; Gordon and Ross, 1936; Gordon, 1939a; 1939b; 1939c; Lapage, 1940; McEwen, 1940; Singer and Baker, 1940; Boddie *et al.*1941; Porter *et al.*1941; Gordon and Whitten, 1941; Stewart and Crofton, 1941; Willman and Baker, 1943; Martin, 1944; Eveleth and Goldsby, 1950).

Comparative trials with a new anthelmintic (phenothiazine) were carried out and efficacy was based on either weight gain, FEC reductions or individual worm counts on some animals that had died during the trial. The CuNic mixture was less effective in sheep (Lapage, 1940; McEwen, 1940; Boddie *et al.*1941; Willman and Baker, 1943) or cattle (Stewart and Crofton, 1941) than phenothiazine. CuNic was found to have anthelmintic action against *H.contortus*, *Ostertagia* spp. and *Nematodirus* spp., but no effect on *Trichostrongylus* spp., *Cooperia* spp., or *Trichuris ovis*, based on worm counts or FEC reductions (Singer and Baker, 1940; Stewart and Crofton, 1941; Willman and Baker, 1943; Gordon, 1935; Levine and Garrigus, 1962; Boddie *et al.*1941). However,

in a trial involving artificial infection of sheep with *Trichostrongylus* spp. larvae, dosing with copper sulphate/nicotine sulphate or copper sulphate/tetrachloroethylene solutions at three weekly intervals was quite efficacious (Gordon and Ross, 1936). The CuNic mixture is more effective against immature *H.contortus* then copper sulphate alone (Gordon, 1939a). Treating animals with a combination of copper sulphate/nicotine sulphate and phenothiazine was less effective than the copper sulphate/nicotine sulphate solution alone (Stewart and Crofton, 1941).

In generating this information, several different CuNic concentrations have been used by various workers. The formulation used most frequently in the field was 5% copper sulphate and 5% of a 40% nicotine sulphate, given in doses of 8-20 ml in lambs and 20-30 ml in sheep (Gibson, 1975).

Sodium arsenite has been used alone or in combination with copper sulphate. On its own at concentrations of 50 or 90 mg in 25 ml of water, it was found to have low efficacy against an artificial infection of *H. contortus* (Ross and Gordon, 1935; Gordon, 1935), possibly due to failure of the oesophageal groove to close (Ross and Gordon, 1935). Combining sodium arsenite with 200 mg of copper sulphate considerably increased efficacy against adult *H. contortus* (Gordon, 1935). This combination was also fully effective against 10-day-old *H.contortus* larvae, but was only partially effective against 15-day-old larvae (Gordon, 1939a). Another combination using half a tablet containing 0.93g of nicotine arsenate and 1g of copper sulphate, either given whole or dissolved in water, was up to 100% effective against *H.contortus* but variable against *Ostertagia* spp. and *T. axei*. There was no effect on *Nematodirus* spp., *T. colubriformis* or *Chabertia* sp.(Eveleth and Goldsby, 1950).

A mixture of copper sulphate and carbon disulphide did give good results against *Trichostrongylus* spp., but because of difficulty of administration and untoward effects, this combination could not be recommended (Gordon, 1935). Another combination trialed involved 2ml of 5% copper sulphate followed by 10ml of tetrachloroethylene in 10ml of mineral oil. There was a decrease in the number of *Haemonchus* and *Ostertagia* spp., but no effect in the number of *Trichostrongylus* spp. (Singer and Baker, 1940).

With the development of oral copper oxide wire boluses for the treatment of copper deficiency in sheep, a trial was undertaken to evaluate if these had any anthelmintic effect. Following pure artificial infections with either *H.contortus*, *O.circumcincta* or *T.colubriformis*, 10-week-old lambs were treated with a higher than recommended dose of intra-ruminal copper oxide wire particles (5g) (a normal therapeutic dose is 0.1 g/kg). There was a 96% and 56% decrease for the *H.contortus* and *O. circumcincta* groups respectively. However, no effect was seen against *T. colubriformis* (Bang *et al.*1990). *Trichostrongylus* larvae (L3 and L4) were shown to be very tolerant of copper concentrations 100 times those observed in the abomasum in this study (Bang *et al.*1990). This would explain some of the lack of efficacy of copper against *Trichostrongylus* spp. seen in earlier

trials (Gordon and Whitten, 1941). It was felt that the mechanism for the increased anthelmintic effect of copper wire compared with copper sulphate solution, was due to a more consistent elevation of soluble ionic copper from the wire particles once they reach the acid part of the abomasum.

2.4 SIDE EFFECTS AND TOXICITY

The use of normal doses of copper sulphate has not been associated with toxicity (Gibson, 1975). However (Rose, 1933) reported on 12 sheep treated with 200-400 grains of copper sulphate (a normal dose is 17.5 grains), this was between 12-23 times the recommended dose. Five animals died within 48 hours and the remainder over the next week. Miraculously four animals survived. The author showed that animals had been dosed with up to 32 grains with no ill-effects, but references Theiler, who found that 45 grains or more killed sheep. Excessive copper is locally irritating to the alimentary mucosa and may cause vomiting, excessive salivation, acute abdominal pain, purging, melaena, conclusions, paralysis, coma and death (Oeheme, 1987).

The combination copper sulphate/nicotine sulphate drench has a narrower safety margin. Care needs to be taken with sheep in poor condition whereby the dose of nicotine sulphate should be decreased (Gordon, 1939a). There were 42 deaths out of 500 lambs following dosing with twice the recommended dose of copper sulphate and nicotine sulphate and 24 hours starvation. Animals showed inco-ordination, muscle tremors and extreme prostration before death 2-3 hours after treatment (Rose, 1936). A similar situation occurred in South Africa where lambs received between one and a half to twice the dose of nicotine sulphate (3.6-4.8 grains instead of 2.4 grains) and 18/42 died over 2-60 hours. The clinical signs were the same as Rose, (1936) and on postmortem there was intense haemorrhagic congestion of the abomasum and small intestine with submucosal oedema, lung oedema and pleural effusion (Crawshaw, 1944). A personal communication by Steyn (1943) said that a fatal dose of nicotine in adult sheep was 2-3 grains and 10-15 grains of nicotine sulphate and the results of this accident confirmed the toxic dose of nicotine sulphate to be between 3.2-4.8 grains (Crawshaw, 1944). Another personal communication by Watermeyer (1943) in the same paper, showed that similar clinical signs of toxicity were seen in cattle dosed with 5 ounces of copper sulphate/nicotine sulphate mixture.

The use of sodium arsenite or lead arsenate is also not without risk (Gordon, 1939a; Eveleth and Goldsby, 1950). The toxicity of copper sulphate/nicotine arsenite tablets was evaluated with animals dying in 48-72 hrs after 2-5 tablets or 3-5 tablets dissolved in water (Eveleth and Goldsby, 1950).

Nicotine sulphate acts on all the nicotine receptors in the nervous system and symptoms seen are those of central nervous system stimulation, excitement and increased respiration. There is irritation

of the gastrointestinal tract with increased peristalsis and diarrhea. Following the initial stimulation there is depression, inco-ordination and ataxia with flaccid paralysis and death within a few hours, usually during a terminal convulsion from paralysis of the respiratory muscles. Treatment is usually not successful due to the rapidity of onset, though oral administration of laxatives, tannic acid or potassium permanganate may have helped (Oeheme, 1987).

3.0 TETRACHLORETHYLENE

3.1 INTRODUCTION AND PHARMACOLOGY

Tetrachlorethylene is a volatile, unsaturated halogenated hydrocarbon, almost insoluble in water but very soluble in fat (del Castillo, 1969; Vanden-Bossche, 1985). There is no direct experimental evidence, but it is thought to work like other halogenated hydrocarbons by becoming dissolved in lipids in muscle cells, particularly those in the muscle innervation processes. This interferes with muscle function ((del Castillo, 1969) citing Mueller 1929) and theoretically would cause paralysis of the parasite, resulting in expulsion due to the peristaltic movements of the intestinal tract.

3.2 EFFICACY

3.2.1 Sheep

The first published use of tetrachlorethylene was by (Schlingman, 1926). Based on the results of a limited critical trial, he concluded that doses of 5ml were effective against abomasal nematodes of sheep, but that there was no effect on whipworms. Further work by the same author was only able to show an anthelmintic effect against *Oesophagostomum* spp.(Schlingman, 1929). It was suggested that the variable effects seen with this anthelmintic could be due to it initially entering the rumen rather than the abomasum (Ross and Gordon, 1935; Gordon, 1935). Ross, (1934) and Monnig and Quin, (1935) had previously shown that pre-treatment with a solution of copper sulphate would cause short-term closure of the oesophageal groove, which would result in deposition of other oral liquids into the abomasum. Critical and controlled trials by Gordon and Ross, (1936) and Ortlepp and Monnig, (1936) using pre-treatment with copper sulphate solution, confirmed the hypothesis that tetrachlorethylene needed to be deposited directly into the abomasum for maximum efficacy against abomasal worms (Table 3.1). Results from controlled trials (Gibson, 1955; Fritts *et al.*1958) using tetrachlorethylene without pre-treatment with copper sulphate supported this, with poor efficacy against *H.contortus*, *O.circumcincta* and *T.colubriformis*. Gibson, (1955) also showed from his controlled trial that faecal egg count reductions were an invalid method of gauging anthelmintic efficacy of tetrachlorethylene, as the drug appeared to inhibit egg production by female worms.

3.2.2 Cattle

Early work using a critical trial with 5 calves and 1 control was inconclusive about its efficacy as no worms were found in either the control or treated calves on post-mortem (Schlingman, 1926).

However, it was assumed to be as effective in this species as it had been shown to be against hookworms and roundworms of other species (Schlingman, 1926). Later, Roberts, (1955) found variable efficacy against natural infections using faecal egg count reductions and larval culture, as did (Gibson, 1956) in a controlled trial using an artificial infection of *T.axei*. It was concluded that the anthelmintic action of tetrachlorethylene was variable and efficacy much less than phenothiazine (Gibson, 1956) (Table 3.2).

3.2.3 Other Species

The only critical efficacy trial published on the use of tetrachlorethylene in horses appeared to use animals which were worm-free before treatment, though there was some effect on the limited number of bots present (Schlingman, 1926). This anthelmintic was also shown to be effective against "roundworms" and mature and immature hookworms after critical (dogs and cats) (Schlingman, 1926; Schlingman, 1929) and controlled trials (dogs) (Miller, 1966a) (Table 3.3). Further work in dogs found that the use of purgatives (Schlingman, 1926) or the feeding of fat (Schlingman, 1929) would reduce the drug's efficacy. In the case of fat, efficacy was reduced to zero (Schlingman, 1929). This would seem to contradict the recommended formulation used in sheep where it is combined with equal parts of liquid paraffin oil. However, the drug still appears to have some efficacy (Gordon and Ross, 1936; Ortlepp and Monnig, 1936; Singer and Baker, 1940; Roberts, 1955) following pretreatment with copper sulphate solution, but this could partly explain the lack of efficacy found in some trials (Ross and Gordon, 1935; Gordon, 1935; Gibson, 1956).

3.3 SIDE EFFECTS AND TOXICITY

Tetrachlorethylene is hepatotoxic (Schlingman and Gruhzit, 1927). There appears to be varying inter-species susceptibility to toxic effects as well as individual variation within species (Schlingman, 1929). The order of increasing species susceptibility to toxic effects is dogs, cats, sheep, cattle and horses (Schlingman, 1929). A wide variety of clinical signs were seen in affected animals such as coughing and choking, ataxia, loss of appetite and bloating (Schlingman, 1926; Schlingman, 1929; Ortlepp and Monnig, 1936; Southcott, 1951; Roberts, 1955; Gibson, 1956; Snow, 1973). The coughing and choking seen after dosing appear to be related to the volatility of the drug, even when given in mineral oil as a vehicle, and the sudden onset of giddiness/ataxia within a few minutes appears to be due to the rapid absorption from the small intestine (Ortlepp and Monnig, 1936). In South Africa, to help overcome some of these problems, the tetrachlorethylene and liquid paraffin mixture was reformulated into an emulsion using soft soap (Ortlepp and Monnig, 1936). To help prevent bloating in sheep, animals were starved overnight and this seemed to help decrease the incidence (Ortlepp and Monnig, 1936). However, in later trials on cattle (Gibson, 1956), it was felt that tetrachlorethylene could not be regarded as a satisfactory anthelmintic for this species because of a lack of efficacy and toxic signs and the availability then of phenothiazine as an anthelmintic.

TABLE 3.1 PERCENTAGE EFFICACY OF TETRACHLORETHYLENE AGAINST GASTROINTESTINAL NEMATODES OF SHEEP

	Abomasum			Small Intestine					Large Intestine			References
Dose	*H. contortus*	*Ostertagia* spp.	*T. axei*	*Nematodirus* spp.	*Trich.* spp.	*Cooperia* spp.	*Bunostomum* sp.	*S. papillosus*	*Oesoph.* spp.	*Chabertia ovina*	*T.ovis*	
Tetrachlorethylene (2.5-5 ml/animal)	0-100	0-40	0-22	0	44	No data			Some		0%	(Schlingman, 1926; Schlingman, 1929; Ross and Gordon, 1935; Gordon, 1935; Ortlepp and Monnig, 1936; Singer and Baker, 1940; Fritts *et al.*1958)
Pre-treatment with copper sulphate (2.5-10 ml/animal)	50-100	Some	0-100	Some	0	No data	50-100	No effect	Some	Some		(Ortlepp and Monnig, 1936; Singer and Baker, 1940; Gibson, 1955)

TABLE 3.2 PERCENTAGE EFFICACY OF TETRACHLORETHYLENE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine			References
Dose	*H. placei*	*Ostertagia* spp.	*T. axei*	*T. colubriformis*	*Nematodirus* sp.	*Cooperia* spp.	*Bunostomum* sp.	*Trichuris* sp.	*Oe. radiatum*	*Chabertia* sp.	
15-30 ml/animal (Controlled trial)		No data	15ml 0-68% 30ml 68-77%	No data	No data			No data	No data	No data	(Gibson, 1956)
Pre-treatment with sodium bicarbonate 10-15 ml/100lb[1]	10ml Highly effective			No data		No effect	No effect				(Roberts, 1955)

[1] Efficacy based on faecal egg count reduction & larval cultures

TABLE 3.3 PERCENTAGE EFFICACY OF TETRACHLORETHYLENE AGAINST GASTROINTESTINAL NEMATODES OF DOGS AND CATS

			Small Intestine						Large Intestine	References
Species		Dose	Ascarids	*T.canis*	*T.cati*	*T.leonina*	Hookworms	*A.caninum*	*T.vulpis*	
Dog	Adults	0.2 ml/kg (gelatine capsules)	100%	No data	No data	No data	100%		0%	(Schlingman, 1926)
	Puppies	0.22-0.48ml/kg						Artificial infection 39-74% 4th stage 91% immatures 94-98% adults		(Miller, 1966a)
Cat	Adults	0.25-0.5ml/kg	100%	No data	No data	No data	100%			(Schlingman, 1926; 1929)

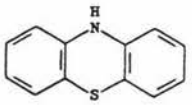
4.0 PHENOTHIAZINE

4.1 INTRODUCTION and PHARMACOLOGY

Phenothiazine has been known to chemists since 1885 (Swales, 1939). It is obtained by fusing diphenylamine with sulphur in the presence of iodine. This results in a substance that is insoluble in water, but extremely soluble in organic solvents and mineral oil (Figure 4.1) (del Castillo, 1969). It was initially used by entomologists in 1935 for the treatment of codling moths (Swales, 1939), but it was not until 1938 when its anthelmintic properties in pigs were first described (Harwood *et al.*1938). However, its insolubility in water made it difficult to formulate for easy administration. This meant both a high dose and large volume of the drug needed to be given to achieve an anthelmintic effect eg. lambs and sheep required between 15-20 grammes of phenothiazine per animal. In early trials, it was formulated to be used as an enema, or in the feed or as an effervescent tablet (Swales, 1939). As the anthelmintic's popularity increased, it has been further formulated as a wettable powder, suspension, paste, tablet (bolus) and incorporated into salt, mineral or combined salt-mineral licks.

After many overseas trials had shown its considerable advantages over the current anthelmintics, it was introduced to New Zealand in 1941(Whitten and Filmer, 1947).

Figure 4.1 Structure of Phenothiazine



To this day, the mode of action of phenothiazine is still unknown. Several trials by various authors have shown a potent anthelmintic action from a very low concentration of the drug (Taylor and Sanderson, 1940; Lazarus and Rogers, 1951; Broome, 1962). *In vitro* trials showed the drug is absorbed via both the mouth and the cuticle of nematodes, and became irreversibly bound especially in the intestine, ovary and cuticle (Lazarus and Rogers, 1950; 1951; Knapp *et al.*1959). There was debate as to whether the drug was absorbed as a saturated solution (Taylor and Sanderson, 1940; Lazarus and Rogers, 1950; Rogers, 1951) or via direct solution from the solid state into the cuticle (Douglas *et al.*1956; 1959). From pharmacological principles, the saturated solution theory is the most likely. Because parasites not susceptible to the drug (*Nippostrongylus muris*) also absorbed phenothiazine, it was proposed that the difference in toxicity to the parasite may be due to the dependence of the susceptible parasites on anaerobic energy sources which phenothiazine was inhibiting (Lazarus and Rogers, 1951). However, this has never been proved. Another conundrum found with the use of this drug was that parasites exposed to phenothiazine *in*

vivo were alive when expelled from the host, and would stay alive *in vitro* as long as untreated worms (Lazarus and Rogers, 1950). As had been shown in *in vitro* trials, these parasites would also retain the phenothiazine they had absorbed when placed in phenothiazine free medium. This further confirmed that the phenothiazine was irreversibly bound (Lazarus and Rogers, 1950). Lazarus and Rogers, (1951) showed that parasites that had absorbed phenothiazine permanently lost their egg-laying capacity and this supported the field results that showed that a single therapeutic dose in horses would inhibit faecal strongyle egg production for up to five weeks as well as inhibit faecal larval development (Gibson, 1945).

Work on the pharmacokinetics of phenothiazine was carried out by (Whitten and Filmer, 1947) and (Clare, 1947) when they were researching the cause of phenothiazine associated keratitis in calves. Their work showed that peak blood levels were reached at 24-27 hours after calves were dosed with 10-30g. Farrington *et al.*,(1962) and Farrington and Thomson, (1962) showed that most of this absorption was from the rumen rather then the abomasum, and absorption increased with decreasing particle size (Table 4.1). Work by several authors on phenothiazine particle size showed a direct relationship between surface area and anthelmintic efficacy (Douglas *et al.*1956; 1959; Gordon, 1956; Thomas and Elliott, 1957; Forsyth, 1959).

TABLE 4.1 EXCRETION OF VARYING PARTICLE SIZES OF PHENOTHIAZINE IN SHEEP FOLLOWING INTRA-RUMINAL OR INTRA-ABOMASAL INJECTION (MODIFIED FROM (Farrington *et al.*1962; Farrington and Thomson, 1962)).

Particle size (cm^2/g)	Method of administration	Faecal Excretion		Urinary Excretion		Miscellaneous excretion
		Percent Faecal Excretion	Days Post Treatment	Percent Urinary Excretion	Days Post Treatment	Percent Miscellaneous Loss
1,000	Intra-ruminal	70-72	0-3	16-22	0-1	6-9
11,500	Intra-ruminal	39-45	1-2	41-54	0-2	7-12
	Intra-abomasal	75-83	0-3	9-12	0-2	7-15
21,100	Intra-ruminal	26-37	1-2	52-63	0-2	9-11

In both calves and sheep, the ingested phenothiazine is converted into phenothiazine sulphoxide in the alimentary tract and absorbed into the portal blood system where it is converted in the liver to form leucophenothiazine ethereal sulphate. This substance is then excreted in the urine and the bile. Some unconjugated leucophenothiazine and leucothionol is also excreted in the urine along with an unknown conjugate of phenothiazine (Clare, 1947). These derivatives are initially colourless

when excreted, but are oxidised in the atmosphere to the colour-fast red-brown dyes phenothiazone and thionol (Gordon, 1939d; 1941; Clare, 1947). It was found that the nature of these excretion products varies with species, age and dose rate (Clare, 1947).

4.2 EFFICACY

4.2.1 Sheep

Swales undertook early dose titration trials in sheep to evaluate the anthelmintic effect of phenothiazine. Though worm numbers were low in the treated animals, he concluded that 0.3-0.5 grammes per pound body weight was effective against *Haemonchus contortus*, *Oesophagostomum spp.* and *Nematodirus spp.*(Swales, 1939). Since then, many other efficacy trials have been carried out in sheep and these are summarised in Table 4.2.

Initially, phenothiazine was used similarly to then current anthelmintics ie. a period of starvation before dosing and the concurrent use of copper salts to close the oesophageal groove to avoid the anthelmintic entering the rumen (Roberts, 1939). However it was found that therapeutic doses of phenothiazine worked even if swallowed into the rumen (Gordon, 1939d), and in fact it was more efficacious if animals were not starved beforehand (Roberts, 1939).

After a period of field use, several verbal reports were received in the early 1950's suggesting that phenothiazine was becoming "less efficient" for the treatment of parasitic gastritis in sheep (Gibson, 1951). It was suggested that "drug-resistance" to phenothiazine was occurring (Sinclair, 1953; Gibson, 1951). Sinclair, (1953) was unsuccessful in trying to induce resistance in a strain of *T. axei* in sheep over six parasite generations, though Drudge *et al.*,(1957a) did isolate an ovine strain of *H. contortus* in Kentucky (Kentucky strain B) which appeared to be more "tolerant" to phenothiazine then other field strains. This strain required between 1-2g/day to inhibit faecal larval development compared with 0.5g/day for other field strains (Drudge *et al.*1957a; 1957b).

Gibson, (1951) postulated that this "resistance" was most likely due to either a reduction in the dose below the therapeutic level for *Trichostrongylus* spp. or that the particle size of current formulations was different to earlier preparations. Earlier trials found that the use of unconditioned phenothiazine was no less efficacious then recrystallised phenothiazine (Habermann *et al.*1940) and this was reconfirmed during a controlled trial with commercial formulations of varying particles size (Gibson, 1951). However this was refuted when further work did show that particle size was important for efficacy (Douglas *et al.*1956; Whitten, 1956; Gordon, 1956). Fine particles (<15-20 microns) were more effective at removing abomasal and small intestinal worms, but the coarser particles (20-30

microns) removed large intestinal worms. Once the importance of this was recognised, more work was undertaken and confirmed a direct relationship between drug purity, surface area and anthelmintic efficacy (Thomas and Elliott, 1957; Kingsbury, 1958; Fritts *et al.*1958; Douglas *et al.*1959; Baker *et al.*1959; Forsyth, 1959; Forsyth *et al.*1961). It was recommended that an optimal preparation could consist of either 70% <5 microns or 90% <10 microns, and the remainder containing particles up to 30 microns (Thomas and Elliott, 1957). This was consistent with recommendations from other trial work for a minimum purity of 85% and a surface area of 12-25,000 sq. cm/g (Forsyth, 1959; Douglas *et al.*1959; Forsyth *et al.*1961). When several commercial formulations were compared against these recommendations, it was considered the variation in particle size of these formulations could possibly explain some causes of anthelmintic failure in the field (Baker *et al.*1959).

Work with both the Kentucky strain B and another "tolerant" strain identified as the "Animal Husbandry Research Division" strain concluded that some isolates of *H.contortus* from sheep differed in their response to various phenothiazine preparations, and these differences were associated largely with the purity of the phenothiazine test product (Colglazier *et al.*1967). Subsequently this "tolerance" was not thought to be a resistance in the classical sense as the refractory strains were responsive to therapeutic doses of purified phenothiazine (Colglazier *et al.*1967; Drudge *et al.*1959).

Other dosing factors were also investigated. Phenothiazine was shown to be as efficacious in removing parasites when the dose was divided over consecutive days (Gordon, 1939d). It also lowered faecal egg counts and inhibited larval development in the faeces for up to four days when used daily as a salt-lick, but animals needed to ingest a minimum of 0.5g/day (Britton *et al.*1942; Martin, 1944; Peterson *et al.*1944). There were problems due to variable acceptance of different salt lick formulations, and being unable to regulate individual animal intakes (Elliott, 1970; De Chaneet and Lewis, 1973). To obtain the maximum anthelmintic benefit from using these salt-licks, it was important that the animals were moved to pasture which had low levels of infective parasite larvae, otherwise clinical disease could still occur (Peterson *et al.*1944).

4.2.2 Cattle

There are fewer publications on the efficacy of phenothiazine in cattle than in sheep. A summary of published trial work is presented in Table 4.3. The cattle parasite species this anthelmintic is most effective against are *H. placei, O. ostertagi, T. axei* and *Oe. radiatum.*

Trial work with regular low dose phenothiazine treatment was also undertaken in cattle, but as with sheep, there was wide individual variation both in the volume and regularity of the intake such that

the 0.5g/day necessary to control faecal larval development and subsequent pasture contamination was often not ingested (Mayhew, 1959; Mayhew *et al.*1959).

4.2.3 Horses

Much of the early efficacy work in horses relied on faecal egg count reduction and pre- and post-treatment larval culture and differentiation. Few critical trials were published and these are summarised in Table 4.4. When phenothiazine was used at doses of 30-45g per animal, good efficacy was achieved against the small strongyles (Cyathostomes, *Triodontophorus* spp. and *Oesophagodontus* spp.), less against the large strongyles (*S. edentatus, S. equinus* and *S. vulgaris*) and no effect on the ascarid *Parascaris equorum* (Harwood *et al.*1940; Grahame *et al.*1942; Gibson, 1950; Poynter, 1958). The large strongyles were more susceptible when doses of over 50g/animal were used (Harwood *et al.*1940; Habermann *et al.*1941). The published FEC reduction work agreed with the findings of these critical trials. Some authors also claimed high efficacy against *T. axei* using doses of 45g (Howell and Britton, 1940). Even when the drug was incorporated at these dose rates in the horse's feed, there were no problems of animals not ingesting the full dose (Howell and Britton, 1940; Roberts, 1941; Howell and Britton, 1942).

Low dose treatment of between 1-5 grammes given daily or over several days was also successful for lowering FEC in the horse (Howell and Britton, 1942; Foster and Habermann, 1944; Gibson, 1945a; 1945b). Gibson, (1945a) was the first to show that this FEC reduction was due to inhibition of egg production by adult worms as opposed to removing the adult worms, and this inhibition of egg production would last up to 35 days. When the trial was repeated with ponies on pasture, Gibson, (1945a) could show over a twelve-month period the corresponding benefits of lowering pasture contamination with infective larvae. This resulted from both a decrease in the ponies FEC, and inhibition of larval development in faeces (Gibson, 1945b). This raised two potential concerns from these trial results (Gibson, 1945b). First that this low level dosing could potentially induce the development of resistance to this drug, and secondly that animals maintained free of nematodes might not develop any resistance to future infection with strongyle worms if they were exposed to these parasites later in life.

4.3 SIDE EFFECTS AND TOXICITY

Phenothiazine is an anthelmintic that has quite a marked difference in toxic effects between species. For those species that are more susceptible, there can be quite variable individual responses.

Sheep and goats are the most insensitive to the toxic effects of phenothiazine (Roberts, 1939; Taylor and Sanderson, 1940; Vanden-Bossche, 1985; Costa *et al.*1982). It was thought that as the

particle size became smaller and more drug was absorbed by the sheep (Farrington *et al.*1962; Farrington and Thomson, 1962), there would be an increase in toxicity (Farrington *et al.*1962). Sheep treated with a single dose up to nine times the therapeutic dose of ultrafine phenothiazine (surface area 40,000 cm^2/g) showed no ill effects (Arundel, 1962), but earlier trials (Taylor and Sanderson, 1940) did show that lambs dosed with 10g daily would show toxic signs after one week. Overseas there were sporadic reports of flock losses after therapeutic doses of phenothiazine (Hebden and Setchell, 1962; Ames and Robinson, 1965). In New Zealand, eighty-one cases of lamb losses after therapeutic dosing were reported between 1958-1962 (Salisbury, 1969). These were associated with hot dry weather and dehydration. Salisbury could replicate this by exposing lambs on pasture to periods of slow dehydration before treatment (Salisbury *et al.*1969). He showed histological evidence of renal failure, with renal papillary necrosis being seen in the acute stage, which then moved towards the medullary region of the kidney with chronicity. This suggested local tissue anoxia, most probably due to vascular stasis (Salisbury *et al.*1969). It was felt that the slight diuretic action of phenothiazine could also help accentuate this toxic effect (Salisbury *et al.*1969). However, when these toxicity figures are compared with the millions of sheep dosed over the twenty plus years plus this drug has been available, the incidence of toxic side-effects is really quite low.

One of the other side effects seen with sheep after therapeutic dosing was the staining of wool by the colourfast reddish-brown dye's phenothiazone and thionol. These are formed by the oxidation of the metabolites leucophenothiazine and leucothionol once they are excreted in the urine (Clare, 1947). To reduce this problem, it was suggested that sheep should be separated soon after dosing to limit wool staining (Gordon, 1941).

There has only been one report of phenothiazine being associated with reproductive side-effects. Some ewes produced stillborn lambs after they had been dosed in the last 10 days of gestation. This suggested there may be a period late in pregnancy when the drug may cause abnormal parturition (Warwick *et al.*1946). A trial by Blackwell and Allen, (1955) showed no detrimental effects on the fertility of ewes when they were dosed the day before breeding.

Cattle on the other hand are more susceptible to toxic side-effects from phenothiazine. Some calves dosed with 0.5-0.8g/lb body weight showed clinical signs of depression, appetite loss, ataxia, diarrhoea and in some case's death (Taylor and Sanderson, 1940; Swanson *et al.*1940; Roberts, 1941; Riek, 1951; Cauthen, 1953). At postmortem examination, there was extensive ulceration of the pyloric end of the abomasum (Taylor and Sanderson, 1940; Roberts, 1941). In other cases it was found that lower doses of 0.1-0.2/lb would still be efficacious and limit these side-effects. However it was recommended that doses should be either decreased or spread over several days in anaemic, sick or debilitated animals (Riek, 1951; Cauthen, 1953; Gibson, 1975)

(Whitten and Filmer, 1947) first reported on another side-effect in cattle with the occurrence of an associated photosensitive induced keratitis. This was first seen in 1943 in young cattle in Taranaki and occurred 36-48 hours after treatment with phenothiazine (Whitten and Filmer, 1947). Development of an animal model showed it was due to the presence of phenothiazine sulphoxide in the aqueous humour of the anterior chamber of the eye, and subsequent exposure of this chemical to bright sunlight that had a wavelength up to 360 nanometres (Whitten and Filmer, 1947; Clare *et al.*1947). The phenothiazine sulphoxide was present in the anterior chamber due to overloading of the metabolic capacity of the calf's liver (Clare *et al.*1947). Treated calves could be protected from the keratitis by providing shelter from bright sunlight on the day following treatment (Whitten and Filmer, 1947). This side-effect was also able to be produced in sheep (Clare *et al.*1947), but apart from the one other report (Gordon and Green, 1951), no other field cases in sheep have been reported.

In 1942, Taylor, (1942) and Howell and Britton, (1942) reported on several toxicities in horses after normal therapeutic doses of phenothiazine. There appeared to be no relationship between the condition, age and weight of the horse. Clinical signs such as colic, diarrhoea or constipation, jaundice, anaemia and in some case's deaths were seen (Taylor, 1942; Howell and Britton, 1942). It appears that phenothiazine may accelerate the effect of serum lysins such as lysolecithin on the normal haemolysis of horse red blood cells (Collier and Allen, 1942; Gordon, 1945). Subsequently, treated animals may show anaemia, and bronzed or yellow mucous membranes along with haemoglobinuria (Grahame *et al.*1940; Swales, 1940). As shown by several published reports, this side-effect does not occur in all horses. For example, there were no ill effects reported after giving therapeutic doses of 20-100g to several foals, weanlings, mares, mares in foal (25 in the last three months of pregnancy) and stallions (Grahame *et al.*1940; Howell and Britton, 1940; Howell and Britton, 1942; Grahame *et al.*1942; McNally, 1943; Gibson, 1945). Neither were side-effects seen in ponies kept on long term low dose therapy (1g/day for up to 12 months (Gibson, 1945) nor up to 5g three times weekly for six months (Foster and Habermann, 1944). It would appear that some animals have an exceptional sensitivity to phenothiazine that is not identifiable before dosing (Taylor, 1942). Treatment of affected animals is aimed at replenishing lost red blood cells and this may include the use of whole blood transfusions and intravenous fluids (Baird *et al.*1970).

Dogs, cats and pigs are particularly sensitive to toxicity from phenothiazine and this compound should not be used in these species (Brander *et al.*1982).

TABLE 4.2 PERCENTAGE EFFICACY OF PHENOTHIAZINE AGAINST GASTROINTESTINAL NEMATODES OF SHEEP

	Abomasum			Small Intestine					Large Intestine			References
Dose	*H. contortus*	*Ostertagia* spp.	*T.axei*	*Nematodirus* spp.	*Trich.* spp.	*Cooperia* spp.	*Bunostomum* sp.	*S. papillosus*	*Oesoph.* spp.	*Chabertia ovina*	*T.ovis*	
15-25 g /animal (0.3-0.7 g/kg)	96-100	96-100	86-98	0-100	26-98	0-99	57-90	No data	51-93	70-99	0	(Swales, 1939; Gordon, 1939d; Taylor and Sanderson, 1940; Habermann *et al.*1940; Gordon, 1941; 1956; Gibson, 1949; 1959a; 1962b; Douglas *et al.*1956; Whitten, 1956; Thomas and Elliott, 1957; Fritts *et al.* 1958; Kingsbury, 1958; Cairns, 1961; Hebden, 1961; Shelton, 1962; Arundel, 1963)

TABLE 4.3 PERCENTAGE EFFICACY OF PHENOTHIAZINE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		References
Dose	*H. placei*	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Bunostomum* sp.	*Capillaria* sp.	*S. papillosus*	*Trichuris* sp.	*Oe. radiatum*	
0.1-0.5 g/lb (0.2-1.1 g/kg)	99-100	17-100	85-100	0 (one trial)	0-49	Slight[1]	0 (one trial)	0	0	99-100	(Swales, 1940; Porter, 1941; Cauthen, 1953; Gibson, 1956; Alicata, 1960; Quinlan, 1986; Grzywinski and Kliszewski, 1983)

[1] Efficacy based on faecal egg count reduction & larval cultures

TABLE 4.4 PERCENTAGE EFFICACY OF PHENOTHIAZINE AGAINST GASTROINTESTINAL NEMATODES OF HORSES

	Stomach	Small Intestine	Large Intestine		References
Dose rate	*T. axei*	*P. equorum*	Large strongyles	Small Strongyles	
25-45 g/animal	100[1]	0	13-85	70-100	(Grahame *et al.*1942; Gibson, 1950; Poynter, 1958)
80-90 g/animal			95	100	(Harwood *et al.*1940)

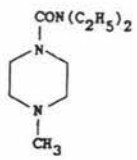
[1] Efficacy based on faecal egg count reduction and larval culture of faeces (Howell and Britton, 1940)

5.0 DIETHYLCARBAMAZINE

5.1 INTRODUCTION

Diethylcarbamazine was developed in the mid 1940's during a search for drugs to combat filariasis in troops in the endemic regions of the Pacific theatre of war (Hewitt *et al.*1947a). Early research used dogs infected with *Dirofilaria immitis* as an animal model, but these required too much of the test compounds and another animal model using *Litosomoides carinii* a filarial parasite of the cotton rat (*Sigmodon hispidus*) was developed. Using this model, more than 500 organic nonmetallic compounds including 112 piperazine compounds were tested (Hewitt *et al.*1947a). The best balance between activity and toxicity was found in the piperazine compounds. The first piperazine that showed filaricidal activity was 1-carbethoxy-4-methylpiperazine hydrochloride (Hewitt *et al.*1947a). When trialed in the dog, this compound was effective against *D. immitis* microfilariae but had less efficacy against adult worms. Side effects in the dog such as nausea, muscular weakness, profuse salivation and prostration resulted in a better tolerated compound being used. This was 1-diethyl-carbamyl-4-methylpiperazine also known as diethylcarbamazine (DEC) (Figure 5.1) (Hewitt *et al.*1947b).

Figure 5.1 Structure of Diethylcarbamazine



Trials in humans showed the same microfilaricidal effect of DEC seen in cotton rats and dogs (Hewitt *et al.*1947c; Santiago-Stevenson *et al.*1947; Hawking and Laurie, 1949). A further benefit of the piperazine compounds was that they could be administered orally. This was unlike many of the currently available treatments for parasitic disease in humans (Hewitt *et al.*1947b).

5.2 PHARMACOLOGY

Publications on the pharmacokinetics and pharmacodynamics of DEC in animals are sparse. What is published is on research completed in the cotton rat. Indirect evidence indicates that DEC is rapidly absorbed and excreted (Hewitt *et al.*1947c; Prichard, 1978; Harned *et al.*1947). Excretion is predominately renal with up to 63% of a single dose being removed within twenty-three hours (Harned *et al.*1947).

The drug DEC is thought to have more than one mode of action when used for treatment of filariae

and ascarids (Martin, 1997). After treatment of cotton rats, adult filariae were recovered alive and showed no external or internal changes (Hewitt *et al.*1947c). *In vitro* studies did not result in any gross effects seen in adult *Litosomoides carinii* placed in solutions of 100-500mg/100ml (Hawking *et al.*1950). However, microfilariae from treated rats were shown to be removed very rapidly from the circulation (ie. within minutes) and with a proportional increase in microfilariae numbers in the liver. Histopathology of the livers of treated animals showed that within six hours of treatment, phagocytes surrounded the microfilariae that had localised there (Hawking *et al.*1948; 1950). In contrast, when microfilariae were exposed to the drug either in serum from treated animals or varying concentrations of DEC, there was no lethal effect with microfilariae remaining alive for several days (Hawking *et al.*1948; 1950; Hawking and Laurie, 1949). This suggested that neither DEC nor any unidentified derivative of it in the body had a direct lethal action on either the adult worms or microfilariae (Hawking *et al.*1950).

It was proposed that DEC acts like an opsonin modifying the microfilariae so that they are attacked by the phagocytes of the reticulo-endothelial system and destroyed (Hawking *et al.*1948; 1950; Hawking and Laurie, 1949). This action may be due to alterations of arachidonic acid metabolism in microfilariae and vascular tissues (Kanesa-thasan *et al.*1991; Maizels and Denham, 1992). This may result in vasoconstriction and an increase in endothelial adhesion causing immobilisation of microfilarial parasites, enhanced adherence and cytotoxic activity by host platelets and granulocytes (Maizels and Denham, 1992). If correct, this would explain why DEC has no action *in vitro* against microfilariae and yet is effective in animals not exposed to previous infections of the parasites (Martin, 1997).

DEC's other mode of action is thought to be on the nematodes neuromuscular system. When DEC was applied to *Ascaris* neuromuscular preparations, it caused an increase in muscle tone and spontaneous activity of the preparation (Broome, 1962). However, using *in vitro* whole and vertically split worm preparations, applying DEC resulted in an initial increase in activity followed by a reversible flaccid paralysis (Natarajan *et al.*1973; Kaushik *et al.*1974). More recent electrophysiological experiments using *Ascaris suum* preparations revealed that the compound has a depolarising action. However, unlike piperazine, this is not due to activation of cholinergic or gamma amino butyric acid (GABA) receptors, but due to inhibition of the outward flow of potassium from the cells (Martin, 1982).

5.3 EFFICACY

5.3.1 Ruminants

Following a clinical report that DEC was shown to be efficacious against microfilariae of *D. immitis*

in a dog, an editorial comment proposed that the drug might also be efficacious against lungworms in cattle (Purchase, 1950). Parker, (1957) had initially expressed reservations about DEC being effective against worms in the bronchioles when the drug was present in the blood stream. However, treatment of calves during a field outbreak of verminous bronchitis caused by *Dictyocaulus viviparus* was successful in both preventing deaths and suppressing faecal lungworm excretion (Parker, 1957). These results initiated a further series of controlled and field trials (Parker and Roberts,1958; Parker *et al*, 1959; Parker and Vallely, 1960; Rubin and Tillotson, 1962; Jarrett *et al*, 1962; Cornwell, 1963; Parker,1963; Kendall, 1965). Treatment of animals at varying intervals following artificial infection showed that the parasites were most susceptible between days fourteen and seventeen post-infection. Titration of the dose regime confirmed that 10mg/lb given either orally or intramuscularly (IM) for three days, or a single treatment at 20mg/lb IM were the most efficient treatment regimes (Parker *et al.*1959; Parker and Vallely, 1960).

A concern raised was that calves treated for lungworms would not develop immunity against future infections (Parker and Roberts, 1958). Variable results were found following calves being artificially infected calves with lungworm larvae, treated with DEC and reinfected with lungworm larvae Parker et al (Parker and Vallely, 1960; Cornwell, 1963; Kendall, 1965). Cornwell, (1963) felt that treatment did not interfere with immunity in field situations. This was because by the time the worms had developed to the stage where they are susceptible to the drug, the host would have been stimulated by antigenic materials capable of causing immunity. Kendall, (1965) disagreed with the conclusions of Cornwell, (1963), as previously exposed calves in his trial did show a lower immune response associated with chemotherapeutic interference of infection. Earlier work using oral vaccination of calves with X-irradiated larvae had indicated that the developing stages could provide calves with significant immunity against moderate field challenge (Jarrett *et al.*1958; Jarrett *et al.*1959). This later trial provided further evidence that the young stages in the development of *D. viviparus* have a potent effect on the development of resistance, but showed that they are not solely involved in the development of the immune response (Kendall, 1965).

As well as aiding in the prevention of patent infection, another concern was whether treatment of cattle with DEC would result in the removal of field infections of adult worms (Jarrett *et al.*1962). DEC was highly effective against L5 or immature adult stages during the prepatent period of the disease, but doubts were raised about the beneficial efficacy of DEC against adult worms when used against heavy patent infections (Jarrett *et al.*1962).

Most trials of the efficacy of DEC against lungworms in sheep have been carried out in Eastern Europe (Kurtpinar and Kalkan, 1960; Olteanu, 1959; Umov, 1957) cited by (Parker, 1963)). It appears that unlike in cattle where the drug is more effective against the immature worms, in sheep it may be more effective against adult than larval *Dictyocaulus filaria* (Skerman *et al.*1968).

Diethylcarbamazine is also effective against *Haemonchus contortus* (Wood *et al.*1959). Combining it with morantel increased the spectrum of activity in sheep to include lungworm.

5.3.2 Horses

Overall, DEC has been shown to have limited efficacy against equine nematodes. Based on faecal egg count reductions there was limited if any effect on ascarids (Poynter, 1955a). Neither high daily doses of DEC intravenously nor daily dosing in the feed for up to 200 days prevented the development of anterior mesenteric artery lesions due to the larval stages of *Strongylus vulgaris* (Todd *et al.*1949; Hofing and Bennett, 1982). The lack of efficacy against small and large strongyles has prevented the routine use of DEC in this species (Poynter, 1955a; Todd *et al.*1949; Hofing and Bennett, 1982).

5.3.3 Dogs and Cats

In the initial screening of anti-filarial compounds in dogs infected with *D. immitis*, DEC caused up to 90-100% reduction in blood microfilariae numbers (Hewitt *et al.*1947c; Hewitt *et al.*1948). Clinical reports confirmed that DEC would cause a reduction in microfilariae, but there was no standard dosing regime for treatment (Purchase, 1950; Alley, 1950). Dose determination trials in dogs indicated that 10mg/lb three times daily for 14-65 days would result in most animals remaining negative for *D. immitis* microfilariae for six months to a year (Foley, 1950; Ziegler, 1950). McGaughey, (1952) (citing Burkhardt (1951), Lederle Laboratories, pers. comm.) recommended that where infection with this parasite is common, prophylactic administration of DEC at 10mg/lb to puppies at intervals of three months following weaning may be better for prevention of *D. immitis* infection. Once the epidemiology of heartworm disease in dogs and cats was better understood, animals were routinely maintained on prophylactic daily low dose DEC therapy. However, with the arrival of the macrocyclic lactones, prophylactic treatment is now only required once a month.

Early work showed that dogs with many heartworms prior to treatment may be at risk of death from pulmonary embolism caused by dead worms once they were killed. It was proposed that high microfilariae counts indicate high number of adult heartworms indicating that the animal may be a poor risk for DEC treatment (Hewitt *et al.*1947a). Foley confirmed this, and proposed that the disintegration of adult worms may cause anaphylactic shock (Foley, 1950). Care needs to be taken when treating animals for adult heartworms.

A summary of trials using DEC against other nematodes is given in Table 5.1. These have shown the drug to be 90% effective against ascarids in single doses or administered daily in food (Hewitt

*et al.*1948; Kanegis, 1948). Daily low-dose administration in food also interfered with the development of experimental *T. canis* and *T. leonina* infections (Wallace *et al.*1956).

5.4 SIDE EFFECTS AND TOXICITY

Diethylcarbamazine appears to have a very high therapeutic index. When used in cotton rats up to 91 times the therapeutic dose could be used before side effects were seen (Hewitt *et al.*1947b). In sheep treated with doses of 20mg/kg, Prichard, (1978) cites Kassai and Hollo, (1963) as recording abortion in heavily pregnant ewes. However, a more recent report suggests that DEC has no effect on pregnant sheep, their offspring or in rams used for breeding. When combined with morantel citrate, up to eight times the therapeutic dose could be given before clinical signs such as depression and recumbency occurred (Cornwell *et al.*1971). These signs were also seen in cattle dosed with up to 20 times the therapeutic dose rate (200mg/lb)of DEC and up to eight times the therapeutic dose rate of the morantel tartrate/DEC combination. All affected animals recovered within 24 hours (Cornwell *et al.*1971; Cornwell *et al.*1972). While limited efficacy was shown in horses, animals treated with an intravenous dose of 4-8mg/lb showed immediate but transitory hypernea, expiratory dyspnea, pronounced vertigo and muscular incoordination resulting in a complete loss of balance. Oral dosing with 25mg/lb did not result in similar side effects (Todd *et al.*1949).

The drug generally has very low toxicity though occasionally some vomiting may be seen. Dogs seem more prone to vomiting after treatment than cats (Hewitt *et al.*1948; Kanegis, 1948; Ziegler, 1950) though if administered with food this side-effect is reduced (Ziegler, 1950).

TABLE 5.1 PERCENTAGE EFFICACY OF DIETHYLCARBAMAZINE (DEC) AND DIETHYLCARBAMAZINE AND STYRYLPYRIDINIUM AGAINST GASTROINTESTINAL NEMATODES OF DOGS AND CATS

			Small Intestine				References
	Species	Dose	Ascarids	*T.canis*	*T. leonina*	Hookworms	
DEC	Dog	25mg/kg bid	100				(Hewitt *et al.*1948)
		50mg/kg sid	98.7				(Hewitt *et al.*1948)
DEC HCl[1]	Puppies	2mg/kg base equivalent in feed daily		Eliminated majority in 3-5 days			(Wallace *et al.*1956)
		5mg/kg base equivalent in feed daily		Interfered with development	Interfered with development		(Wallace *et al.*1956)
DEC & Styrylpyridinium	Dog	2.2mg/kg DEC 5mg/kg Styrylpyridinium in feed daily				93-98% FEC reduction after 1 week	(Berger *et al.*1969)
DEC HCl	Cat	25mg/kg once daily	91.5				(Kanegis, 1948)
		25mg/kg twice daily	98.5				(Kanegis, 1948)
		50mg/kg once daily	100				(Kanegis, 1948)
	Kittens	10mg/kg	95-100				(Kanegis, 1948)

[1] HCl Hydrochloride salt

6.0 PIPERAZINE

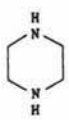
6.1 INTRODUCTION

Early work using diethylcarbamazine in animals gave good results against canine ascarid infections (Hewitt *et al.*1948). However, the high cost of the drug meant it was impractical for routine use in farm animals, and cheaper derivatives were sought (Sloan *et al.*1954). Fayard (1949) first reported on the anthelmintic activity of piperazine when he published a paper on its efficacy against ascarids in humans (cited by Leiper, (1954)). Further trials in laboratory animals (Standen, 1953; Brown *et al.*1954), dogs and cats (Sloan *et al.*1954), horses (Sloan *et al.*1954), poultry, (Leiper, 1952; Sloan *et al.*1954), and pigs (Leiper, 1954; Sloan *et al.*1954) indicated that piperazine base and several of its salts had good activity against ascarids, hookworms, small strongyles and oxyurids.

6.2 PHARMACOLOGY

Piperazine itself is deliquescent and so is not normally available as the base, but rather as the hexahydrate or as a salt (Figure 6.1). The salts most commonly used have been adipate, citrate, hydrochloride, phosphate, sulphate, and tartrate (Prichard, 1978). Early work showed that the physical nature of the hexahydrate and the nauseating taste associated with it detracted from its value in the treatment of dogs and cats (Sloan *et al.*1954). The advantages of the salts were that they were less distasteful, and they were more soluble than the piperazine base (Sloan *et al.*1954; Leiper, 1954; Davies *et al.*1954; del Castillo, 1969; Roberson, 1988). In order to be able to compare the efficacy of different salts, dosages were expressed in terms of the piperazine hydrate equivalent eg. 100mg of piperazine hydrate is equivalent to 120mg of piperazine adipate, 125mg of piperazine citrate, and 104mg of piperazine phosphate (Roberson, 1988). Along with the numerous piperazine salts, a variety of formulations such as tablets, powders, liquids and pastes were subsequently made commercially for the treatment of dogs, cats, and horses.

Figure 6.1 Structure of Piperazine



Early *in vitro* work (Goodwin and Standen, 1954; Standen, 1955; Poynter, 1955a; 1956) showed that 1:1000 dilutions of various piperazine salts induced flaccid paralysis of the worms within 5-6 hours. Worms then placed in anthelmintic free medium recovered in 2-3 hours. There was no difference in this recovery response between any of the various salts. It was concluded that the anthelmintic

action was due to paralysis of the worms, after which the normal peristaltic actions of the intestinal tract resulted in their expulsion.

Initially it was thought that the specific mechanism of action of piperazine was inhibition of the formation of succinate used for energy in the parasite. However, this was shown to be secondary to the myoneural block that caused the paralysis (Bueding *et al.*1959). Further work pointed to the myoneural block being due to hyperpolarisation of the muscle cell membrane. This hyperpolarisation was greatest at the part of the somatic muscle cell containing the nucleus ("nuclear bags") which were close to the nerve cord (del Castillo, 1969). The resting potential of cells is due to the difference in the concentration of K^+ and Cl^- ions between the cytoplasm and the extracellular fluid. Changes in the concentration of extracellular Cl^- ions markedly influenced the action of piperazine (when extracellular Cl^- was reduced, the hyperpolarising effect of piperazine was considerably decreased). It was concluded that activation of the inhibitory receptors by the drug increases the permeability of the syncytial membrane to chloride ions and the change seemed to take place exclusively at the post-synaptic inhibitory receptors (del Castillo, 1969).

More recent studies have confirmed that the piperazine-induced hyperpolarisation is from piperazine acting as a low potency gamma aminobutyric acid (GABA) agonist on the extra-synaptic GABA receptors located in the nuclear bag region of the ascarid muscle cell (Martin, 1980; 1982).

6.3 EFFICACY

6.3.1 Sheep

From the outset it was found that piperazine showed variable and generally low efficacy against gastrointestinal nematodes in ruminants. When sheep were dosed with 4g into the rumen, the drug was shown to be effective only against *Oesophagostomum* spp. (Gordon, 1955; Marquardt and Fritts, 1956). Higher doses of 10-25g piperazine salts dosed directly into the rumen or abomasum were not effective against, *Trichostrongylus* spp. or *Chabertia ovina* (Gordon, 1955). Combining piperazine with other anthelmintics such as copper sulphate, nicotine sulphate, carbon disulphide and the organophosphate haloxon, did improve efficacy against *H. contortus*, but not other trichostrongyles (Gordon, 1955; 1957; Kingsbury and Heffer, 1967). Even higher efficacy against *Haemonchus* was seen when piperazine was combined with haloxon, but this was most likely due to the effect of the haloxon (Kingsbury and Heffer, 1967).

Medicating drinking water to give an estimated intake of 380-450 mg/kg over 24-48 hours resulted in better efficacy against *Nematodirus* spp., *Bunostomum*, *Oesophagostomum* and *Chabertia*. However, *Haemonchus*, *Trichostrongylus* and *Trichuris* spp. were still not removed (Shumard and

Eveleth, 1957). Interestingly, drenching animals with the same dose as in the drinking water had no effect on *Nematodirus* or *Chabertia* and only a partial efficacy against *Bunostomum* (Shumard and Eveleth, 1957). Because of this variable response and the lack of efficacy against most of the more pathogenic parasites, this anthelmintic was never marketed for use in sheep. A summary of the efficacy of piperazine in sheep is provided in Table 6.1.

6.3.2 Cattle

In cattle, some efficacy was shown against *Ostertagia* spp. (72-81%), *Cooperia* spp. (60-97.6%), and *Oesophagostomum radiatum* (80-100%) at doses of 0.07-0.2mg/lb, but this efficacy was less for the other major parasite species (Swanson *et al.*1957; Riek and Keith, 1958a; Riek, 1958) (Table 6.2). Unlike in sheep, when piperazine-1-carbodithioic acid was given directly into the abomasum, there was increased efficacy against *H. placei* and *Cooperia* spp. (Riek and Keith, 1958a). Rather than an increase in efficacy of piperazine, this was probably due to the full dose of the drug coming in contact with the abomasal acid resulting in a larger volume of carbon disulphide being released than if the drug had been given orally, deposited into the rumen and more slowly released into the abomasum (Riek and Keith, 1958a). Subsequently as with sheep, there was little if any field use of piperazine anthelmintics in cattle.

6.3.3 Horses

Early work using faecal egg count (FEC) reduction showed that 0.1- 0.2 g/lb piperazine adipate was effective against ascarids and small strongyles even when given with food (Sloan *et al.*1954; Poynter, 1955a; 1955b). However, it had limited effect on large strongyles, especially *S.edentatus*. Efficacy against adult female *Oxyuris equi* was greater at 400mg/kg than against males and larval stages (Sloan *et al.*1954). Results of critical trials at a dose of 0.1g/lb confirmed the results against ascarids and *Oxyuris equi* adults (Downing *et al.*1955). Combining piperazine with carbodithioic acid (a precursor of carbon disulphide) was aimed at providing a combination product which would also remove bots (*Gasterophilus* larvae). Doses of 75-200mg/kg of this combination were no more effective at removing large strongyles than piperazine alone and the efficacy against bots was less than that for carbon disulphide (Drudge *et al.*1957c). The same trial also showed the limitation of basing efficacy against certain worm species from FEC reduction. The marked reduction in strongyle egg counts reflected the high level of anthelmintic activity against small strongyles, but were misleading as an index of action against the large strongyles which were less effectively removed (Drudge *et al.*1957c). Further critical trials (Drudge *et al.*1962) comparing different piperazine salts and doses of 5, 10, 20 mg piperazine base/lb showed that piperazine-carbon disulphide complex was most effective against ascarids at the 5 & 20 mg/lb (100%), but that the

other piperazine salts had variable activity against mature and immature ascarids (0-100%) and mature *Oxyuris equi* (7-100%).

To assist in the removal of the large strongyles, piperazine (88mg base/kg) was combined with levamisole hydrochloride (8mg/kg). This combination resulted in increased efficacy against *S.vulgaris* (33-97%) and *S.edentatus* (63%) as well as removing all mature and immature ascarids (Drudge *et al.*1974; Lyons *et al.*1975). But adding phenothiazine and carbon disulphide to piperazine considerably increased efficacy against the large strongyles, up to 99.5% for *S. vulgaris* and 97% for *S. edentatus* (Drudge *et al.*1966). Other combinations with fenbendazole also showed improvement in the removal of mature and immature small strongyles (Wescott *et al.*1982). A summary of the efficacy of various piperazine salts in horses is given in Table 6.3.

Resistance to the benzimidazole anthelmintics was identified as a management problem in horses soon after the introduction of this class of anthelmintic. Piperazine with its different mode of action was used in combination with various benzimidazoles to remove benzimidazole resistant small strongyles (Slocombe and Cote, 1977; Kelly *et al.*1981; Webster *et al.*1981; Griffin *et al.*1983; Barger *et al.*1985). However, this was not successful in all cases. A mebendazole and piperazine combination resulted in only a small additional decrease in the faecal egg count when compared to mebendazole alone, and it was recommended that this combination not be used in benzimidazole-resistant cases (Britt and Clarkson, 1988).

6.3.4 Dogs and Cats

As in horses, piperazine salts are highly efficacious against all ascarid species in dogs and cats, but less so against other nematodes (Table 6.4) (Sloan *et al.*1954; Mann *et al.*1955; Bradley *et al.*1956). Both species of hookworms (*Ancylostoma caninum* and *Uncinaria stenocephala*) showed a variable response to either single or successive doses ranging from 25-300mg piperazine adipate/kg, but there was no effect on whipworms in the dog (Sloan *et al.*1954; Bradley *et al.*1956; Colglazier *et al.*1959). In one trial, the higher doses resulted in vomiting and decreased efficacy (Colglazier *et al.*1959), but this response was not reported in earlier work (Sloan *et al.*1954).

Different salts and formulations of piperazine were shown to have equivalent efficacy against different stages of artificial infections of *T.canis* in puppies (English and Sprent, 1965; Jacobs, 1987). Fisher *et al.*(1993) found that treating puppies at 2, 4 and 6 weeks of age resulted in a decrease in the number of *T.canis* worms, but failed to reduce the output of eggs significantly. More parasites were removed when treated with either 200 or 400mg/kg piperazine adipate or citrate at 14 days of age (71%) compared to 21 days (86%) (English and Sprent, 1965). This indicates a need for repeat treatments of young animals to ensure complete removal of infection. The anthelmintic

has little effect on the developing larval forms so, as the adult worms are eliminated, they are quickly replaced by new egg-laying adults (Fisher *et al.*1993). In an effort to reduce the ascarid burden of new-born puppies, doses of 20mg were given daily for the duration of gestation to bitches artificially infected with *T.canis*. This was unsuccessful in preventing the intra-uterine ascarid infection of puppies (Hayes and McDaniel, 1959).

6.4 SIDE EFFECTS AND TOXICITY

The poor efficacy of piperazine salts in sheep and cattle meant that there was limited use of this anthelmintic in these species although there were few reports of toxic effects when combined with other drugs. Of 10 sheep treated with a piperazine arsenic combinations (piperazine glycolarsanilate), 3 died and analysis of the livers showed lethal levels of arsenic (Marquardt and Fritts, 1956). This combination was not used any further. Undesirable side effects such as inco-ordination were seen in sheep dosed with piperazine-nicotine sulphate and these resembled those seen after administration of copper sulphate-nicotine sulphate mixture (Gordon, 1957). Other combinations of piperazine with copper and arsenic or acetoarsenite to improve efficacy had low therapeutic indices of around 3 (Kingsbury and Heffer, 1967)

In horses, piperazine used alone appears to have very low toxicity. The only signs seen in animals treated with five times the recommended dose (1200-1500 mg/kg) was a slightly laxative effect (Downing *et al.*1955). When three times the therapeutic dose of the levamisole/piperazine product was given there was mild hyperexcitation. Further doubling this to six times the recommended dose resulted in severe side effects in one mare resulting in death 11 days later and two other treated horses showed cardiac failure, pulmonary oedema and hepatic changes on post-mortem (Drudge *et al.*1974). These were the result of the toxic effects of levamisole.

Trials in cats and dogs dosed with 100mg/kg daily for 10 days did not result in any side-effects, even in pregnant animals (Mann *et al.*1955). Other trials have shown that dogs are susceptible to dose-dependent vomiting following treatment. In work on the effectiveness of piperazine citrate against ascarids, it was found that a dose of 0.2 g/lb liveweight resulted in vomiting in 39% of animals. Decreasing this dose to 0.17 g/lb in puppies under 10lb decreased the prevalence of vomiting from 39% to 1.5% (Greenberg *et al.*1958). Doses of 75-100mg/kg piperazine citrate or sulphate in dogs after an 18-36 hour fast caused vomiting within an hour if the drug was given in a gelatin capsule, but this was delayed for 3-4 hours if the drug was given in food (Colglazier *et al.*1959). There were no other side-effects such as nausea, depression or inappetance. Kittens are more tolerant of higher doses of piperazine citrate than puppies (Greenberg *et al.*1958).

In the late 1970's and mid 1980's there were reports of toxicity in kittens and puppies associated

with accidental overdosing. The most common clinical signs were neurological and gastrointestinal. Animals showed ataxia, hindlimb weakness, vomiting and diarrhoea. Most animals recovered within 48 hours of the overdose (Stoffman and Braithwaite, 1976; Hartigan and McGilligan, 1976; Goddard and Johnston, 1986; Woolliscroft, 1987; Bownass, 1987; Darke, 1987). The only animal not to recover had severe and recurring epilepsy, and on post-mortem was found to have pituitary hypoplasia. This may have caused the unusual clinical signs (Hartigan and McGilligan, 1976).

TABLE 6.1 PERCENTAGE EFFICACY OF PIPERAZINE AGAINST GASTROINTESTINAL NEMATODES OF SHEEP

		Abomasum			Small Intestine			Large Intestine			References
Piperazine Salt	Piperazine dose	*H.contortus*	*Ostertagia* spp.	*T.axei*	*Nematodirus* spp.	*Trichostrongylus* spp.	*Bunostomum*	*Oesoph.* spp.	*Chabertia ovina*	*T.ovis*	
adipate	33-40 mg/kg							27-97			(Kingsbury and Heffer, 1967)
citrate	450 mg/kg	0			0-28		25-100			0	(Shumard and Eveleth, 1957)
	450 mg/kg Drinking water (24 - 48 hours)	0-50	1	0	0-100	0 - 27	0-100	100	0-100	0	(Shumard and Eveleth, 1957)
adipate & Copper/Arsenic	40 mg/kg base							58-100			(Kingsbury and Heffer, 1967)
adipate & Copper/Antimony	55 mg/kg							76			(Kingsbury and Heffer, 1967)
adipate & Haloxon	62.5-80 mg/kg							93-100			(Kingsbury and Heffer, 1967)

TABLE 6.2 PERCENTAGE EFFICACY OF PIPERAZINE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

		Abomasum			Small Intestine			Large Intestine		References
Piperazine salt	Dose	*H. contortus*	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Bunostomum phlebotomum*	*Trichuris discolor*	*Oe. radiatum*	
citrate	0.07-0.14 g/lb	8-15	72-81	2-16	56	76-98	12-34	0-12	100	(Swanson *et al.*1957)
hydrate[1]	0.03-0.2 g/lb	oral <60		<60		oral <60	<60		80-100	(Riek and Keith, 1958a)
		intra-abomasal 80-100		<60		intra-abomasal 80-100	<60		80-100	(Riek and Keith, 1958a)
Piperazine & Carbon disulphide	0.1-0.2 g/lb	oral <60		<60		<60	<60		80-100	(Riek and Keith, 1958a)
		intra-abomasal 80-100		<60		<60	<60		80-100	(Riek and Keith, 1958a)

[1] Based on FEC reduction and larval differentiation

TABLE 6.3 PERCENTAGE EFFICACY OF PIPERAZINE AGAINST GASTROINTESTINAL NEMATODES OF HORSES

					Large Strongyles			Small Strongyles					References
Piperazine salt	Dose	*T.axei*	*Parascaris equorum*	*Parascaris equorum* immature	*S. edentatus*	*S. equinus*	*S. vulgaris*	Small strongyles	*"Trichonema"* adults (Cyathostomes)	*"Triodontophorus"* adults (Cyathostomes)	*O.equi* Adults	*O.equi* Immature	
adipate	220 mg/kg		100	100	0	0	50		89-97	55-76	80	poor	(Downing *et al.*1955; Gibson, 1957)
adipate	22 mg base/kg		0-100	70							25-100		(Drudge *et al.*1962)
citrate	10 mg base/lb		52-89	100							7-21% (2 horses)		(Drudge *et al.*1962)
	20 mg base/lb		100	100							48 (1 horse)		(Drudge *et al.*1962)
Piperazine & Carbon disulfide	75-200 mg/kg	0	100	100	0-38 (200mg dose)		36-45	89-98			64-100 (3 horses)		(Drudge *et al.*1957c)
Piperazine base & Levamisole HCl	88 mg/kg 8 mg/kg		100	100	63		33-97	97			70-76	33	(Drudge *et al.*1974; Lyons *et al.*1975b)
Piperazine base & Levamisole HCl	alfalfa pellet		100	100	90		97	98			17	76	(Lyons *et al.*1975b)

TABLE 6.4 PERCENTAGE EFFICACY OF PIPERAZINE AGAINST GASTROINTESTINAL NEMATODES OF DOGS AND CATS

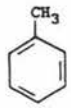
				Small Intestine					Large Intestine	References
Piperazine salt	**Species**	**Dose**	**Ascarids**	*T.canis*	*T. leonina*	Hookworms	*Ancylostoma caninum*	*Uncinaria stenocephala*	*T.vulpis*	
citrate	**Dog**	**80mg/lb (gelatine capsule)**	100			50			0	(Bradley *et al.*1956)
citrate or sulphate	**Dog**	**75-100 mg/kg**					41	86-100%		(Colglazier *et al.* 1959)
adipate	**Puppies**	**100mg/kg**		30-38 (immature)	36-73 (immature)				0 (adult & immature)	(Fisher *et al.*1993)
adipate	**Puppies (14-21 days)**	**200-400 mg/kg**		71 - 86						(English and Sprent, 1965)
citrate	**Puppies**	**220 mg/kg**		17-100						(Robinson *et al.* 1976)
citrate	**Puppies (21 days)**	**400 mg/kg**		81						(English and Sprent, 1965)
Piperazine base	**Puppies (10-49 days)**	**100 mg base/kg**		83-97						(Jacobs, 1987)
citrate	**Cat**	**100 mg/kg (for 10 days)**	96				18			(Mann *et al.*1955)

7.0 TOLUENE

7.1 INTRODUCTION AND PHARMACOLOGY

Toluene, also called methyl benzene, is an aromatic hydrocarbon which is poorly soluble in water (Figure 7.1) (The Merck Index, 1989). It is unclear how toluene exerts its anthelmintic effect, but it has characteristics which suggest a couple of possibilities. It is markedly irritant, and also has a pronounced depressant effect on tissue cells. Either of these two actions in other anthelmintics is known to be detrimental to the survival of a nematode in the gastrointestinal tract and to subsequently promote the parasite's death and/or expulsion (Jones, 1956).

Figure 7.1 Structure of Toluene



7.2 EFFICACY

The first recorded use of toluene in animals was by (Hall and Wigdor, 1926) when they evaluated a number of hydrocarbons in dogs. The results from that trial did not show that toluene had a significant advantage over the current anthelmintics chenopodium and santonin (Hall and Wigdor, 1926), and subsequently no further reports were published 1947, when toluene and a number of halogenated toluene derivatives were evaluated by critical test in the dog (Enzie, 1947). The results of these trials are shown in Table 7.1. It was found that giving the toluene in gelatine capsules after starving animals for 18-24 hours gave the best results (Enzie, 1947; Enzie and Colglazier, 1953; Miller, 1966b; Jordan and Freeny, 1974). Dichlorophen was combined with toluene to provide a product with efficacy against both nematodes and cestodes (Miller, 1966b; Sharp *et al.*1973).

Todd and Brown, (1952) published the first critical test on the efficacy of toluene in horses in 1952 (Table 7.2). Doses of 0.2 ml/lb given by stomach tube showed very good efficacy (99-100%) against mature and immature ascarids (*Parascaris equorum*), but no appreciable effect was seen against strongyles (Todd and Brown, 1952; Sinclair and Enzie, 1953; Smith, 1955). There was also some anthelmintic effect on bots, but this varied with the species, *Gasterophilus intestinalis* was more susceptible than *G. nasalis*. The advantages of toluene compared to carbon disulphide, the then most common equine anthelmintic, was that no starvation was needed before treatment and toluene was also effective against immature ascarids. It is unclear why in horses the presence of food has no effect on toluene's efficacy compared with the decrease in efficacy in unstarved dogs (Enzie,

1947; Enzie and Colglazier, 1953; Miller, 1966b; Sharp *et al.*1973), cats (Enzie and Colglazier, 1953; Hass and Collins, 1975) and cattle (Riek and Keith, 1957). Possibly the use of a stomach tube in horses allows a bolus of concentrated toluene to come in direct contact with the parasites in the gastrointestinal tract.

There is only one major trial published on the use of toluene in cattle, and its anthelmintic efficacy was based on faecal egg count reduction, faecal culture and larval differentiation (Riek and Keith, 1957). Efficacy was improved if the anthelmintic entered the abomasum directly and as in cats and dogs, better efficacy was obtained by starving animals overnight. Good results were found against *H.placei, O.ostertagi, T.axei, Cooperia spp.* and *B.phlebotomum*, but there was no effect on *Oesophagostomum radiatum* (Riek and Keith, 1957).

7.3 SIDE EFFECTS AND TOXICITY

Toluene is regarded as a relatively safe anthelmintic and is well tolerated in domestic animals (Hall and Wigdor, 1926). However aromatic hydrocarbons can have an anaesthetic action on the brain if inhaled or absorbed in high concentrations (Hall and Wigdor, 1926). Clinical signs of this were seen infrequently as ataxia, depression and occasionally collapse in treated horses, calves, dogs and cats, and tended to be more common in young animals or those in poor condition (Todd and Brown, 1952; Enzie and Colglazier, 1953; Smith, 1955; Riek and Keith, 1957; Miller, 1966a). Occasionally cats and dogs would vomit 30-60 minutes after treatment even if they had been starved overnight (Enzie and Colglazier, 1953; Jordan and Freeny, 1974). This was most likely due to a dissolution of the gelatine capsule and release of the toluene which then had an irritant effect on the gastric mucosa. However, affected animals did not show any other clinical signs such as nausea or inappetence (Enzie and Colglazier, 1953).

Repeated exposure to toluene is reputed to be toxic to bone marrow, but no effect was seen in the haematopoietic system of a horse over the week following a single treatment of 0.1ml/lb, or in the haematocrit of a dog dosed with 0.1ml/lb daily for five days (Sinclair and Enzie, 1953; Enzie and Colglazier, 1953). Responses to chronic treatment or high doses vary within and between species. For example, one horse dosed chronically with 10 ml toluene daily for two weeks then increased to 20ml/day for one week, became lethargic but recovered once treatment stopped; whereas a second horse showed no ill effects after 20ml daily for 10 days followed by 40ml/day for two days (Sinclair and Enzie, 1953). In a calf, a dose of 0.4ml/lb after 24 hours starvation caused a short period of collapse for a couple of hours, but there was no evidence of hepatic pathology on liver biopsies, whereas another calf showed no side-effects after 0.3ml/lb was injected into the abomasum (Riek and Keith, 1957). Treatment of seven pregnant mares with 200ml of toluene resulted in one mare foaling within four hours of treatment, but no other adverse effects were seen in the other mares that could be directly attributed to the toluene (Todd and Brown, 1952).

TABLE 7.1 PERCENTAGE EFFICACY OF TOLUENE AGAINST GASTROINTESTINAL NEMATODES OF DOGS AND CATS

Species	Dose	Ascarids	*T.canis*	Hookworms	*A.caninum*	*A.tubaeforme*	Whipworms *T.vulpis*	References
Dog	0.1ml/kg toluene	14-97		87			39	(Hall and Wigdor, 1926; Enzie and Colglazier, 1953)
	0.2ml/kg toluene (18-24 hr starve)	100		100			85	(Enzie, 1947)
	264mg/kg toluene & 220mg/kg dichlorophen		93		96			(Sharp *et al.*1973)
Cat	0.1-0.22 mg/kg (18-24 hr starve)	93-99		89-100		61		(Enzie and Colglazier, 1953; Hass and Collins, 1975)
	264mg/kg toluene & 220mg/kg dichlorophen	92				91		(Hass and Collins, 1975)

TABLE 7.2 PERCENTAGE EFFICACY OF TOLUENE AGAINST GASTROINTESTINAL NEMATODES OF HORSES

	Small Intestine	Large Intestine		References
Dose	*Parascaris equorum*	Large Strongyles	Small strongyles	
0.1ml/lb	47.5	No effect	No effect	(Todd and Brown, 1952)
0.2ml/lb	99.8-100	No effect	No effect	(Todd and Brown, 1952; Sinclair and Enzie, 1953)

8.0 CYANACETHYDRAZIDE

8.1 INTRODUCTION

In the search for an effective treatment for lungworm disease in livestock, many different drugs have been tried (Rose, 1973). Walley (1957c) screened more than 3000 compounds using a guinea pig model of lungworm infection. Using this model, it was possible to establish infections with the three major species of lungworms affecting livestock, *Dictyocaulus viviparus*, *Dictyocaulus filaria* and *Metastrongylus apri*. Compounds which showed evidence of anthelmintic activity were then further tested in cattle, sheep, goats and pigs. From this work, it was discovered that the compound cyanacethydrazide had activity against lungworms (Walley, 1957c).

8.2 PHARMACOLOGY

Cyanacethydrazide is relatively soluble in water, but requires a stabiliser to keep it in solution. One of the practical difficulties of field use was that the solutions needed to be made up immediately prior to use (Walley, 1960). Cyanacethadrazide is efficacious against adult lungworm when used orally, subcutaneously or intramuscularly (Walley, 1957a; 1957c). The recommended dose rate for parenteral treatment is 15mg/kg up to a maximum dose of 5g for cattle, 1g for sheep, goats and pigs. For oral administration, a dose of 17.5mg/kg is required up to the same maximum doses identified for parenteral administration (Walley, 1957c). The mode of action of cyanacethydrazide on lungworms has never been published, though it has been noted that the drug does not kill the adult lungworms *in vivo* (Walley, 1957a).

8.3 EFFICACY

The ideal anthelmintic for use in a lungworm infection would be active against migrating larvae and both mature and immature worms (Walley, 1960). With cyanacethydrazide, it was found that the drug was only effective against adult worms, not larvae (Walley, 1957c; Rubin, 1959; Swanson *et al.*1959; Rubin and Tillotson, 1960). Only those worms living in the air passages such as *Dictyocaulus viviparus* and *Protostrongylus rufescens* were affected (Walley, 1957c). Worms were not killed *in situ*, but somehow become dislodged and removed, possibly because of some damage to them so that they were unable to resist the muco-ciliary flow in the airways, and are carried up the trachea and swallowed (Walley, 1957a). Following removal of the adult worms, larvae disappear from the faeces by the fourth day after treatment (Walley, 1957a). This may assist in decreasing pasture contamination but, as pointed out by (Swanson *et al.*1959), lungworm larvae are being ingested continuously so that worms are likely to be in various stages of development when the host

is treated and removing only adults reduces the value of the treatment (Swanson *et al.*1959). Because of this and the development of anthelmintics with a wider spectrum of activity, cyanacethydrazide was only used for a short time as a commercial lungworm treatment.

8.3.1 Sheep & Goats

In sheep and goats, cyanacethydrazide is effective against *Dictyocaulus filaria* (Walley, 1957a; Dorrington, 1958; O'Donoghue, 1958; Vodrazka *et al.*1960; Gibbs and Pullin, 1960; Skerman *et al.*1968; del Castillo, 1969; Rose, 1973) a. but there have been conflicting results over its efficacy against *Protostrongylus rufescens* (Walley, 1957a; Vodrazka *et al.*1960). The first trials using doses of 15-25mg/kg for three consecutive days resulted in the removal of most *D. filaria* in the lungs within 24 hours (Walley, 1957a). A series of critical trials in Eastern Europe helped confirm that the drug had some efficacy against *D. filaria,* (Table 8.1) (Vodrazka *et al.*1960). No efficacy was seen against lungworm species located within the lung tissue such as *Muellerius capillaris* (Walley, 1957a; 1957b; 1957c; Gibbs and Pullin, 1960; Rose, 1973).

8.3.2 Cattle

A level of efficacy similar to sheep and goats was seen in calves treated with three daily doses of 15 mg/kg (Walley, 1957a). There was, however, some dispute about whether this drug was as efficacious as claimed (Baxter, 1957; Rubin, 1959; Swanson *et al.*1959; Rubin and Tillotson, 1960; 1962). Field trials using a treatment regime of 26 mg/kg once a week on either two or three occasions failed to show any clinical distinction between treated and control animals. Both lost their cough and depressed appearance with some general improvement in condition (Baxter, 1957). A more robust series of trials using an artificial infection with a dose regime of 16mg/kg for three days also failed to show any clinical benefit (Swanson *et al.*1959). However, animals in this trial were being treated while larvae were still immature and migrating, and it had been shown that the drug does not work on immature cattle *Dictyocaulus* (Walley, 1957c). Further work confirmed that unlike diethylcarbamazine which is most efficacious on days 14-16 after ingestion of infective larvae, cyanacethydrazide was ineffective when givev on these two days. Efficacy increased to 79% on days 21-22 post-ingestion, and increased to 86% 5-6 days after the infection became patent (Rubin and Tillotson, 1960).

Other work supported the use of the drug in the field. When used monthly as a prophylactic treatment, calves still developed immunity against lungworm and the regular treatment limited pasture contamination (Harrow, 1959; Walley, 1960). It was proposed that because of the spectrum of activity against only adult and immature adult worms, the drug was of definite but limited value

in the treatment of bovine verminous pneumonia (Rubin and Tillotson, 1960), but possibly better control of lungworm infection would be by biologic eg. vaccination, rather than chemical means (Rubin and Tillotson, 1962).

8.4 SIDE EFFECTS AND TOXICITY

Cattle, sheep and goats have been treated with up to 30 subcutaneous injections of a concentrated solution (25% w/v cyanacethydrazide) resulting in only some slight and temporary irritation at the injection site. Any reaction normally subsided within 20 seconds, though in some cases it lasted up to a minute. Following subcutaneous or intramuscular injection, 15-30% of cattle showed a short lived increase in lacrimation and watery nasal discharge (Walley, 1957b).

However, the drug appears to have a narrow safety margin as when animals were given twice the therapeutic dose, some animals (30% after an oral dose, 60% after a parenteral dose) showed symptoms ranging from depression and inappetance, through to isolated mild convulsions five hours after receiving a subcutaneous injection. At doses of 40-50mg/kg subcutaneously, treated animals developed fatal convulsions. The clinical signs appeared very similar to strychnine poisoning with death from asphyxia due to respiratory embarrassment. The most effective antidote is vitamin B6 (pyridoxine hydrochloride) when given at the same dose rate as the cyanacethydrazide (Walley, 1957b).

Other trials have shown that therapeutic doses of the drug had no effect on pregnant animals and results in only a slight drop in milk production when given to lactating dairy cattle (Walley, 1957a; 1957b; Groves, 1958).

TABLE 8.1 EFFICACY OF CYANACETHYDRAZIDE AGAINST PULMONARY NEMTAODES IN SHEEP

Dose of 10% solution by subcutaneous injection	Cumulative percentage of adult and immature adult *D. filaria* removed over time following the first treatment		Total percentage effectiveness	Percentage of animals completely cleared of adult and immature adult *D. filaria*
	24 hours	48 hours		
15mg/kg single treatment	56%	73%	80%	60%
15 mg/kg for 3 successive daily treatments	49%	78%	94%	51%
30 mg/kg, single treatment	65%	76%	77%	13%
40 mg/kg single treatment	88%	98%	98%	78%

9.0 BEPHENIUM and THENIUM

9.1 INTRODUCTION AND PHARMACOLOGY

Information on the bephenium family of compounds was first published in 1958 by (Copp *et al.*1958). They are quaternary ammonium compounds which are weakly soluble in water and poorly absorbed from the gut (Broome, 1962; del Castillo, 1969; Vanden-Bossche, 1985). The solubility of the drug appears to affect its anthelmintic efficacy which is optimal with the hydroxynaphthoate salt (Broome, 1962). In both *in vivo* and *in vitro* trials in dogs and cats, bephenium was shown to have a very rapid mode of action (Burrows, 1958). *Ancylostoma caninum* died within 20-30 minutes, but *Toxocara cati* was more resistant taking between 1-7 hours (Burrows, 1958). Further work on the drug's mode of action showed it caused a contraction of nematode muscle similar to that caused by acetylcholine but at a fifth of the concentration (Broome, 1962). This action was able to be blocked by both piperazine and d-tubocurarine (Broome, 1962). As seen in Figure 9.1, the drug is structurally similar to acetylcholine (Broome, 1962).

Figure 9.1 A comparison of the structural similarity between Bephenium and Acetylcholine

Acetylcholine Bephenium

9.2 EFFICACY, SIDE EFFECTS AND TOXICITY

Early efficacy work by Copp *et al., (*1958) showed that bephenium was highly effective against *Nematodirus spp.*, *H.contortus*, *T.axei*, *Cooperia curticei* and *Ostertagia spp.* in the sheep, *A.caninum* in the dog and cat, with lower activity against *U.stenocephala, T.canis* and *T.cati.*

9.2.1 Sheep

The embonate and hydroxynaphthoate salts were the two compared in field trials. A number of authors showed that in sheep 250mg/kg bephenium hydroxynaphthoate was as efficacious against the adult and larval stages of *Nematodirus spp.* as the embonate salt, but was more active against *Ostertagia spp.* and slightly more active for *H. contortus* and *T. axei* (Table 9.1) (Rawes and Scarnell, 1958; 1959; Gibson, 1959b; 1960; Banks and Korthals, 1960; Dunsmore, 1960).

Increasing the dose to 500 mg/kg and injecting it into the rumen was highly effective against *H.* contortus, *T. axei*, and Oe. *columbianum* as was 500mg/kg injected into the abomasum to remove *T. colubriformis* (Gordon, 1958). Bephenium hydroxynaphthoate was more efficacious against *Ostertagia spp* (mature and immature) and *T.axei* when given into the abomasum than into the rumen (Dunsmore, 1960). No toxic side effects were seen in sheep after single doses of up to 2000mg/kg (Rawes and Scarnell, 1959).

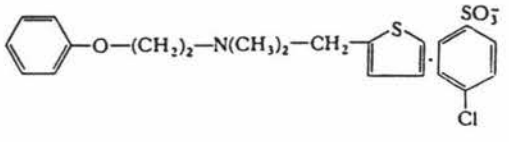
9.2.2 Cattle

Work in cattle (Armour and Hart, 1960; Eisa and Rubin, 1961; Ames *et al.*1963) using critical and controlled trials showed that 225-250mg/kg bephenium hydroxynaphthoate would kill a number of gastro-intestinal parasites as shown in Table 9.2. At therapeutic dose levels, there were no side effects seen either macro- or microscopically (Armour and Hart, 1960; Eisa and Rubin, 1961).

9.2.2 Dogs and Cats

In dogs and cats, Burrows, (1958) found that various bephenium salts (chloride, bromide, iodide, hydroxynaphthoate, and combined chloride and hydroxynaphoate) would remove up to 97% of hookworms at doses of 11.5mg of base/kg, but dose rates up to 50mg of base/kg had limited effect on lumen-dwelling parasites such as *T.canis* and *T.cati.* Rawes, (1961) evaluated a number of doses and dose regimes. His results disagreed with Burrows' recommended dose rate of 11.5mg base/kg, and found that two doses of 50mg base/kg of bephenium hydroxynaphthoate twelve hours apart was the most effective against *A.caninum* and *U.stenocephala*. One of the major side-effects from the use of bephenium were the large number of dogs (up to 40%) which would vomit after dosing, even with doses as low as 10mg base/kg (Burrows, 1958; Rawes, 1961; Brown, 1962a; Burrows and Lillis, 1962). It was clear that another drug was needed with the same level of efficacy, but caused less vomition.

Figure 9.2 Structure of Thenium Closylate



Another related compound by the name of thenium fitted these criteria Figure 9.2 (Burrows *et al.*1960). This anthelmintic was highly effective against natural and artificial infections of *A.caninum* in the dog and cat, and *U.stenocephala* in the dog, when given either a total single dose of 250mg

thenium base, or 25mg base/kg twice daily or once a day for two days (Table 9.3) (Rawes and Clapham, 1961; Burrows and Lillis, 1962; Brown, 1962b). It was more effective then bephenium against *T.canis* and *T.leonina,* but less against some of the gastrointestinal trichostrongyles in sheep (Burrows *et al.*1960). At therapeutic doses in dogs and cats, it caused less emesis then bephenium (Burrows *et al.*1960; Rawes and Clapham, 1961; Burrows and Lillis, 1962). Thenium was also been combined with piperazine salts in order to improve its efficacy against *Toxocara spp.*, and doses of 250mg thenium and 500mg piperazine were found to be the most efficacious (Rawes and Clapham, 1962; Corwin and Miller, 1978).

Besides emesis in dogs and cats, a report by (Rettig, 1981) indicated that Airedales and collies may be more susceptible to thenium toxicosis. Some members of these two breeds when treated with thenium closylate would present with a flaccid paralysis. This was thought to be due to the thenium competitively binding at the neuromuscular junction of both smooth and skeletal muscle (Rettig, 1981).

TABLE 9.1 PERCENTAGE EFFICACY OF BEPHENIUM SALTS AGAINST GASTROINTESTINAL NEMATODES OF SHEEP

	Abomasum			Small Intestine			Large Intestine			References
Dose	*H. contortus*	*Ostertagia* spp.	*T. axei*	*Nematodirus* spp.	*Trich.* spp.	*Cooperia* spp.	*Oesoph.* spp.	*Chabertia ovina*	*T.ovis*	
250 mg/kg hydroxynaphthoate	96	75-98	74-88	99-100	32-55	97				(Rawes and Scarnell, 1959; Gibson, 1959b; 1960; Banks and Korthals, 1960; Dunsmore, 1960)
500mg/kg hydroxynaphthoate	Highly effective (Intra-ruminal injection)		Highly effective (Intra-ruminal injection)		Effective (*T.colubriformis*) (Intra-abomasal injection)		Highly effective (Intra-ruminal injection)			(Gordon, 1958)
250 mg/kg embonate	70	0-98	0-77	98	30-40	99				(Rawes and Scarnell, 1959; Gibson, 1959b; 1960; Dunsmore, 1960)

TABLE 9.2 PERCENTAGE EFFICACY OF BEPHENIUM HYDROXYNAPHTHOATE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine			References
Dose	*H. placei*	*Ostertagia* spp.	*T. axei*	*T. colubriformis*	*Nematodirus* spp.	*Cooperia* spp.	*Bunostomum* spp.	*Trichuris* spp.	*Oe. radiatum*	*Chabertia ovina*	
225-250 mg/kg	81-100%	27-85	59-88	No data	100	89-100	67-90	No data	99-100	100	(Armour and Hart, 1960; Eisa and Rubin, 1961; Ames *et al.*1963)

TABLE 9.3 PERCENTAGE EFFICACY OF BEPHENIUM AND THENIUM AGAINST GASTROINTESTINAL NEMATODES OF DOGS AND CATS

			Small Intestine					Large Intestine	References
Anthelmintic	Species	Dose	*T.canis*	*T.cati*	*T.leonina*	*A.caninum*	*U. stenocephala*	*T.vulpis*	
Bephenium	Dog	25-50 mg base/kg bid[1]	11-27		100[2]	65-75	9-97	0	(Burrows and Lillis, 1962)
	Cat	25-50mg base/kg bid		50-56		94-97			(Burrows and Lillis, 1962)
Thenium	Dog	25-50 mg base/kg bid	92-100		100[1]	91-92	93-100		(Burrows and Lillis, 1962)
	Cat	25-50 mg base/kg bid		46-75		96-100			(Burrows and Lillis, 1962)
Thenium & Piperazine	Dog	250 mg thenium & 500 mg piperazine bid	94-98			96			(Rawes and Clapham, 1962; Corwin and Miller, 1978)

[1] bid Dosed twice daily

[2] For 50 mg/kg bid dose only

10.0 ORGANOPHOSPHATES

10.1 INTRODUCTION

Organophosphates (OP's) have been used as insecticides since the late 1930's (Prichard, 1978). In the 1950's, there was an increased interest in their use because of the development of resistance in arthropod parasites to the chlorinated hydrocarbons (Radeleff and Woodard, 1957). At that time, it was postulated that these compounds may have efficacy against internal parasites and possibly this may be better than that currently obtained with phenothiazine. Nematode screening tests showed that several OP's had efficacy against equine strongyle larvae (Levine *et al.*1956). However, the problem was that some earlier OP's such as parathion were too toxic for warm-blooded animals to be considered for internal administration (Levine *et al.*1956). There was a need for OP anthelmintics that were effective at low concentrations. Further screening against horse strongyle larvae *in vitro* identified sixty nine OP's that were active at a concentration below 0.01M (Levine *et al.*1958). At the same time, (Herlich and Johnson, 1957) reported on a critical trial evaluating the *in vivo* anthelmintic efficacy of orally administered Dow ET-57. This work was followed up with several faecal egg count reduction tests and critical trials confirming the efficacy of OP's against internal nematodes of sheep and cattle (Herlich and Porter, 1958; Riek and Keith, 1958b; Gordon, 1958a; Schad *et al.*1958). This paved the way to develop this group of compounds for the control of parasitic nematodes in sheep & cattle. The major problems were the relatively high dose levels needed to give a broad anthelmintic spectrum, and the drugs narrow therapeutic index (Hebden and Hall, 1965). Further research led to the development of OP's such as Haloxon and Naphthalphos requiring lower therapeutic doses and with fewer toxic side effects (Hebden and Hall, 1965; Schrader, 1965; Douglas and Baker, 1968).

10.2 PHARMACOLOGY

The OP's are very poorly soluble in water but are quite lipid soluble. The more lipid soluble agents are well absorbed orally and percutaneously. After absorption, most are excreted almost entirely as hydrolysis products in the urine, with plasma and tissue enzymes being responsible for hydrolysis to the corresponding phosphoric and phosphonic acids (Taylor, 1992b).

It was well known that the principal pharmacological action of the organophosphates in mammals was due to their binding and subsequent formation of a stable phosphorylated bond with acetylcholinesterase. This resulted in inhibition of the enzyme. However, there was no evidence that this was the mechanism of action in nematode parasites. Using histochemical techniques, which were later confirmed by others (Knowles and Casida, 1966; Hutchinson and Probert, 1972), Lee

(1962) identified the presence and location of esterase enzymes in *Ascaris* spp. Many of these enzymes were found in the innervation processes and sheaths of muscles and the reproductive organs. Using *H. contortus* as a model, Lee and Hodsden(1963) showed in this parasite that there was no evidence for the existence of more than one cholinesterase. The subsequent reaction of an OP (haloxon) with this cholinesterase was bimolecular, and reversed very slowly if at all. This was unlike the reversible reaction seen with sheep erythrocyte cholinesterase. This helped explain the In the same experiment, when *Trichuris ovis* were exposed to the same OP, the inhibitory effect was considerably less. This suggested that the efficacy of OP anthelmintics on different parasite species may be due to the sensitivity of the various parasite's cholinesterase enzymes (Lee and Hodsden, 1963; Hart and Lee, 1966).

By inhibiting the enzyme responsible for the breakdown of the neurotransmitter acetylcholine there is an accumulation of acetylcholine at the nerve endings. The effect of this is flaccid paralysis of the parasite (Rew, 1978). As intestinal nematodes must be able to maintain an appropriate feeding site and actively ingest and move food through their intestinal tract, any effect on proper neuromuscular coordination will result in them being paralysed and removed from the gastrointestinal tract (Rew, 1978). This occurs quite rapidly, most worms are eliminated within 24-36 hours after treatment.

The OP's have been used in a variety of oral and parenteral formulations and routes of administration among the different species. These include oral drenches, gels, pastes, boluses, pellets, powder and in feed premixes plus subcutaneous or intra-ruminal injection and topical pour-on formulations. As alluded to previously, several of this group of compounds have a narrow therapeutic index. Some formulations have been designed to take this into account. For example, dichlorvos can be quite toxic when used in liquid form and so it has been included as a plasticiser in vinyl resin pellets. This allows control of the rate of release and subsequent duration of exposure which increases efficacy and assists in reducing toxicity (Prichard, 1978; Barragry, 1984).

10.3 EFFICACY

The narrowness of the therapeutic index and subsequent development about the same time this class was discovered of safer and broader spectrum anthelmintics such as the benzimidazoles resulted in few OP's being developed for therapeutic use. Some are still used, but for specific efficacy against certain parasites not removed by some of the broad spectrum anthelmintics .

10.3.1 Sheep

The earliest OP anthelmintics used in sheep were Trolene (Dowco ET 57, Ronnel) (Figure 10.1),

Dowco 105 and Neguvon (Bayer L13/59, Trichlorphon) (Figure 10.2) (Table 10.1). Critical and controlled tests with Trolene showed very limited efficacy against any of the gastrointestinal nematodes even at doses of up to 110mg/kg (Schad *et al.*1958). Dowco 105 had improved efficacy at 200 mg/kg against abomasal and small intestinal parasites, with removal of between 94-100% of *Ostertagia*, *Nematodirus* and *Trichostrongylus* spp. (Douglas *et al.*1959). This compound was never developed any further. Dowco 105 was an ethyl phosphoramidate and it was the methyl derivative Ruelene (Crufomate) (Figure 10.3) that became the commercialised compound.

Figure 10.1 Structure of Trolene

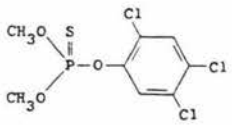
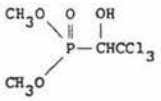


Figure 10.2 Structure of Neguvon



There were conflicting reports on the efficacy of Neguvon against the various gastrointestinal nematodes. At low doses (55mg/kg) it removed up to 100% of *H. contortus* (Gordon, 1962) but did not affect other parasites. Using controlled tests, doses of 100mg/kg had no effect against *T. axei* (Gibson, 1960). Based on faecal egg count reduction, doses of up to 5g/100lb worked against *Ostertagia* and *T. axei* only when injected or swallowed directly into the abomasum (Gordon, 1958b; Dunsmore, 1960). Another OP, Asuntol (Coumaphos) (Figure 10.4), had proved efficacious against *H. contortus*, *O. circumcincta*, *T. axei*, *T. colubriformis*, *C. curticei* and *S. papillosus* when used at low doses (25 mg/kg), but had a very narrow therapeutic index (Herlich and Porter, 1958). It was also trialed for lambs in feed and drinking water and only showed high efficacy by this means for *Haemonchus* (95-96%) (Train *et al.*1968). As with any drug fed by this means, there would be no control over ingestion and in a field situation animals could easily ingest toxic doses. Combining Neguvon with Asuntol in a 10:1 ratio gave similar results in a FECR test to Asuntol alone without the toxic side effects or the need for copper sulphate to stimulate the oesophageal reflex (Stampa, 1959). A controlled trial by Dunsmore (1960) confirmed these results.

Figure 10.3 Structure of Ruelene

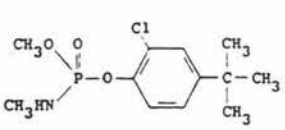
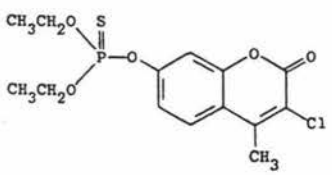


Figure 10.4 Structure of Asuntol



Ruelene was another early OP. At doses of 200mg/kg in controlled trials, it had very good efficacy against *Haemonchus* and *Cooperia*, and 85 and 67% for *Ostertagia* spp. and *T. axei* respectively. The small intestinal *Trichostrongylus* spp. were removed more efficiently (up to 92%) (Douglas and Baker, 1959; Gibbs and Pullin, 1961). Efficacy against the large intestinal nematodes

Oesophagostomum, *Chabertia ovina* and *Trichuris ovis* was generally low (64-82%), but this was higher than any of the other marketed OP's (Douglas and Baker, 1959). Only doses of 150 mg/kg showed any effect on immature *Haemonchus* (Galvin *et al.*1962). However, this was not unusual as the early OP's in general were not very efficacious against any of the immature abomasal or intestinal nematodes.

The development of Haloxon in 1962 provided another relatively broad spectrum OP anthelmintic (Figure 10.5) (Brown *et al.*1962). The advantages it had over the earlier compounds was a wider spectrum, lower therapeutic dose (50 mg/kg) and a wider therapeutic index (Brown *et al.*1962; Armour *et al.*1962; Nunns *et al.*1964; Baker and Douglas, 1965; Kingsbury and Curr, 1967). It also had high efficacy against the 4th and 5th larval or immature adult stages of *H. contortus*, intestinal *Trichostrongylus* spp., *Cooperia curticei* and the 5th larval stages of *Ostertagia* and *Nematodirus* spp. (Armour *et al.*1962; Gibson and Parfitt, 1968). The only major worm species with variable susceptibility to Haloxon were *Oesophagostomum columbianum*, *Chabertia ovina* and *Trichuris ovis* (Kingsbury and Curr, 1967).

Figure 10.5 Structure of Haloxon

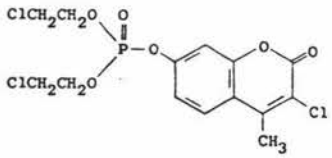
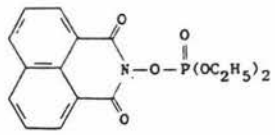


Figure 10.6 Structure of Naphthalphos



Naphthalphos was another broad spectrum OP (Figure 10.6), and at doses of 75mg/kg had better activity than Haloxon against immature *Ostertagia circumcincta, T. axei* and *T. colubriformis* (Thomas, 1964). When used as a bolus rather than a drench, efficacy increased for *Nematodirus* spp. (12% to 50%), and *S. papillosus* (50% to 83%) (Train *et al.*1968). But like other OP's there was poor effect against *Oesophagostomum* species (0-54%) (Hebden and Hall, 1965; Federmann, 1965; Train *et al.*1968). More recently this compound has made a resurgence in the control of benzimidazole- (BZ) and levamisole-resistant *H. contortus* (Jambre *et al.*1979; Cooper *et al.*1996). When combined with albendazole, it gave greater than 95% efficacy against all parasite species including BZ resistant *O. circumcincta* and *Trichostrongylus* spp. (Cooper *et al.*1996).

10.3.2 Goats

Naphthalphos and coumaphos in feed have also been used in goats with similar levels of efficacy to sheep (Table 10.2) (McDougald *et al.*1968).

10.3.3 Cattle

As with sheep, Trolene (Dowco ET-57), Dowco 105, Neguvon (Bayer L13/59) and Asuntol (Bayer 21/199) were some of the first OP's used as anthelmintics in cattle. It was the initial use of Trolene against cattle grub that inspired Herlich and Johnson (1957) to evaluate its efficacy in critical trials against gastrointestinal parasites in sheep and cattle. In cattle, there was good efficacy against *H. placei* (85-100%) and variable, but enough efficacy to be encouraging against the other nematodes (Table 10.3) (Herlich and Johnson, 1957).

Asuntol was the next OP to be trialed. It was initially used as a topical and contact insecticide. It was effective against nematode larvae in *in vitro* trials, and at doses of 25 mg/kg it was rapidly effective against *H. placei*, *O. ostertagi*, *T. axei*, *Cooperia* spp., and *Oe. radiatum*, expelling nearly all nematodes within 48 hours (Herlich and Porter, 1958). More recently, it proved quite effective against all the major species where it was trialed as either a low dose (2mg/kg), short (6 days) or long term (24 days) continuous in-feed anthelmintic in feedlots (Cox *et al.*1967; Ciordia and Baird, 1968; Ciordia, 1972b; Zeakes *et al.*1976).

Using FECR and larval differentiation, Riek and Keith (1958b) and Riek (1958) compared Asuntol with another OP, Neguvon (Bayer L13/59). Neguvon appeared to remove a broader range of adult and immature parasites as well as being less toxic (Riek and Keith, 1958b; Riek, 1958), though there was some disagreement about the lack of toxicity (Gordon, 1958b). Neguvon was further trialed under controlled and field outbreak conditions using oral and subcutaneous routes of administration. Doses greater than 60 mg/kg by subcutaneous injection were needed for efficacy against *O. ostertagi* and *Cooperia* spp. in a critical (Lee and Shonekan, 1959) and controlled trials (Banks and Michel, 1960), but oral doses of 80 mg/kg gave good clinical results in a field outbreak (Banks and Mitton, 1960). Unlike in sheep, there was reasonable efficacy against the large intestinal parasite *Oesophagostomum radiatum*, especially when the subcutaneous route was used.

The results with Ruelene in cattle were less positive than had been shown for sheep, though the drug did show some promise. Unlike Neguvon, Ruelene at doses of 40-60 mg/kg was more efficacious against *Cooperia* spp. when used orally (Alicata, 1960; Herlich *et al.*1961; Bourke, 1962; Shelton, 1962). Trials using topical application gave erratic results for all nematode species (Herlich *et al.*1961; Brown, 1962c).

As with sheep, the arrival of Haloxon provided a more broad spectrum OP for cattle with less toxicity. Oral doses of 35-59 mg/kg gave very high efficacy against adult worms of the genera *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia* and *Strongyloides*. There was variable efficacy against *Nematodirus* and *Bunostomum* (Brown *et al.*1962; Hart, 1964; Bosman, 1965; Baker *et al.*1969; Benz, 1972). Like other OP's, Haloxon was not effective against the histotrophic

stages of *Ostertagia* even if the dose was raised to 100 mg/kg (Armour, 1964). It did have good efficacy against the larval stages of *Cooperia* spp. either as an oral drench or bolus (Hart, 1964; Benz, 1972).

Trial work on the use of Naphthalphos in cattle was first published in 1965 (Federmann, 1965). In controlled and critical trials there was good efficacy at 50-75 mg/kg against *Ostertagia*, *Cooperia* and *Trichostrongylus* spp. (Federmann, 1965; Stober and Ende, 1965; Rubin and Hibler, 1967; Ciordia, 1972a; Zeakes *et al.*1976). This compound was not as consistently effective against immature *Cooperia* spp. as Haloxon (Rubin and Hibler, 1967) and like most OP's, there was variable efficacy against the large intestinal nematodes (Ciordia, 1972a; Zeakes *et al.*1976).

Dichlorvos was also used as a low dose in-feed treatment. Efficacy was as effective against *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Nematodirus* spp. at doses of 10mg/kg for 29 days or 25 mg/kg for 2 days, as a single drench of 50 mg/kg (Poeschel and Todd, 1972).

10.3.4 Horses

Organophosphates have been of special interest as potential broad spectrum agents for the control of internal parasites of the horse, because of the desirable combination of insecticidal and anthelmintic activities. The four OP's which have been used in horses are Butonate, Dichlorvos, Haloxon and Trichlorphon. All of these have very good efficacy (99-100%) against adult *Parascaris equorum* and *Oxyuris equi*. Efficacy against the small strongyles is more variable (0-100%) with *Triodontophorus* species being the most susceptible. Of the large strongyles, *S. vulgaris* seems the most susceptible to Haloxon and Dichlorvos (Table 10.4.) (Bosman, 1966; Voss and Hibler, 1971; Drudge and Lyons, 1972; Cook, 1973a; 1973b; Voss and Hibler, 1973; Drudge *et al.*1975; Lyons *et al.*1976; Lyons *et al.*1976; Lyons *et al.*1981).The volatility and narrow therapeutic index of Dichlorvos has resulted in it being formulated into a resin pellet. The pellets have the potential of an optimal release rate that would not only reduce toxicity, but also enhance antiparasitic activity during the passage through the digestive tract. Dichlorvos in this formulation was trialed as a low dose in feed treatment. The standard therapeutic dose for a single treatment was 25 mg/kg, whereas dosing animals at 10mg/kg for three or five days was as efficacious. One difficulty was encouraging all animals to consume the treated ration (Drudge and Lyons, 1972). Butonate in feed at a dose of 39 mg/kg was not very efficacious against the oxyurids, or small or large strongyles (Voss and Hibler, 1973).

The two OP's still currently in use today are Dichlorvos and Trichlorphon. Both have been combined with other anthelmintics such as thiabendazole, mebendazole, oxfendazole or morantel. The intention was to broaden the anthelmintic spectrum to aid in the removal of ascarids, oxyurids and

Gasterophilus larvae (Lyons *et al.*1977; Drudge *et al.*1984; Presson *et al.*1984a; Seibert *et al.*1986b).

10.3.5 Dogs and Cats

Dichlorvos has been the OP anthelmintic most used in dogs. Against *T. vulpis*, single doses of 30 mg/kg removed 72% of parasites, with a repeat of the dose in two weeks removing 92% (Olsen *et al.*1977). Dichlorvos resin pellets have been added to canned and dry dog food in both slow and fast release formulations. Doses of 20 mg/kg twice daily or a single dose of 31.5 mg/kg in canned dog food, were 100% effective against ascarids, hookworms and whipworms (Batte *et al.*1966). When added to dry feed, whipworms were best eliminated by slow release formulations used for 5-25 days. Ascarids and hookworms were less affected by the release rate, but needed Dichlorvos concentrations of greater than 50-100 ppm in the feed. The optimal efficacy against the three canine nematodes was obtained with feed containing slow release preparation for 5 days (Roberson *et al.*1977). Haloxon has also been used in the dog and the cat. Doses of 17-50 mg/kg achieved 100% against mature and immature intestinal stages of *T.canis* and *T. leonina* in dogs. Two doses of 20-33mg/kg 24 hours apart achieved more than 80% efficacy in 8/9 dogs and 88.5% efficacy against *T.cati* in the cat (Kingsbury *et al.*1977). The results of these trials are presented in Table 10.5.

10.4 SIDE EFFECTS AND TOXICITY

The OP's act by inhibiting several enzymes. This includes acetylcholinesterase which they inhibit by phosphorylating the esterification site. This phosphorylation can occur in mammals and nematodes. The result of this enzyme inhibition is to block cholinergic nerve transmission, and stimulate the parasympathetic nervous system. This inhibition is usually reversible, but the rate at which this inhibition can be reversed and the rate of inactivation of the various OP's determine their relative toxicity to different animals. (Radeleff and Woodard, 1957; Prichard, 1978; Prichard, 1986b). The earlier OP's were too toxic for mammals and had a very narrow therapeutic index. Later compounds such as Haloxon and Naphthalphos were a lot less toxic (Radeleff and Woodard, 1957; Armour *et al.*1962; Malone, 1964). This was thought to be because they are less selective for the mammalian acetylcholinesterase. There was also variation in animals' abilities to metabolise organophosphates. In sheep, this was been shown to be genetically determined (Lee, 1964).

Cholinesterase is normally present in blood, and changes in the level were thought to be an indicator of the level of toxicity. However, blood cholinesterase is composed of plasma cholinesterase (pseudo-cholinesterase) and erythrocyte cholinesterase (true cholinesterase). Different species have different proportions of these in their blood (Kruckenberg and Vestweber,

1973). Because of the extent of inter- and intra-species variation, it was not possible to correlate the level of toxicity with the level of exposure. Low cholinesterase levels therefore only indicate that animals have been exposed to an OP (Radeleff and Woodard, 1957; Radeleff, 1960). The reaction to treatment with an OP varies with the animal and the particular compound. Some exposed animal's have been shown to have a rapid reduction followed by a slow increase in plasma cholinesterase (Albert and Stearns, 1973; Bello *et al.*1974; Shmidl *et al.*1983). For example horses dosed with therapeutic doses of Dichlorvos had a marked decrease within 24 hours and a slow recovery of the enzyme with it being less than 50% pretreatment levels nine days after treatment, and still not complete recovery by 49 days (Snow, 1974). In another case, sheep exposed to five times the therapeutic dose of Haloxon showed only a slight depression of red blood cell cholinesterase that returned to normal within one week (Brown *et al.*1962).

Animals respond differently to the toxic effects of OP poisoning. Acute clinical signs reflect stimulation of the parasympathetic nervous system and these can vary between species eg. ruminants and horses. Frequently, animals showed signs within 3-4 hours after dosing and recovered within 24-36 hours. Ruminants such as sheep, cattle and goats initially show muscarinic signs such as profuse salivation, and gastrointestinal hypermotility with diarrhoea. Following these are nicotinic signs such as twitching of the musculature of the face and body, stiffness, ataxia and subsequent recumbency (Lee and Shonekan, 1959; Skerman, 1962; Kerr *et al.*1987). The predominant clinical signs in the horse differ from ruminants and are characterised initially by colic and diarrhoea followed by muscular weakness, salivation and dyspnea (Younger, 1965; Bello *et al.*1974). The literature is sparse on the effects of OP's on dogs, but they tend to show parasympathetic signs with slight vomiting and intermittent diarrhoea. Clinical cases of OP poisoning in this species are reasonably common (Batte *et al.*1966; Kingsbury *et al.*1977).

Animals may also show clinical signs of chronic toxicity that may be seen 2-6 weeks after dosing. This is characterised by delayed neurotoxicity, as a result of peripheral nerve damage, ataxia and paralysis (Kingsbury and Curr, 1967; Cook, 1973a; Kerr *et al.*1987). These effects are apparently not due to inhibition of cholinesterase, but rather due to inhibition of a separate enzyme, neurotoxic esterase (Taylor, 1992b).

Atropine sulphate is effective as an antidote in the treatment of livestock poisoned with OP compounds. Atropine is generally considered to block the central and muscarinic effects of excess acetylcholine, but not the nicotinic effects. However care must be taken using this compound as large doses may induce extreme depression or restlessness followed by death.

Another antidote is pyridine-2-aldoxime methiodide (2-PAM, Pralidoxime). This compound was designed as a specific antidote against alkylphosphate intoxication. It is capable of reactivating acetylcholinesterase inhibited by certain OP compounds. The chloride salt of 2-PAM was used

rather than the iodide to increase solubility. The 2-PAM chloride treatment was usually effective against mild to moderately severe poisoning produced by several different OP compounds. Response to the 2-PAM varied with the compound, the degree of poisoning when treated, and the animal species. Severely poisoned sheep, cattle and goats did not respond to the uniform doses of 2-PAM (Younger and Radeleff, 1964). This was most likely because the phosphorylated site of the acetylcholinesterase has become more stable due to loss of an alkyl or alkyoxy group. This prevents the oxime from cleaving off the phosphate group and reactivating the enzyme (Taylor, 1992b). In these severe cases, animal's usually responded after atropine administration. Where 2-PAM was effective, relief of poisoning occurred within 10-30 minutes. Toxic side effects were not seen with 2-PAM. The combination of the two drugs may provide a more reliable therapeutic effectiveness against many OP compounds than either drug alone (Younger and Radeleff, 1964).

TABLE 10.1 PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF SHEEP.

		Abomasum			Small Intestine					Large Intestine			References
	Dose	*H. contortus*	*Ostertagia* spp.	*T.axei*	*Nematodirus* spp.	Trich. spp.	*Bunostomum*	*Cooperia spp.*	*S. papillosus*	*Oesoph. spp.*	*Chabertia ovina*	*T.ovis*	
Trolene (Dow ET-57)	100mg/kg	0	0		0	0		0			0		(Schad *et al.* 1958)
Trolene	110 mg/kg	31		0									(Gibson, 1960; 1961)
Dowco 105	75 mg/kg		52	0	62-89	0-100			69				(Douglas *et al.* 1959)
Dowco 105	200 mg/kg		94	38	96-98	96-100			95				(Douglas *et al.* 1959)
Neguvon (Bayer L13/59)	75mg/kg		60-73	37	0	32-43		62	75				(Knapp and Mosher, 1963)
Neguvon	100 mg/kg oral	100		0*									(Gibson, 1960; 1961)
Neguvon	111 mg/kg intra-ruminal		Adult- 0 L4 - 76[1] L5 - 76	0									(Dunsmore, 1960)
Neguvon	111 mg/kg - oral post CuSO4 swab		Adult 93-97 L4 - 73 L5 - 91	95-98									(Dunsmore, 1960)
Neguvon & Asuntol (10:1)	50mg/kg oral		80-85	82	72	44-91		99	92				(Knapp and Mosher, 1963)

[1] L4 4th stage larvae
[2] L5 5th stage larvae/immature adult

TABLE 10.1 CTD. PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF SHEEP.

		Abomasum			Small Intestine					Large Intestine			References
	Dose	*H. contortus*	*Ostertagia* spp.	*T.axei*	Nematodirus spp.	*Trich.* spp.	*Bunostomum* sp.	*Cooperia* spp.	*S. papillosus*	*Oesoph* spp.	*Chabertia ovina*	*T.ovis*	
Asuntol	25 mg/kg	100	100	100	35	95-100		100				100	(Herlich and Porter, 1958)
Asuntol	2mg/kg/day 6 days - feed	94	51		4	0			0	0		48.6	(Train *et al.* 1968)
Asuntol	2mg/kg/day 6 days water additive	96	59.3		49	18			50	58.3		45.1	(Train *et al.* 1968)
Ruelene	75mg/kg	91											(Gibson, 1961)
Ruelene	100mg/kg	100	31-60.3	24-38	0	0-80	0	76	0		0	0	(Douglas and Baker, 1959; Gibbs and Pullin, 1961)
Ruelene	125 mg/kg	98 imm 89[1]	49 imm 54	46 imm 0	0 imm 0	58 imm 0			65	0 imm 0			(Galvin *et al.* 1962)
Ruelene	150 mg/kg	99-100 imm 85	0-97 imm 0	36-85 imm 0	0-96 imm 0	64-96 imm 0		97-99	45	0 imm 0		0	(Galvin *et al.* 1962; Shelton,1962; Skerman, 1962)
Ruelene	200mg/kg	100	85-96.5	67-91	75-84	56-99.8	100	100	0-64	69	63	82	(Douglas and Baker, 1959; Gibbs and Pullin, 1961)

[1] Imm Immature worms

TABLE 10.1 CTD. PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF SHEEP.

		Abomasum			Small Intestine				Large Intestine			References
	Dose	*H. contortus*	*Ostertagia* spp.	*T.axei*	*Nematodirus* spp.	*Trich.* spp.	*Cooperia* spp.	S. *papillosus*	*Oesoph.* spp.	*Chabertia ovina*	*T.ovis*	
Haloxon	30-50 mg/kg	100	40-100	92-100	51-100	86-100	100	100	70-82	0-82	23-71	(Armour *et al.*1962; Nunns *et al.*1964)
Haloxon	50 mg/kg		95-99.7 d2 - 37.4[1] d7 - 11 d14 - 67.5		28%	98%						(Baker and Douglas, 1965; Gibson and Parfitt, 1968)
Haloxon	50mg/kg intra-ruminal		84-94		83	72-87						(Armour *et al.*1962)
Naphthalphos	34.5 mg/kg		74		79	95						(Cooper *et al.*1996)
Naphthalphos	50 mg/kg	99.5 - 100 d7 - 99.7 d14 - 100	86-100	100	12.3-83	73.4-9.5 d7 - 81.9 d14 - 95.6			0-53.8		35.3	(Hebden and Hall, 1965; Federmann, 1965; Train *et al.*1968)
Naphthalphos	50 mg/kg bolus	100	96		50.3	79.7		83.3	36.5		83.2	(Train *et al.*1968)
Naphthalphos	75 mg/kg	100	99.5	100	37.5	100						(Hebden and Hall, 1965)
Naphthalphos + ABZ	34.5 mg/kg & 3.6 mg/kg	100	77-100		74-100	81-100	100		100			(Cooper *et al.*1996)
Trichlorphon	70mg/kg SQ[2]	90	42-100	40	2-51		25-100	59			100	(Lyons *et al.*1967)

[1] Indicates 2-day-old larvae and percentage efficacy

[2] SQ - subcutaneous injection

TABLE 10.2 PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF GOATS

		Abomasum			Small Intestine				Large Intestine			References
Salt	Dose	*H. contortus*	*Ostertagia* spp.	*T.axei*	*Nematodirus* spp.	*Trich.* spp.	*Bunostomum* sp.	*Cooperia* spp.	*Oesoph.* spp.	*Chabertia ovina*	*T. ovis*	
Naphthalphos	25 mg/kg	97	74.3		72.6	48.1			0		0	(McDougald *et al.*1968)
Naphthalphos	50 mg/kg	99.9	88.5		63.9	84.8			0		0	(McDougald *et al.*1968)
Coumaphos	0.86 mg/kg in feed - 6 days	69.8	23.1		72.4	58			0		0	(McDougald *et al.*1968)

TABLE 10.3 PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		References
	Dose	*Haemonchus* sp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* sp.	*Oe. radiatum*	
Trolene (Dowco ET-57)	100mg/kg	85-100	23-75	0	0	42-86	0		1-35	8-88	(Herlich and Johnson, 1957)
Dowco 105	120-130mg/kg		66	100		87	96				(Baker *et al.*1959)
Neguvon (Bayer L13/59)	66mg/kg		d7-38[1] d15 - 0 d21 - 64 d28 - 96								(Banks and Michel, 1960)
Neguvon	110mg/kg		d15 - 0 d21 - 65 d28 - 98.5								(Banks and Michel, 1960)
Asuntol (Bayer 21/199)	12.5-25 mg/kg	100	30-100	81-100	32-93	100		10-100	95-100	100	(Herlich and Porter, 1958)
Ruelene	17mg/kg in feed premix for 3 consecut days		68 L4 - 34[2]	26	29 L4 - 25	96-99 L4 - 87	L4 - 9	67 (*S.papillosus* 72)	T. ovis 28 (*D. viviparus* 0%)	26 L4 - 0	(Lyons *et al.*1982)
Ruelene	38.5mg/kg		0-80		0-54	98-100					(Bourke, 1962)
Ruelene	40-60 mg/kg	100	81-88	0-98		95-100	91-100			90-100	(Herlich *et al.*1961; Shelton, 1962)

[1] Indicates 7-day-old larvae and percentage efficacy
[2] L4 4th stage larvae

TABLE 10.3 CTD. PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		References
	Dose	*Haemonchus* sp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich* spp.	*Bunostomum phlebotomum*	*Trichuris* sp.	*Oe. radiatum*	
Haloxon	15 mg/kg	100				100	61-77	0		0	(Hart, 1964)
Haloxon	30 mg/kg	100 d7 - 81[1] d14 - 100				100 d7 - 14 d14 - 99	91-99 d7 - 32 d14 - 50	0		24-35 d7 - 0 d14 - 0	(Hart, 1964)
Haloxon	34-48 mg/kg	100	89-99.3	99.9-100	74	99.7-100	99-100	17-82	100 (*Ascaris* spp. 97)	99.1-100 (*Chabertia* spp. 100)	(Bosman, 1965)
Haloxon	40mg/kg		94-100 d7 - 0 d14 - 1-81 d21 - 94-100								(Armour, 1964)
Haloxon	49 mg/kg bolus	100	94.3 Larvae - 0[2]	99.1 Larvae - 0		99.2-100 Larvae- 99.3	100				(Benz, 1972)
Haloxon	50 mg/kg	100 d7 - 98 d14 - 100				100 d7 - 43 d14 - 100	90-100 d7 - 46 d14 - 73	36015		61-100 d7 - 0 d14 - 0	(Hart, 1964)
Haloxon	50 mg/kg (intra-ruminal)	99	90.5	100		99.9-100					(Baker *et al.*1969)
Haloxon	52 mg/kg	100	89.3 Larvae - 0	97.2 Larvae - 0		96.6-97.6 Larvae- 98.9	99.4				(Benz, 1972)
Haloxon	54 mg/kg feed premix	96.4	89.5 Larvae - 0	98.3 Larvae - 0		92.2-96.1 Larvae- 98.5	93.5				(Benz, 1972)
Haloxon	100 mg/kg		d7 - 0-62								(Armour, 1964)

[1] Indicates 7-day-old larvae and percentage efficacy

[2] Percentage efficacy against larval stages

TABLE 10.3 CTD. PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		References
	Dose	*Haemonchus* sp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich* spp.	*Bunostomum phlebotomum*	*Trichuris* sp.	*Oe. radiatum*	
Naphthalphos	50 mg/kg	99.8-100	78-97 d10 - 0-23[1] d25 - 0-99	68-100	87-97	72-100 d10 - 6-100 d25 - 0-96	74-100				(Federmann, 1965; Stober and Ende, 1965; Rubin and Hibler, 1967; Ciordia, 1972a; Zeakes *et al.*1976)
Naphthalphos	75 mg/kg		d25 - 95-99 d10 - 0-37			d25- 0-99 d10 - 0-100					(Rubin and Hibler, 1967)
Coumaphos	2 mg/kg in feed for 6 days	99.4-99.8	86.7-89.7	98.1		99.8-100	95.9-96.3		90.9	74.8	(Zeakes *et al.*1976; Ciordia, 1972b)
Coumaphos	0.32% in crumbles 2mg/kg 6 days	98	80	84.3		100	77.8			61.5	(Ciordia, 1972b)
Dichlorvos	2mg/kg in feed 29 days	100	18		65	0					(Poeschel and Todd, 1972)
Dichlorvos	10 mg/kg in feed 29 days	100	98		100	100					(Poeschel and Todd, 1972)
Dichlorvos	25 mg/kg in feed 2 days	100	100		100	100					(Poeschel and Todd, 1972)
Dichlorvos	50 mg/kg in feed 1 day	100	100		100	100					(Poeschel and Todd, 1972)

[1] Indicates 10-day-old larvae and percentage efficacy

TABLE 10.4 PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF HORSES

				Large Strongyles			Small Strongyles					References
	Dose	*Parascaris equorum*	*Parascaris equorum* immature	*S. edentatus*	*S.equinus*	*S.vulgaris*	Small strongyles	*Trichonema* adults	*Triodontophorus* adults	*O.equi* Adults	*O.equi* Immature	
Haloxon	56-75 mg/kg oral	100		50-70	50-70		94-100	95-99	100	100		(Bosman, 1966)
Haloxon	60mg/kg stomach tube	100	100	2-79		99-100	67-92			100	99-100	(Lyons *et al.*1981a)
Haloxon	60 mg/kg feed	100		47-80		100	78-87			100	100	(Lyons *et al.*1981a)
Haloxon	60 mg/kg powder gun	99-100		17-100		100	88			100	100	(Lyons *et al.*1981a)
Haloxon & Trichlorfon	60 mg/kg & 20 mg/kg			30-38		100		79-83	100			(Cook, 1973b)
Haloxon & Trichlorfon	60 mg/kg & 10 mg/kg			37-74		100		80-92	100			(Cook, 1973b)
Butonate	28mg/kg stomach tube	91-100										(Voss and Hibler, 1971)
Butonate	39 mg/kg in feed	92-100		*Strongylus* spp. 0-100 (average 8)			0-64			0-100		(Voss and Hibler, 1973)

TABLE 10.4 CTD. PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF HORSES

				Large Strongyles			Small Strongyles					References
	Dose	*Parascaris equorum*	*Parascaris equorum* immature	*S.edentatus*	*S. equinus*	*S.vulgaris*	Small strongyles	*Trichonema* adults	*Triodontophorus* adults	*O.equi* Adults	*O.equi* Immature	
Dichlorvos	10mg/kg oral gel	100										(Hass *et al.*1973)
Dichlorvos	14mg/kg oral gel	95										(Hass *et al.*1973)
Dichlorvos	20 mg/kg oral gel (Adult Horses)	100										(Hass *et al.*1973)
Dichlorvos	20 mg/kg oral gel (Foals)	82										(Hass *et al.*1973)
Dichlorvos	28 mg/kg oral gel (Adult Horses)	100										(Hass *et al.*1973)
Dichlorvos	40 mg/kg oral gel (Adult Horses)	100										(Hass *et al.*1973)
Dichlorvos	36.3 mg/kg paste			*Strongyloides westeri* 71-100								(Drudge *et al.*1982)

TABLE 10.4 CTD. PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF HORSES

				Large Strongyles			Small Strongyles					References
	Dose	*Parascaris equorum*	*Parascaris equorum* immature	*S. edentatus*	*S.equinus*	*S.vulgaris*	Small strongyles	*Trichonema* adults	*Triodontophorus* adults	*O.equi* Adults	*O.equi* Immature	
Dichlorvos	25 mg/kg in feed	100	100	0-100		100	95-99			100	100	(Drudge and Lyons, 1972)
Dichlorvos	33 mg/kg (restrict water)	100	100	96-100	33-100	96-100				100		(Drudge and Lyons, 1972)
Dichlorvos	33 mg/kg (no water restrict)	100	100	96-100	0-60	100				100		(Drudge and Lyons, 1972)
Dichlorvos	33-43 mg/kg in feed	100	100	25-81		88-100	87-96			100	100	(Drudge and Lyons, 1972)
Dichlorvos	50 mg/kg in feed	100		93-97		99-100	97-100				100	(Drudge and Lyons, 1972)
Dichlorvos	100 mg/kg in feed			100		100	100			100		(Drudge and Lyons, 1972)
Dichlorvos	12.5 mg/kg/day for 2 days in feed	100	100	89-92		93-100	68				100	(Drudge and Lyons, 1972)
Dichlorvos	10 mg/kg for 3 days in feed		100	90		99-100	90-99				100	(Drudge and Lyons, 1972)
Dichlorvos	10 mg/kg for 5 days in feed			96		100	100					(Drudge and Lyons, 1972)

TABLE 10.4 CTD. PERCENTAGE EFFICACY OF ORGANOPHOSPHATES AGAINST GASTROINTESTINAL NEMATODES OF HORSES

				Large Strongyles			Small Strongyles					References
	Dose	*Parascaris equorum*	*Parascaris equorum* immature	*S. edentatus*	*S.equinus*	*S.vulgaris*	Small strongyles	*Trichonema* adults	*Triodontophorus* adults	*O.equi* Adults	*O.equi* Immature	
Trichlorfon	40mg/kg liquid	100	100	0-12		0-28				99-100		(Drudge *et al.*1975)
Trichlorfon	40mg/kg paste	100	100	0		0-50				100		(Drudge *et al.*1975)
Trichlorfon	40mg/kg paste & feed	100	100	0-5		0-83				11-100		(Lyons *et al.*1976a)
Trichlorphon & TBZ[1]	44mg/kg & 40 mg/kg	100	100	82-100	72-100	89-100				100		(Lyons *et al.*1977)
Trichlorfon and OFZ[2]	40 mg/kg & 2.5 mg/kg	100		100		100	97			100	100	(Presson *et al.* 1984a)
Trichlorphon & Morantel	30 mg/kg & 6 mg/kg	100		72		100				100		(Drudge *et al.*1984)

[1] TBZ Thiabendazole
[2] OFZ Oxfendazole

TABLE 10.5 PERCENTAGE EFFICACY OF ORGANOPHOPHATES AGAINST GASTROINTESTINAL NEMATODES OF DOGS AND CATS

				Small Intestine					Large Intestine	References
	Species	Dose	Ascarids	*T.canis*	*T. leonina*	Hookworms	*Ancylostoma caninum*	*Uncinaria stenocephala*	*T.vulpis*	
Dichlorvos - medicated feed	Dog	Fast Release 200ppm 2d[1]		85			80	100	89	(Roberson *et al.*1977)
		Fast Release 200ppm 5d		100	100		100		68	(Roberson *et al.*1977)
		Fast Release 300ppm 2d 8.7-12.5 mg/kg			100		100	100	80	(Roberson *et al.*1977)
		Fast Release 400ppm 2d 6.5-14.5 mg/kg					100	100	64	(Roberson *et al.*1977)
		Slow Release 50ppm 25d		80			92		100	(Roberson *et al.*1977)
		Slow Release 100ppm 5d		34			99	100	73	(Roberson *et al.*1977)
		Slow Release 200ppm 5d		90-100			88-92	100	98-100	(Roberson *et al.*1977)
Dichlorvos -	Dog	30 mg/kg (oral capsule)							1 dose - 78 2 doses - 92	(Olsen *et al.*1977)

[1] Dose of anthelmintic in parts per million and number of days the anthelmintic was fed to the animals

TABLE 10.5 CTD. PERCENTAGE EFFICACY OF ORGANOPHOPHATES AGAINST GASTROINTESTINAL NEMATODES OF DOGS AND CATS

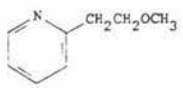
				Small Intestine					Large Intestine	References
	Species	Dose	Ascarids	*T. canis*	*T. leonina*	Hookworms	*Ancylostoma caninum*	*Uncinaria stenocephala*	*T.vulpis*	
Haloxon	Dog	17-50 mg/kg		100	100					(Kingsbury *et al.*1977)
	Dog	20-33mg/kg 2 doses 24 hrs apart						80		(Kingsbury *et al.*1977)
	Cat	20-33mg/kg 2 doses 6-24 hrs apart		*T.cati* 88.5						(Kingsbury *et al.*1977)
		33-83mg/kg		*T.cati* 67			*A. tubaeforme* 35			(Kingsbury *et al.*1977)
		54-81mg/kg					*A. tubaeforme* 67			(Kingsbury *et al.*1977)

11.0 METHYRIDINE

11.1 INTRODUCTION

In the search for new anthelmintic compounds it was found that a few of the substituted pyridines had a degree of activity against internal parasites. When efficacy was evaluated using a laboratory model in mice, 2-(beta-methoxyethyl)-pyridine (methyridine) was the substituted pyridine shown to have the greatest activity against nematodes throughout the intestinal tract (Figure 11.1). This was unusual, as other anthelmintics used in animals tended to be effective only against nematodes in specific locations in the gastrointestinal tract eg. abomasum, small intestine, large intestine. Methyridine was also unusual in that initially it appeared more efficacious when given subcutaneously than orally, and was as effective against both adult and immature stages of mouse parasites. Further trials were carried out in sheep, cattle and other domestic species (Broome and Greenhalgh, 1961).

Figure 11.1 Structure of Methyridine



11.2 PHARMACOLOGY

Methyridine is colourless and water soluble. Solutions have been developed for both oral (30%) and subcutaneous (90%) administration. Early development work showed that 200mg/kg by either route of administration was the most efficacious dose in sheep and cattle (Walley, 1961; Broome and Greenhalgh, 1961). Later work added intraperitoneal injection as another route of administration (Walley, 1962).

Following oral or subcutaneous administration, peak blood levels (125 mcg/ml) of methyridine are reached in 1-3 hours in both sheep and calves (Broome, 1961b; Burns *et al.*1967) b. This decreases to 45-60 mcg/ml at six hours, with most of the drug removed by 24 hours (Burns *et al.*1967). The drug is rapidly distributed throughout the tissues (Burns *et al.*1967), with a greater concentration being found in the fore-gut after oral dosing and in the small and large intestines after subcutaneous dosing (Broome, 1961a). Being a basic compound, after passing into the abomasum, methyridine becomes ionised which subsequently results in trapping of the compound (ion-trapping) (Broome, 1961b). A close association has been found between concentrations in the blood and in the small and large intestines, with the direction of drug movement being concentration dependant (Broome,

1961a). This close contact with the gastrointestinal tissue allows intimate contact of the drug with the parasites embedded in the intestinal mucosa or lying beneath the mucus lining of the digestive tract (Broome, 1961a). However, although the time of maximum concentration is short, it is sufficiently long to produce paralysis in the parasites (Broome, 1961a). Metabolism is rapid with 98-99.5% of the methyridine being metabolised to compounds with short half lives (Burns *et al.*1967). None of the metabolites have shown activity *in vitro* or *in vivo* (Broome, 1961a). Excretion is predominantly renal (Burns *et al.*1967).

Methyridine causes a depolarising neuromuscular block similar to that of a decamethonium. This block cannot be reversed by acetylcholine (Broome, 1961a). *In vitro* experiments with intact nematodes (including *Ostertagia* spp. from sheep, *Nematospiroides dubius* and *Heterakis spumosa* from mice, and *Nippostrongylus muris* from rats) have shown that methyridine passes through the cuticle and results in a partial spastic paralysis in the worms. This develops within one hour at concentrations similar to peak levels reached in the alimentary canal following the administration of therapeutic doses (Broome, 1961a; 1961b). *In vitro* work with varying concentrations of methyridine have shown that the paralysis is reversible in third stage *Ostertagia circumcincta* larvae and does not result in any long term damage. This could allow elimination of the worms attached to the mucosa of the gastrointestinal tract. However, those buried in its wall, although temporarily immobilised, might not be eliminated by the host and could later recover and resume development (Denham, 1970).

The amount of methyridine entering ascarids is pH-dependant, with much greater drug concentrations in the parasite at pH 8 than pH 5 or 3. The anthelmintic activity of the compound *in vitro* was greatest in alkaline solutions, which probably accounts for the differences in anthelminthic activity noted in various parts of the alimentary canal. As methyridine is a basic compound, the very low pH of the abomasum results in almost complete ionisation and the drug is unable to enter the parasites. Subsequently this results in poor efficacy against parasites inhabiting this area of the gastrointestinal tract (Broome, 1961b).

11.3 EFFICACY

11.3.1 Sheep

An early series of development and field trials showed good efficacy against worms of the abomasum and small intestine, especially mature and immature *Ostertagia spp.*, *Trichostrongylus* spp., *Cooperia* spp., and *Nematodirus* spp., and also against *Trichuris* in the caecum and large intestine. There was only limited activity against *Haemonchus* and *Ostertagia* in the abomasum, but efficacy was better against the mature and immature *Trichostrongylus* (Walley, 1961).

Further trials involving both artificial and field infections confirmed these results (Tables 11.1 & 11.2) (Gibson, 1962a; Gordon, 1962; Gibbs and Pullin, 1962; Walley, 1962; Dale *et al.*1962; Broome and Walley, 1963; Thomas and George, 1967; Gibson and Parfitt, 1973). Efficacy against abomasal parasites was more variable for the reasons outlined above (Walley, 1961; Broome, 1961b; Gibson, 1962a; Gordon, 1962; Gibbs and Pullin, 1962) and increased if methyridine was swallowed directly into the abomasum (Gordon, 1962). Adult worms were the most susceptible (86-99%) stage of *Dictyocaulus filaria*, but there was still some useful activity against immature forms (Walley, 1963). Early migratory forms were little affected, but at 10 days postinfection about 50% of the developing lungworms were eliminated and this increased to 66% for 15 day old worms (Walley, 1963). Removing the early stages helped prevent the pathological changes seen with large numbers of migrating larvae (Walley, 1963) and lowered faecal larval excretion for up to 30 days (Skerman *et al.*1968). Methyridine also has some inhibitory effect on larval development in the faeces (Gibbs and Pullin, 1962). Up to 80% efficacy was shown against *Protostrongylus rufescens* (Walley, 1963).

The route of administration did not affect efficacy and methyridine could be given by the oral, subcutaneous or intraperitoneal routes (Walley, 1961; 1962).

11.3.2 Cattle

As with sheep, efficacy of methyridine in cattle is also very good against small and large intestine worms using doses of 200mg/kg (Table 11.3). It was less efficacious against abomasal parasites and very early *Dictyocaulus viviparus* larvae (Groves, 1958; Walley, 1961; 1963). Field trials confirmed these results (Hamilton, 1961; Young, 1961; Macrae, 1961). There was no decrease in efficacy when the intraperitoneal route of administration was added to the oral and subcutaneous routes (Walley, 1962).

11.3.3 Dogs

Only one trial is reported on the efficacy of methyridine in dogs. Using the standard 200mg/kg dose subcutaneously the compound was 100% efficacious against *Trichuris vulpis*, but only had moderate activity against *Toxocara* (64%) and *Ancylostoma caninum* (76%) (Colglazier *et al.*1966).

11.4 SIDE EFFECTS AND TOXICITY

The two major side effects from the routine use of methyridine were reactions at the site of subcutaneous or intraperitoneal injection (Walley, 1961; 1962; Thorpe, 1962; Gracey and Kerr,

1961; Groves, 1961; Macrae, 1961; Gibson, 1962a; Colglazier *et al.*1966) and neurological clinical signs (Groves, 1958; Walley, 1961; Thorpe, 1962; Gracey and Kerr, 1961; Macrae, 1961; Reeves, 1962).

The size of the subcutaneous injection reaction was proportional to the size of the dose. Oedema was present within 24 hours and healed within 7-28 days leaving no trace in most animals. If the injection was not truly subcutaneous but into muscle, the reaction tended to be more severe (Groves, 1961; Thorpe, 1962; Walley, 1962). If the subcutaneous injection was too close to the shoulder joint, animals could be lame for up to four days (Groves, 1958).

Methyridine has a narrow therapeutic margin and occasionally therapeutic doses were found to result in depression, inco-ordination and collapse (Groves, 1958; Macrae, 1961). The intraperitoneal route appeared slightly more toxic than the oral or subcutaneous routes (Walley, 1962; Reeves, 1962). Higher doses of 400mg/kg in sheep and cattle resulted in a neuromuscular block, respiratory depression and death within hours of treatment (Walley, 1961).

There are reports of severe illness and sometimes death in cattle when dosed simultaneously with methyridine and diethylcarbamazine. This has occurred irrespective of the route of administration and the dose. Animals could die within two hours of treatment and on post-mortem showed severe visceral congestion and oedema. Such occurrences were sporadic and unpredictable and could vary in severity within a group of animals (Harrow, 1962).

TABLE 11.1 PERCENTAGE EFFICACY OF METHYRIDINE AGAINST ABOMASAL AND SMALL INTESTINE NEMATODES OF SHEEP

		Abomasum			Small Intestine					References
Dose rate	*H. contortus*	*Ostertagia* spp.	*T.axei*	Immature	*Trich.* spp.	*Cooperia* spp.	*Bunostomum* spp.	*Nematodirus spp.*	Immature	
200 mg/kg Sub-cutaneous (SQ)	18-100	1-99 d2 - 53[1] d7 - 39 d14 - 98.3 d28 - 73.5	0-100	86-95	18-100	8-100	0-100	93-100		(Walley, 1961; Gibson, 1962a; Gibbs and Pullin, 1962; Gibson and Parfitt, 1973)
200 mg/kg oral	0-100	0-99	0-100	54-100	94-99	91-100	50-100	95-100 d3 - 77 d10 - 96 (late 4th stage) d12 - 99.9 (5th or preadult)	58-100	(Walley, 1961; Broome and Walley, 1963; Thomas and George, 1967)
200 mg/kg Intra-peritoneal	78-100	73-100	95-100	74-90	92-100	89-100	71-83	99-100	66-93	(Walley, 1962; 1963)

[1] Indicates 2-day-old larvae and percentage efficacy

TABLE 11.2 PERCENTAGE EFFICACY OF METHYRIDINE AGAINST LARGE INTESTINE AND PULMONARY NEMATODES OF SHEEP

	Large Intestine			Lungs	References
Dose rate	*Oesophagostomum* spp.	*Trichuris* spp.	*Chabertia ovina*	*D. filaria*	
200 mg/kg SQ	7-100	91-100	0-100	41-59	(Walley, 1961; Gibson, 1962a; Gibbs and Pullin, 1962; Gibson and Parfitt, 1973)
200 mg/kg Oral	75-93	100	-	20	(Walley, 1961; Broome and Walley, 1963; Thomas and George, 1967)
200 mg/kg Intra-peritoneal	75-93	99-100	82-100	d1 - 0[1] d15 - 36-67 d20 - 47-67 d25 - 93 d26 - 82-95	(Walley, 1962; 1963)

[1] Indicates 1-day-old larvae and percentage efficacy

TABLE 11.3 PERCENTAGE EFFICACY OF METHYRIDINE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum				Small Intestine				Large Intestine		Lungs	References
Dose	*H. contortus*	*Ostertagia* spp.	*Trich.* axei.	Immatures	*Trich* spp.	*Cooperia* spp.	*Nematodirus* sp.	*Immatures*	*Trichuris* sp.	*Chabertia* sp.	*D. viviparus*	
200mg/kg Sub-cutaneous (SQ)	63-100	51-100	93-100	40-100	90-100	95-100	89-100	60-100	98-100	60-86	0-84	(Walley, 1961)
200mg/kg Oral	79-93	57-99	90-98	62-100	84-97	91-99	93-100	59-89	97-100	67		(Walley, 1961)
200mg/kg Intra peritoneal (IP)	87-100	63-98	89-100	80-90	90-100	94-100	98-100	76-100	96-100	50-88	57-86	(Walley, 1962)
200mg/kg Oral, SQ, IP											d1 - 16-20[1] d5 - 34-43 d8 - 41 d10 - 42-68 d12 - 49 d14- 68 d15 - 59-70 d16 - 48-61 d17 - 61-78 d20- 27-99 d25 - 92 d26 - 97 d30- 75-96 d32 - 98-99	(Walley, 1963)

[1] Indicates 1-day-old larvae and percentage efficacy

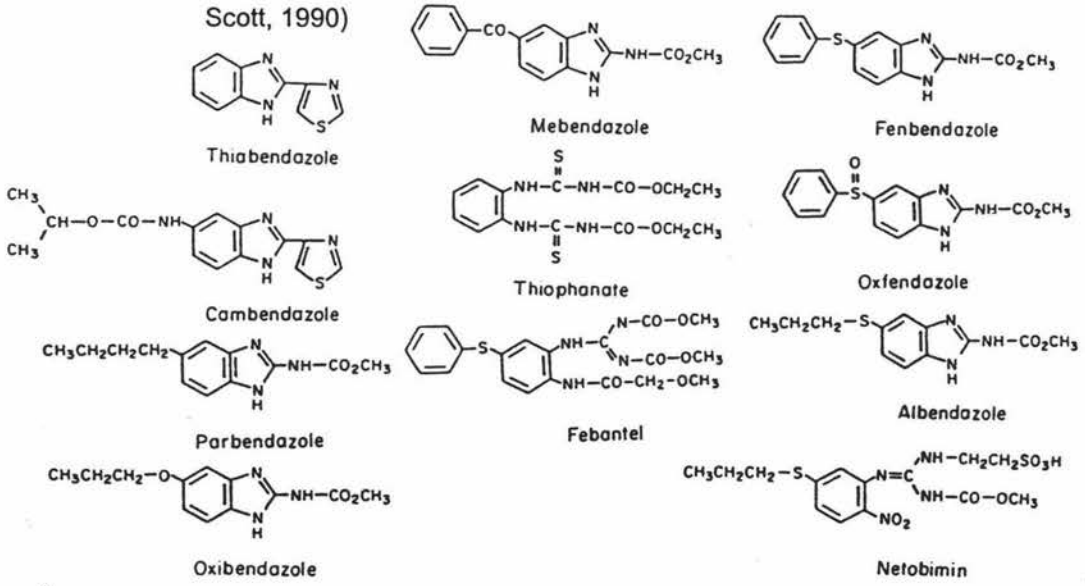
12.0 BENZIMIDAZOLES

12.1 INTRODUCTION

Before the arrival of the benzimidazoles in the early 1960's, most of the available anthelmintics had a limited spectrum of activity. Phenothiazine had been used for many years, and the organophosphates had just started to become popular. However, an initial problem with the organophosphates was their narrow therapeutic index and non-selective inhibition of acetylcholinesterase. The first information on a new and unique compound to replace these was published by Brown *et al.,* (1961). Thiabendazole as it was called, had significant anthelmintic activity against gastrointestinal parasites in sheep, goats, cattle, horses, swine, dogs and poultry. It had complete larvicidal activity *in vitro* at very low doses, and there was no other activity against other microorganisms, and negligible mammalian toxicity. It could be formulated as a drench for therapeutic use, or added to feed or mineral supplements for prophylactic control (Brown *et al.*1961).

Phenzidole, a phenyl benzimidazole was the next compound to be trialed as an anthelmintic in Australia (Forsyth, 1962), but its popularity was limited. It was not until 1967 that parbendazole, the next marketable benzimidazole was produced (Actor *et al.*1967). Over the next 10-15 years a whole series of benzimidazole and pro-benzimidazole compounds were released. These included cambendazole (Egerton and Campbell, 1970), mebendazole (Callear and Neave, 1971), oxibendazole (Theodorides *et al.*1973), thiophanate (Eichler, 1973), fenbendazole (Baeder *et al.*1974), oxfendazole (Averkin *et al.*1975), albendazole (Theodorides *et al.*1976a), febantel (Bankov, 1978) and netobimin ((Nafissi-Varchei, 1983) cited by Delatour *et al* (1986)). The difference among all these compounds was the modification of the side chains attached to the basic benzimidazole ring (Townsend and Wise, 1995).

Figure 12.1 Structures of Benzimidazole and Pro-benzimidazole anthelmintics (McKellar and Scott, 1990)



12.2 PHARMACOLOGY

Benzimidazoles as a class of compounds have the same basic chemical structure, but it was unclear how they exerted their anthelmintic effect. Initially it was thought that the mode of action was the inhibition of the fumarate reductase system (Prichard, 1970; Brander *et al.*1982). Parasites have anaerobic metabolic pathways of energy production dissimilar to those in their aerobic hosts. The essential component of this is the fumarate reductase reaction (Prichard, 1973). Inhibition of this pathway results in a decrease in the anaerobic uptake of glucose by the parasites, and a subsequent decrease in parasite glycogen reserves (Walker and Knight, 1972; Van den Bossche, 1972a; Prichard, 1973; Van den Bossche and De Nollin, 1973; Van den Bossche *et al.*1974; Duwel, 1977; Coles, 1977; Prichard *et al.*1978). When neither thiabendazole nor cambendazole inhibited the fumarate reductase system of thiabendazole resistant *Haemonchus contortus,* this supported the proposal that this was the site of action (Prichard, 1973; Romanowski *et al.*1975). However, when other parasite species such as *Nematodirus spathiger,* an obligate aerobe without a fumarate reductase system, were also shown to be sensitive to the benzimidazoles, the implication was that more than one mode of action existed (Coles, 1977).

Research conducted about the same time showed that mebendazole was absorbed by the intestinal cells of *Ascaris suum,* resulting in microscopically visible physical changes in the parasites (Van den Bossche and De Nollin, 1973; Van den Bossche *et al.*1974). Further investigation suggested that the primary site of action might be damage to the organelles involved in the secretory mechanisms of the intestinal cells. The underlying mechanism was the deterioration of the cytoplasmic microtubules in the intestinal cells (Borgers *et al.*1975). It was proposed that tubulin maybe the receptor for the benzimidazole mode of action in nematode parasites (Friedman and Platzer, 1978; Behm and Bryant, 1979). *In vitro* and *in vivo,* these compounds were found to bind both bovine brain and nematode tubulin (Friedman and Platzer, 1978; Behm and Bryant, 1979). However, there was a difference in potency between nematode and mammalian tubulin in favour of the nematodes, which was thought to explain the selective toxicity of the benzimidazoles (Behm and Bryant, 1979).

Microtubules are an integral part of the cell and are in constant equilibrium with tubulin. Several discrete functions have been ascribed to microtubules at the cellular level. These are formation of the mitotic spindle in cell division, maintenance of cell shape, cell motility, cellular secretion, nutrient absorption and intracellular transport. The disruption of the tubulin-microtubule equilibrium can be seen as leading to a cascade of direct and indirect biochemical/physiological changes resulting in the loss of cellular homeostasis (Lacey, 1988). When an inhibitor binds to the tubulin, it prevents the self association of subunits onto the growing microtubules. This results in 'capping' at the associating end, while the microtubule continues to dissociate from the opposing end, with a net loss of microtubule length and function (Lacey, 1990).

Benzimidazoles are selective tubulin inhibitors and this may explain the basis for many other changes seen eg. inhibition of glucose uptake. There is a structural requirement for the benzimidazoles for them to bind to tubulin effectively. The presence of a carbamate in the 2-position of the benzimidazole ring is essential for potent activity, but the most potent microtubule inhibitory activity was found to reside in the 5-substituted benzimidazole carbamates. Oxibendazole (OXB), parbendazole (PBZ), mebendazole (MBZ) and fenbendazole (FBZ) have 2-6 times greater binding and inhibition of microtubule polymerisation than thiabendazole (TBZ) which is only substituted at the 2-position with a 4-thiazole group (Friedman and Platzer, 1978; Lacey, 1988). The potency of benzimidazoles (mg/kg dose) based on *in vivo* efficacy studies displayed increasing rank order and was positively correlated to efficacy. This was TBZ < CBZ < PBZ <OBZ <MBZ <FBZ ~ABZ ~OFZ (Lacey, 1988). This helped explain the increase in efficacy and the ever decreasing doses required for the more recent benzimidazoles such as fenbendazole and oxfendazole.

While the mode of action was starting to be shown to be associated with microtubule inhibition, Prichard continued to look for a relationship between this and the inhibition of fumarate reductase (1978). Cambendazole, oxfendazole, thiabendazole and fenbendazole were all shown to inhibit fumarate reduction oxidation in *Haemonchus contortus* mitochondria (Prichard *et al.*1978) but this was a consequence of microtubule inhibition rather than a specific mode of action (Lacey, 1988). These observations suggested that all the benzimidazoles affected parasitic helminths similarly. Prichard (1978) proposed that the variation in the spectrum of activity seen with the various members of this family could be due to their pharmacokinetic behaviour. A comparison was made of the effects of the plasma concentration and duration of thiabendazole, fenbendazole and oxfendazole against benzimidazole resistant *Haemonchus contortus,* and *Trichostrongylus colubriformis,* and was shown to correlate with the period over which high plasma concentrations were maintained. Further support for this was provided when it was shown that infusion or multiple drenching of cattle with thiabendazole was efficacious in removing 85-92% of inhibited *Ostertagia ostertagi* larvae. Normally, a single drench of thiabendazole is quite ineffective against these larvae (Prichard *et al.*1978).

A knowledge of both the mode of action and the pharmacokinetics of the benzimidazoles is important in understanding the differences in efficacy found between the various members of this class. A balance is needed between the duration of exposure of the parasite to the benzimidazole (to allow binding to tubulin), and the pharmacokinetics of the drug in the host (Lacey, 1988). More potent binding and slower elimination rates allow for maximum duration of microtubule binding. However, efficacy is also dependent on the rate of removal of the parasite from its preferred site. If this is slower than the rate of dissociation of the drug with the microtubules, then recovery of the parasite can occur (Lacey, 1990).

The early compounds, thiabendazole and cambendazole, are thiazolyl benzimidazoles and their

anthelmintic activity is due to the parent compound (Townsend and Wise, 1995; Hoff *et al.*1970; McKellar and Scott, 1990). However, one problem was that these compounds were metabolised very quickly to inactive metabolites (Townsend and Wise, 1995). Subsequently, modifications of the benzimidazole ring were made to limit the rate of metabolic inactivation by the liver to increase the persistence of the anthelmintic (Townsend and Wise, 1995). Some of these metabolic reactions produce sulphoxide metabolites more anthelmintically active than the parent compound eg. oxfendazole is fenbendazole sulphoxide (Marriner and Bogan, 1981; Ngomuo *et al.*1984; Short *et al.*1987) and ricobendazole is albendazole sulphoxide. Both compounds have been used as anthelmintics in their own right (Delatour *et al.*1987; Marriner and Bogan, 1981; McKellar and Scott, 1990).

Several pro-drugs have also been developed such as thiophanate (Vonk and Sijpesteijn, 1971), febantel (Delatour *et al.*1985; Courtney *et al.*1988) and netobimin (Delatour *et al.*1986; Lanusse *et al.*1990). These are converted to active benzimidazole compounds within the animal. Thiophanate cyclises into a benzimidazylcarbamate ester, lobendazole (Actor *et al.*1967; McKellar and Scott, 1990). Febantel is a phenylguanidine compound and is metabolised into fenbendazole and subsequently oxfendazole (Courtney *et al.*1988). Netobimin is a nitro phenyl guanidine that following reduction by the microflora of the rumen or intestine is cyclised into albendazole (Delatour *et al.*1986; Townsend and Wise, 1995).

The benzimidazoles as a class are generally very insoluble in water. Their bioavailability and pharmacodynamics are influenced by their formulation and presentation. Formulation changes have been made by various substitutions at the 5-position of the benzimidazole ring. This has resulted in a further decrease in solubility that after oral administration results in delays before peak blood levels are reached. These changes have given each benzimidazole compound its own unique plasma profile. These plasma profiles have been used and abused in the marketing of these compounds (Marriner and Bogan, 1981). The time to peak plasma concentrations of some benzimidazole compounds are provided in Table 12.1.

One other effect of this decrease in solubility is the influence on the partitioning of anthelmintics. More soluble compounds such as TBZ are better absorbed from the rumen than a less soluble compound such as FBZ (Prichard *et al.*1981). This allows more FBZ to remain in the rumen for longer, where it consequently acts as a reservoir. As well, this allows more anthelmintic to pass into the abomasum along with the digesta. However, some FBZ absorbed is also secreted back into the small intestine (Prichard *et al.*1981). This is an important factor in efficacy. A divided dose of oxfendazole given via multiple intravenous injections was as effective against nematodes as when given as a total single oral dose. Most likely once the drug is in the blood stream it cycles across the gutwall between the vascular system and the gastrointestinal tract. This exposes the worms in the mucosa of the abomasums and small intestine to the recycling anthelmintic to a greater extent

than the drug solely contained in passing digesta (Hennessy and Prichard, 1981). Because the less soluble drugs remain in plasma for longer and since it is assumed there is an equilibrium in the drug concentration between the plasma and the gastrointestinal tract, extending the time the parasites are exposed to effective concentrations of the anthelmintic increases efficacy (McKellar and Scott, 1990).

TABLE 12.1 TIME TO PEAK PLASMA CONCENTRATION FOLLOWING BENZIMIDAZOLE ADMINISTRATION TO SHEEP OR CATTLE

Benzimidazole	Species	Route of Administration	Time to Peak Plasma Concentration (hours)	References
Thiabendazole	Cattle	Oral	4 - 7	(Tocco *et al.*1965)
Parbendazole	Sheep	Oral	6	(Actor *et al.*1967; DiCuollo *et al.*1974)
Thiophanate	Not identified	Oral	8	(Brander *et al.*1982)
Oxibendazole	Sheep	Oral	6	(Theodorides *et al.*1973)
Fenbendazole	Cattle	Oral	30-48	(Anon, 1976; Duwel, 1977)
Oxfendazole	Cattle	Oral	24-36	(Ngomuo *et al.*1984)
	Cattle	Intra-ruminal	24-36	(Ngomuo *et al.*1984)
Febantel	Cattle	Oral	12-24	(Delatour *et al.*1985)
Albendazole	Cattle	Oral	15	(Van den Bossche *et al.*1982)
Netobimin	Cattle	Oral	7-10	(Lanusse *et al.*1993)

Several authors have shown that the pharmacokinetics differ between similar species such as cattle, sheep and goats. In some cases, such as with thiabendazole, cattle metabolise the compound more rapidly, which limits the period of anthelmintic exposure to the parasite (Weir and Bogan, 1985). Following the argument above of efficacy being associated with duration of exposure, this may explain why, in cattle, thiabendazole has lower efficacy against *Dictyocaulus viviparus* and inhibited *Ostertagia ostertagi* larvae than against *Dictyocaulus filaria* and inhibited *Ostertagia circumcincta* larvae in sheep (McKellar and Scott, 1990).

The benzimidazoles are extensively metabolised in mammals following oral administration (Gotschall *et al.*1990). This is influenced not only by the species, but also the chemical structure of the compound. Metabolism depends heavily on the substituent present at the C-5 of the benzimidazole nucleus (Gotschall *et al.*1990). Compounds with an aromatic group substituted at the 5-position such as mebendazole, fenbendazole and oxfendazole, undergo oxidation, hydroxylation and conjugation as a glucuronide or sulphate ester. This increases polarity of the compounds which

facilitates their excretion in bile (45-60% of a dose). Aliphatic compounds (those with a water-soluble side chain) such as albendazole and parbendazole undergo simple oxidation and hydroxylation that produce metabolites that are sufficiently water soluble for excretion predominantly in urine (70-80% of a dose), with a smaller proportion in bile (12-15%) (Lacey, 1988; Gotschall *et al.*1990).

Because of microtubule inhibition, some benzimidazoles can also affect the metabolism of other members of the family, and potentiate anthelmintic activity. Parbendazole potentiated the efficacy of oxfendazole against benzimidazole resistant *Haemonchus contortus* and *Trichostrongylus colubriformis* (Hennessy *et al.*1985). However, this has never been developed any further than laboratory trials.

Several other factors have also been identified as influencing the efficacy of benzimidazoles. These include; volume of the dose; lower dose volumes (1ml/10kg live weight) providing better absorption parameters than higher dose volumes (1ml/5kg) (Hennessy *et al.*1997): composition of the diet; animals fed concentrates have larger areas under the plasma concentration versus time curve (Taylor *et al.*1992): and concurrent level of nutrition (Prichard, 1980). Other factors are the route of administration, including the effect of the oesophageal groove (McEwan and Oakley, 1978; Prichard and Hennessy, 1979; Prichard, 1980; McKellar and Scott, 1990) and the level of parasite infection (Smith and Archibald, 1968; Prichard, 1980; McKellar and Scott, 1990). Severe infection can decrease the absorption of anthelmintic and increase gut transit times (Prichard, 1980; McKellar and Scott, 1990).

The most debated of these factors has been the influence of the oesophageal groove on efficacy. It was proposed that because the duration of exposure of the parasite to the anthelmintic is so important, if the anthelmintic were deposited directly into the rumen, it would act as a reservoir for the slow release of anthelmintic into the abomasum (McEwan and Oakley, 1978). The effects of this would be to increase the efficacy of the anthelmintic. Drenching of calves (weighing 125-205 kg) with a combination of fenbendazole and a coloured dye showed that there was evidence of rumen bypass in 50% of animals (McEwan and Oakley, 1978). After dosing sheep with a combination of oxfendazole and glucose, it was found that 29% of animals had high plasma glucose levels, suggesting a complete bypass of the rumen. In this trial, efficacy was reduced against benzimidazole resistant *Haemonchus contortus* (91 to 45%) (Prichard and Hennessy, 1979). A result of this was the development of an oxfendazole intra-ruminal injection designed to preclude the possibility of ruminal bypass due to oesophageal groove closure. However, as others have pointed out, several other factors are also important in achieving adequate plasma benzimidazole concentrations. Ruminal bypass was being overemphasised as a cause of therapeutic failure, (Marriner and Bogan, 1979; Prichard, 1980; Charleston, 1981).

The lack of solubility of the benzimidazole family initially limited the method of administration to a

variety of oral formulations. These include suspensions, pastes, granules, boluses, powder, pellets, feed additives, and continuous or pulse release intra-ruminal devices. As well, a formulation of oxfendazole is available for intra-ruminal injection. More recently, with advances in formulation chemistry, a pour-on formulation of oxfendazole has been released for sale in New Zealand. The development of the pro-benzimidazole netobimin has provided a compound sufficiently water soluble that it can be given as either an oral drench, a parenteral injection or administered in drinking water.

12.3 EFFICACY

12.3.1 Thiabendazole

The year 1961 saw the first of many publications on the remarkable efficacy of benzimidazole compounds against mammalian parasitic nematodes. In laboratory trials, a single oral dose 50 mg/kg of thiabendazole removed more than 95% of worms belonging to ten genera of gastrointestinal parasites (*Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Bunostomum*, *Strongyloides*, *Chabertia*, *Trichuris* and *Oesophagostomum*) (Brown *et al.*1961). This was a considerable improvement on the efficacy seen with other current anthelmintic compounds such as phenothiazine and the organophosphates, with a greater degree of safety. Further laboratory and field trials confirmed the initial efficacy and the results of the published trials in cattle are documented in Table 12.2. The initial recommended dose rate was between 50-67mg/kg which was higher than that for sheep due to the more rapid metabolism in cattle (Bailey *et al.*1961). At this dose, thiabendazole gave good results against adult *Haemonchus*, *Trichostrongylus* spp., *Bunostomum*, *Oesophagostomum* and *Chabertia*, but there was some degree of variation against *Ostertagia*, *Cooperia* and *Nematodirus*. This was particularly noticeable for the immature stages of *Ostertagia* and *Cooperia* (Baker and Douglas, 1962; Keith, 1963; Smith and Archibald, 1968; Rubin *et al.*1965; Brander *et al.*1982). Increasing the dose rate to 100-110mg/kg improved efficacy against adult *Ostertagia* and *Cooperia* (Baker and Douglas, 1962; Bell *et al.*1962; Smith and Archibald, 1968; Smith and Archibald, 1969) but only resulted in 64% mortality of early 4th stage *Ostertagia* (Douglas and Baker, 1968) and 48-69% for 10 day old *Cooperia* spp. Further increasing the dose rate to 136 and 176 mg/kg still did not improve efficacy against early 4th stage or inhibited larvae (Anderson, 1977). No efficacy was seen against adult or larval stages of *Dictyocaulus viviparus* at a dose of 110 mg/kg (Smith and Archibald, 1969). This was in contrast to its efficacy against *Dictyocaulus filaria* in sheep (Smith and Archibald, 1969), and it was proposed that this may be due to a difference in metabolism of the drug between the two species (Tocco *et al.*1965; Weir and Bogan, 1985; McKellar and Scott, 1990) or difference in parasite susceptibility. Other advantages of this compound were its ability to inhibit nematode egg production and interfere with larval development, limiting pasture contamination and future infection(Brown *et al.*1961).

12.3.2 Phenzidole

Phenzidole, a 2' phenyl benzimidazole was trialed in sheep in 1962. Doses of 200-250 mg/kg were shown to be as effective as 50mg/kg of thiabendazole. When combined with phenothiazine at a dose of 100mg/kg of phenzidole and 300mg/kg of phenothiazine, it was claimed that phenzidole potentiated the efficacy of phenothiazine (Forsyth, 1962). However, no synergism was shown with this or other combinations of these two compounds (Egerton *et al.*1963). This compound was not trialed in cattle.

12.3.3 Parbendazole

Benzimidazoles developed after thiabendazole had structural modifications at the 5-position to slow the rate of metabolism and excretion (Prichard, 1978). Parbendazole is a benzimidazole carbamate, like mebendazole, oxibendazole, fenbendazole, oxfendazole and albendazole. It was 1967 when the first results of efficacy trials were published (Actor *et al.*1967). The dose for cattle (20-40 mg/kg) was lower than that for thiabendazole (66-88 mg/kg), but it still had similar levels of efficacy against adult *Haemonchus* and *Oesophagostomum* and adult and immature *Trichostrongylus* spp. (Theodorides *et al.*1968; Rubin, 1968; Rubin, 1969; Ross, 1970; Benz, 1968). At the lower end of the dose range (20 mg/kg), there was good removal of adult *Ostertagia* spp. and adult and larval *Cooperia* spp., but there was still a variable response against the larval stages of *Ostertagia*, especially 10 day-old larvae, *Nematodirus* and *Trichuris* (Theodorides *et al.*1968; Rubin, 1968; Ross, 1970). The use of in-feed medication at doses of 20-40 mg/kg appeared as efficient as the use of oral drenches (Benz, 1968; Bradley, 1968; Rubin, 1969). None of the published trials evaluated efficacy against *Dictyocaulus viviparus*. More specific efficacy details are presented in Table 12.3.

12.3.4 Cambendazole

Cambendazole is the only other thiazolyl benzimidazole besides thiabendazole. The structural modification to this compound was the addition of an acylamino group at C-5 that appeared to inhibit its metabolism (Hoff *et al.*1970). It was released commercially in 1970 as drench, paste, bolus and in-feed formulations. Like the other benzimidazoles, it was extremely potent in preventing the development of helminth eggs and larvae (Hoff *et al.*1970). The lowest efficacious dose was between 22-44 mg/kg (Table 12.4). However, like thiabendazole and parbendazole, adult and larval *Ostertagia*, and adult *Bunostomum* and *Nematodirus* were the dose-limiting species (Egerton *et al.*1970; Baker and Walters, 1971; Ciordia and McCampbell, 1971a; Benz, 1971a; Gibbs and Gupta,

1972). Increasing the dose up to 60mg/kg did improve efficacy against immature *Ostertagia* sometimes (Gibbs and Gupta, 1972) but some trials with lower doses had similar levels of efficacy (Ciordia and McCampbell, 1971a; Cairns *et al.*1975). Doses of 20 mg/kg were highly effective against adult *Dictyocaulus viviparus* (Hoff *et al.*1970; Baker *et al.*1972) though there was some variability seen even at doses as high as 30-40 mg/kg (Gibbs and Gupta, 1972; Rubin, 1972; Cairns *et al.*1975; Lyons *et al.*1978). Like *Ostertagia*, immature *Dictyocaulus* did not respond very well to cambendazole (Baker *et al.*1972; Cairns *et al.*1975).

12.3.5 Mebendazole

Mebendazole at 15 mg/kg was more efficacious against adult and larval forms of *Bunostomum* than either thiabendazole, parbendazole and cambendazole (Table 12.5). It was just as effective for adult and larval *Haemonchus* and *Cooperia*, but only very low efficacy was seen for adult *Ostertagia* (41%). This was unexpected and could not be explained (Westhuizen *et al.*1984).

12.3.6 Oxibendazole

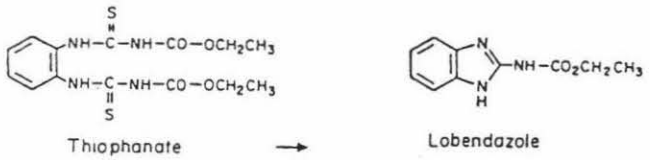
The doses of anthelmintic were continuing to decrease in size as the potency and pharmacokinetics of the drugs was improved. Using both critical and controlled trials, doses of 10 mg/kg removed between 95-100% of *Ostertagia*, *Trichostrongylus* spp., *Cooperia*, *Strongyloides* and *Oesophagostomum* (Table 12.6) (Theodorides and Chang, 1974; Herlich, 1975). It was noticeable that the efficacy against *Haemonchus* dropped off slightly for this anthelmintic (88-90%) at the lower dose rates of 5-7.5 mg/kg (Theodorides and Chang, 1974). Published trials with this drug also concentrated more on artificial infections against larvae of certain ages. There was still limited effect against immature *Ostertagia* whether the drug was given at 10mg/kg as a drench or at 15 mg/kg as a feed premix (Herlich, 1975; Theodorides *et al.*1976b; Crowley *et al.*1976). This drug has been mostly used in horses.

12.3.7 Thiophanate

This was the first pro-benzimidazole. It is converted into lobendazole, a benzimidazylcarbamate ester (Figure 12.2) (Actor *et al.*1967; McKellar and Scott, 1990). Doses of 50 mg/kg were shown to be highly efficacious (97-100%) against *Haemonchus*, *Ostertagia* and *Trichostrongylus* spp., but against *Cooperia* and *Nematodirus* efficacy decreased to between 54-85% (Table 12.7) (Eichler, 1973; Fabiyi *et al.*1979). Increasing the dose to 75 mg/kg and above improved the efficacy against *Cooperia* to >99% (Baines and Bell, 1980; Duncan *et al.*1979). However, like the early

benzimidazoles, efficacy against inhibited *Ostertagia* larvae was low to non-existent when given as a single oral drench (Eichler, 1973). Doses as high as 112 mg/kg only removed 68% of early 4th stage larvae (Duncan *et al.*1979). However, when given in feed at 20 mg/kg for five days, efficacy against inhibited L4 increased to 97% (Duncan *et al.*1979).

Figure 12.2 Structural changes in the metabolic conversion of Thiophanate to Lobendazole



12.3.8 Fenbendazole

The real breakthrough in increased efficacy occurred with the development of fenbendazole (Baeder *et al.*1974). At doses as low as 5 mg/kg, there was greater than 95% efficacy for adult and immature stages of *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Cooperia*, *Bunostomum*, *Oesophagostomum* and *Dictyocaulus* (Table 12.8) (Duwel, 1974; Todd *et al.*1976; Tiefenbach, 1977; Crowley *et al.*1977; Craig and Bell, 1978; Benz and Ernst, 1978a; Samizadeh-Yazd and Todd, 1978; Duwel, 1979; Williams *et al.*1981a; 1983; Duwel and Kirsch, 1981; Yazwinski *et al.*1985; Blagburn *et al.*1986). This was the most broad spectrum anthelmintic known at the time of its introduction. Oral formulations have included drench, paste, feed premixes, feedblocks and slow release boluses.

Initially it was thought that this compound had a high efficacy against inhibited *Ostertagia ostertagi* larvae (Duncan *et al.*1976). When attempts were made to replicate these findings, some authors could (Tiefenbach, 1977), but many were unable to (Anderson, 1977; Lancaster and Hong, 1977; Elliott, 1977; Searson and Doughty, 1977) even when trials were done on the same properties over subsequent years (Williams *et al.*1979b; 1981a; 1984; 1991; Lancaster *et al.*1981). Debate went on in the literature over the next 15 years and the results are summarised in Table 12.9. Duncan and co-workers who had started the initial debate proposed that there were several factors responsible for the variations seen by other researchers (Duncan *et al.*1976; 1977). These were, the time of the year of treatment influencing the metabolic rate of the larvae, host immunity making larvae less susceptible, effect of the oesophageal groove on location of the drench, and age of the animals treated. Duncan *et al.* (1978) could repeat the results of an earlier trial by which 89-97% of inhibited larvae were removed when a dose of 7.5 mg/kg was used either early or late in the season whereas Lancaster *et al.* (1981) still could not get repeatability and concluded that treatment with a single dose of fenbendazole was not an entirely reliable method of preventing winter ostertagiosis. Others also found it difficult to obtain consistent results (Williams *et al.*1979b; 1981a; 1984; 1991). Various explanations were offered (Snider and Williams, 1980; Williams *et al.*1984; Snider *et al.*1985). The

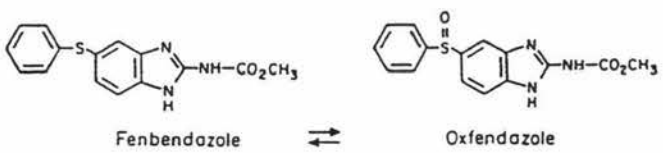
overall conclusion was that while a single dose of 7.5 mg/kg of fenbendazole will remove adult and immature *Ostertagia* larvae in most animals, a small and ill-defined proportion of cattle may harbour substantial numbers of O. *ostertagi*, particularly inhibited early L4 after treatment. These cattle must be regarded as a prime source of infection for the following grazing season. Therefore control involves a combination of pasture management and appropriately timed use of a highly efficient anthelmintic (Williams *et al.*1984).

Feedblocks and in-feed supplements have also been used. Doses in feed as low as 0.25 mg/kg for up to five days would remove 95-100% of *Haemonchus*, *Ostertagia*, *Trichostrongylus* spp., *Cooperia* and *Oesophagostomum* (Crowley *et al.*1977) which was comparable to cattle having access to 1 mg/kg/day of a feedblock for up to 10 days (McBeath *et al.*1977; 1981).

12.3.9 Oxfendazole

This compound, while discovered as an anthelmintic in its own right, is the active sulphoxide metabolite of fenbendazole (Figure 12.3).

Figure 12.3 Structure and metabolic conversion pathway of Fenbendazole to Oxfendazole



Consequently it has almost the same spectrum of action. Both oxfendazole and fenbendazole are very insoluble and along with their increased potency for binding to tubulin, doses a tenth to a twentieth of that of thiabendazole (2.5-5 mg/kg) are highly efficacious (Table 12.10). Use of the intra-ruminal administration route to avoid closure of the oesophageal groove is as efficacious as oral administration (Bairden *et al.*1983). Oxfendazole has also been formulated in slow-release (Anderson and Laby, 1979) and pulse-release intra-ruminal capsules (Jacobs *et al.*1986), but it was the pulse-release formulation that was commercially developed, predominantly for use in the Northern Hemisphere. It is designed to release a dose of 750 mg of oxfendazole at 21 day intervals for five doses. The rationale behind this formulation type is that in the case of lungworm, the anthelmintic would destroy all invading lungworms before they reached maturity. This would suppress larval production and consequently prevent the accumulation of pathogenic numbers of larvae on the pasture. The use of a 21-day interval would allow some migration to occur which might induce some degree of immunity (Jacobs *et al.*1986). Challenge trials have shown calves do develop some level of protective immunity to lungworm (Jacobs *et al.*1986; 1989; Vercruysse *et al.*1987; Fisher and Jacobs, 1995). Several field trial have shown that these products also will reduce faecal egg counts and worm numbers of *Ostertagia* and *Cooperia* while still allowing calves

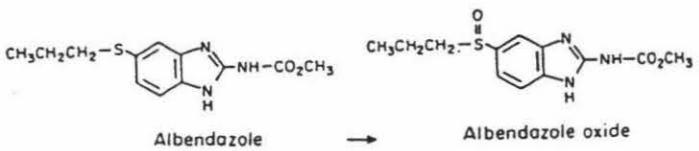
to develop a degree of immunity (Vercruysse *et al.*1987; Mitchell, 1987; Jacobs *et al.*1987a; 1987b; Herbert and Probert, 1987; Borgsteede *et al.*1988; Holmes *et al.*1991; Fisher and Jacobs, 1995).

The same issue about the efficacy against inhibited larvae of *Ostertagia ostertagi* has also been debated. Trials with doses ranging from 0.625-6.25 mg/kg, showed variable degrees of efficacy (Table 12.11).

12.3.10 Albendazole

Albendazole was the last of the benzimidazole carbamates to be released commercially. The parent drug itself does not remain in the body for long, quickly being metabolised to the active sulphoxide (Figure 12.4). Albendazole sulphoxide is slightly more soluble than either fenbendazole and oxfendazole, and has a shorter duration in the bloodstream. However, it still maintains efficacy of 98% and higher at doses of 5 mg/kg for *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Bunostomum*, *Oesophagostomum* and *Dictyocaulus* (Table 12.12) (Theodorides *et al.*1976c; Benz and Ernst, 1977; Williams *et al.*1977b; Theodorides *et al.*1980). Against inhibited larvae of *Ostertagia* it has slightly less efficacy than either fenbendazole or oxfendazole, even at doses of up to 15 mg/kg (Theodorides *et al.*1976c; Herlich, 1977; Theodorides *et al.*1980; Williams *et al.*1979a; 1981b; Williams, 1991).

Figure 12.4 Structure and metabolic conversion pathway of Albendazole to Albendazole Sulphoxide

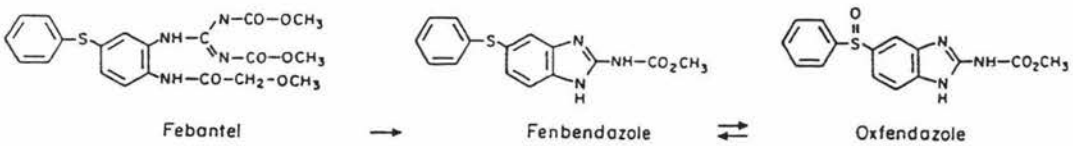


12.3.11 Febantel

This was the second pro-benzimidazole to be released commercially. Febantel is a guanidine derivative and is metabolised into fenbendazole, oxfendazole, a sulphoxide analogue of febantel and a sulphone analogue of oxfendazole (Figure 12.5). Its anthelmintic activity probably results from its metabolism to fenbendazole and oxfendazole (Courtney *et al.*1988). Febantel has good efficacy against all species at doses as low as 5 mg/kg (Table 12.13). Although several trials showed more variability with a 7.5 mg/kg dose, especially against *Haemonchus*, *Ostertagia*, and the larval stages of *Trichostrongylus axei*, *Cooperia* and *Dictyocaulus viviparus* (Grelck *et al.*1978; Hoberg *et al.*1988; Williams *et al.*1988). Increasing the dose to 7.5 mg/kg increased efficacy against these species and stages to >99% (Grelck *et al.*1978; Ciordia *et al.*1982; Blagburn *et al.*1989). Against inhibited

Ostertagia ostertagi larvae, there was as much variability with doses from 2.5-10 mg/kg as had been shown for fenbendazole and oxfendazole (Grelck *et al.*1978; Ciordia *et al.*1982; Hoberg *et al.*1988; Williams *et al.*1988; Blagburn *et al.*1989; Stuedemann *et al.*1990). Febantel is also ovicidal like the other members of the benzimidazole family. Doses as low as 0.5-2.5 mg/kg resulted in complete inhibition of embryonic development of *Haemonchus contortus* eggs. This action is very rapid, with up to 90% decrease in the number of larvae recovered within 3-6 hours after treatment (Thomas, 1979).

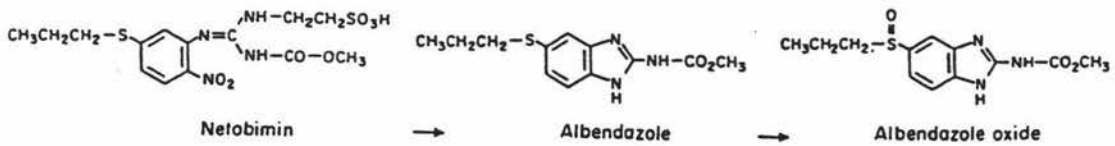
Figure 12.5 Structure and metabolic conversion pathway of Febantel to the active metabolites Fenbendazole and Oxfendazole



12.3.12 Netobimin

Netobimin was the last pro-benzimidazole to be released. It is biotransformed in the animal following reduction of a nitro group by the microflora of the gastrointestinal tract, and then cyclised into albendazole. Albendazole is then rapidly oxidised to albendazole sulphoxide (Figure 12.6) (Delatour *et al.*1986; Lanusse *et al.*1990; 1991; 1993). Because of its solubility, it can be administered either parenterally or orally. Trials showed greater than 95% efficacy at doses of 7.5, 15 and 20 mg/kg against adult and immature *T. axei* and adults of *Haemonchus* and *Ostertagia*, with *Cooperia* efficacy decreasing to 89.5% at the 7.5 mg/kg dose rate (Table 12.14) (Williams *et al.*1985; Duncan *et al.*1986; Prichard, 1986a; Richards *et al.*1987). Only at the higher dose rate of 20 mg/kg was there any efficacy against developing and early 4th stage *Ostertagia* larvae (Prichard, 1986a; Duncan *et al.*1986; Yazwinski *et al.*1992). Administration in drinking water at doses of 2.8mg/kg/day for seven days was shown to suppress *Dictyocaulus* larvae and trichostrongyle egg output. On post mortem, this reduction in larvae and egg output was shown to correlate with a reduction of 99.9% of *Ostertagia*, 96.9% of *Cooperia* and nearly 100% *Dictyocaulus viviparus* (Downey, 1987).

Figure 12.6 Metabolic conversion pathway of Netobimin to Albendazole and Albendazole Sulphoxide



12.4 SIDE EFFECTS AND TOXICITY

The benzimidazoles as a family are very safe compounds. While their mechanism of action is to bind to tubulin, they have a higher avidity for nematode tubulin compared with mammalian tubulin (Lacey, 1990). There are rare reports of acute toxicity in sheep and cattle. In calves treated with 200 and 400 mg/kg thiabendazole, some animals showed dyspnea and excessive salivation that disappeared after 24 hours (Bell *et al.*1962) while other trials at the same dose showed no ill effects (Keith, 1963). In a clinical trial evaluating the pharmacokinetics of thiabendazole, three out of six steers collapsed and died after an oral dose of 700 mg/kg (eight times the therapeutic dose rate) (Hennessy and Prichard, 1979).

Cambendazole has produced fatal pulmonary oedema and hydrothorax in cattle, which has been associated with the feeding of concentrates shortly before dosing (Hogg, 1978; Main and Vass, 1980; Brander *et al.*1982). Doses of up to three times the therapeutic dose rate have also resulted in inappetance and listlessness in cattle (Brander *et al.*1982). High doses of thiophanate (200 mg/kg) have caused renal failure in sheep, though calves dosed with up to 5000 mg/kg showed no clinical effect. This may be associated with the more rapid oxidation of benzimidazoles seen in cattle. Biochemistry suggested there was some level of hepatic dysfunction in all of these calves (Eichler, 1974).

Parbendazole was the compound that first showed that this group of drugs could be teratogenic. Sheep dosed at twice the therapeutic dose during early pregnancy produced lambs with skeletal abnormalities. These tended to be limited to the long bones (Saunders *et al.*1974). Other researchers reproduced the lesions (Szabo *et al.*1974; Middleton *et al.*1974; Shone *et al.*1974), especially when ewes were dosed with twice the therapeutic dose (60 mg/kg) on day 17 of pregnancy (Middleton *et al.*1974). However, these effects could not be reproduced in cattle (Miller *et al.*1974). Oxibendazole also showed no acute toxic effects in ruminants and laboratory animals (Theodorides *et al.*1973; Theodorides *et al.*1977), but in sheep it was embryotoxic during early pregnancy (Delatour *et al.*1976). Albendazole has also been found to produce abnormalities in lambs when ewes are dosed during early pregnancy (Prichard, 1978; Brander *et al.*1982; Theodorides *et al.*1993). In cattle, dosed with 25 mg/kg on day 7 or 14 of gestation, there was a decrease in the conception rate (Theodorides *et al.*1993). Neither mebendazole, oxfendazole nor fenbendazole appear to exert any of these reproductive effects in cattle (Marsboom, 1973; Anon, 1976; Van den Bossche *et al.*1982).

When cattle were dosed with a bromosalan and either fenbendazole (James, 1979) or oxfendazole (Anon, 1978), toxic signs and deaths occurred. It has been recommended there should be a minimum of a week between treatments with these benzimidazoles and a bromosalan (James, 1979; Piercy *et al.*1979).

Overall, many millions of doses of these drugs have been given to animals and the side effects noted above have been negligible when compared with the therapeutic benefits.

TABLE 12.2 PERCENTAGE EFFICACY OF THIABENDAZOLE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	
50-55 mg/kg drench	80-100[1]	13-83	97-100	100	12-99.1					(Baker and Douglas, 1962; Keith, 1963; Smith and Archibald, 1968)
67 mg/kg drench		68-99 d10-0-39[2]			0-98 d10-48-99					(Rubin *et al.*1965)
88 mg/kg drench		66-77 L4 - 24-72								(Anderson, 1977)
90-140 mg/kg bolus		94.7-100		100	5-100					(Smith and Archibald, 1968)
100mg/kg bolus	99-100	99.6	100		93					(Bell *et al.*1962)
100-110mg/kg drench	97	95-100 d10-3-97 Imm-95[3]	99-100	91-100 Imm-100	17-100 d10-64-99 Imm-100					(Baker and Douglas, 1962; Bell *et al.*1962; Ames *et al.*1963 Stober and Ende, 1965; Rubin *et al.*1965; Smith and Archibald, 1969)
132 mg/kg drench		40-87 L4-24-72								(Anderson, 1977)
176 mg/kg drench		59-92 L4-24-72								(Anderson, 1977)

[1] Efficacy based on Faecal Egg Count reduction. Highly effective 80-100%, effective 60-80% and ineffective <60%

[2] Indicates 10-day-old larvae and percentage efficacy

[3] Imm Immature nematodes

TABLE 12.3 PERCENTAGE EFFICACY OF PARBENDAZOLE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	
20mg/kg drench	100	98-99.2 d7-13-71[1] d10-20-62 d14-63-93	100	0	100 d7-100 d10-100 d14-100	100 d7-84-95 d10-100 d14-95-100	*Strongyloides papillosus* 0	83.3	100	(Theodorides *et al.*1968; Rubin, 1968)
20mg/kg feed premix		d7-22-76 d14-44-59			d7-100 d14-100	d7-95-100 d14-89-95				(Rubin, 1968)
30mg/kg drench	100	90-100 d3-89.2 d10-35.9 d14-96	99-100	67-100	99-100 d3-99.6 d10-98.8 d14-100	d14-100	100 low no.		100	(Rubin, 1968; 1969; Benz, 1968; Ross, 1970)
30mg/kg 22% feed premix	100	100	99-100	83-100	98.6-100		100 low no.		98.9-100	(Benz, 1968; Rubin, 1969)
30mg/kg 3% pellets	100	85-100	99-100	83-100	100		100 low no.		100	(Benz, 1968; Rubin, 1969)
40mg/kg drench	100	100	100	95.6	100		*Strongyloides papillosus* 0	83.3	100	(Theodorides *et al.*1968)

[1] Indicates 7-day-old larvae and percentage efficacy

TABLE 12.4 PERCENTAGE EFFICACY OF CAMBENDAZOLE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	
10-11 mg/kg drench	85.3-99	77-93 L4 - 0[1]	97.2	32	82-90	97-99	65			(Egerton *et al.*1970; Benz, 1971a)
13.33 mg/kg drench							*S. papillosus* 100			(Restani and Borrelli, 1971)
15-16.5 mg/kg drench	88-99	0-96 L4-0-69 L5 - 0[2] Imm-77-80[3]	92.2-96		90-99.6 Imm-81-87	98.6-100	71		34-97 Imm-9	(Egerton *et al.*1970; Baker and Walters, 1971; Benz, 1971a; Gibbs and Gupta, 1972; Cairns *et al.*1975)
20 mg/kg drench	84-100	82-99 L3-79 L4-0-52 L5-75 Imm-64-99	97-100	43-81 L4-100 Imm-48	93-100 L4-100 Imm-68-99	99	*S. papillosus* 100		0-100 Imm-53-82	(Baker and Walters, 1971; Benz, 1971a; Restani and Borrelli, 1971; Gibbs and Gupta, 1972 ; Kistner and Lindsey, 1974; Cairns *et al.*1975)
20-23 mg/kg paste	99-100 L4-99*	78-97.2 L4-73 L5-61**	99-100 L4-95		92-99 L4-93 L5-93	99				(Benz, 1973; Cairns *et al.*1975)
22-23 mg/kg drench	98-100	87-98 L4-0	99	77	98-99	99	0	95		(Egerton *et al.*1970; Restani, 1971)

[1] L4 4th larval stages
[2] L5 5th larval stages
[3] Imm Immature stages

TABLE 12.4 CTD. PERCENTAGE EFFICACY OF CAMBENDAZOLE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	
25 mg/kg drench	93-100 Imm-87-99	70.7-99.5 L3-86[1] L4-0-79[2] L5-43[3] Imm-41-99[44]	98.5-100 L4-99	33-87.5 L4-91 Imm-0	95-100 L4-94 Imm-72-98	99			60-100 Imm-4-95	(Baker and Walters, 1971; Benz, 1971a; 1973 Gibbs and Gupta, 1972; Baker *et al.* 1972; Kistner and Lindsey, 1974; Cairns *et al.* 1975)
25mg/kg paste	99 L4-99	92-95 L4-88-97 L5-85	99 L4-95		99 L4-91 L5-93	99			98	(Cairns *et al.*1975)
25mg/kg bolus	100	96.9-97.2 Imm-100	98.7-100		98.3-99.7					(Benz, 1971a; 1971b)
25mg/kg bolus 14days apart	100	99.1 Imm -100	100		99.7					(Benz, 1971b)
25mg/kg feed premix	100	97.4	99.2		95.7					(Benz, 1971a)
25mg/kg feed pellets	96.7	79.2	98.7		95.5					(Benz, 1971a)

[1] L3 3rd stage larvae
[2] L4 4th stage larvae
[3] L5 5th stage larvae/Immature adult
[4] Imm Immature stages

TABLE 12.4 CTD. PERCENTAGE EFFICACY OF CAMBENDAZOLE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	
27-35 mg/kg drench	99-100 Imm-97[1]	94-99 L4-32-95[2] Imm-68-98[3]		81 Imm-32	93-98 Imm-91-99	99	47-59 *S. papillosus* 100		41-95 Imm-41-79	(Egerton *et al.*1970; Restani and Borrelli, 1971; Gibbs and Gupta, 1972; Rubin, 1972; Cairns *et al.*1975)
30mg/kg bolus	100	98.2	97.2		98					(Benz, 1971a)
34mg/kg paste	99 L4 - 99	97 L4-97 L5-97[3]	99 L4-99		99 L4-99 L5-99	99				(Cairns *et al.*1975)
40mg/kg paste									47-90 Imm-0***	(Cairns *et al.*1975)
40-44 mg/kg drench	99-100 Imm -91	93-100 L3-72[4] L4-92 Imm -30-99	99-100	32-94 Imm -47	99-100 Imm -93-99	99			91-99 Imm-7-96	(Egerton *et al.*1970; Baker and Walters, 1971; Rubin, 1972; Gibbs and Gupta, 1972; Cairns *et al.*1975)
60 mg/kg drench		99 Imm -90		99 Imm -96	100 Imm -99				Imm-71	(Gibbs and Gupta, 1972)

[1] Imm Immature stages
[2] L4 4th stage larvae
[3] L5 5th stage larvae/Immature adult
[4] L3 3rd stage larvae

TABLE 12.5 PERCENTAGE EFFICACY OF MEBENDAZOLE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine	Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Oe. radiatum*	*D. viviparus*	
15 mg/kg drench	98.8 L4 - 96.6[1]	41.5 L4 - 27.3			99.7 L4- 100		99.1 L4- 99.9	99.9 L4- 91.5		(Westhuizen *et al.*1984)

[1] L4 4th larval stages

TABLE 12. 6 PERCENTAGE EFFICACY OF OXIBENDAZOLE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	
5, 10, 15, 20 mg/kg drench	80-100	80-100	80-100		80-100	80-100			80-100	(Theodorides *et al.*1973)
5mg/kg drench	65-90	66-100	100		84-85	99-100	100 (1 calf) *S. papillosus* 95		100	(Theodorides and Chang, 1974; Herlich, 1975; Theodorides *et al.*1976)
7.5mg/kg drench	88	100	100		84	100	*S. papillosus* 93		100	(Theodorides and Chang, 1974; Theodorides *et al.*1976b)
10 mg/kg drench	80-100 d3-91[1] d7-94	95-100 d3-34 d7-34	95-100 d3-76 d4-87		95-100 d3-99* d7-96	95-100 d3-99* d7-100	*S. papillosus* 97		95-100 d3-99 d7-100	(Theodorides and Chang, 1974; Herlich, 1975; Theodorides *et al.*1976b)
15-20 mg/kg drench	91-100	91-100	91-100		91-100	91-100			91-100	(Theodorides and Chang, 1974)
15mg/kg feed premix	99-99.2 d3-90.5 d7-91.1	89-100 d3-0-81.5 d7-23-90.9	96-99	83-99.3 d3-95.7 d7-99.3	99-100 d3-92-99.9 d7-96-99.7	99-100	94-99 *S. papillosus* 94	80-100 d3-98.5 d7-99.4	99.7-100 d3-30-84 d7-18-86	(Theodorides *et al.* 1976b; Crowley *et al.*1976; Williams *et al.*1978)

[1] Indicates 3-day-old larvae and percetnge efficacy

TABLE 12.7 PERCENTAGE EFFICACY OF THIOPHANATE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	
20 mg/kg for 5 d in feed		98.2 EL4 - 97.3[2]	94.8		99.6 EL4-100					(Duncan *et al.*1979)
50 mg/kg drench	100 d3-99[1] d7-99 d14-99	97-99 d3-98 d7-99 d14-100	d3-100 d7-100 d14-100 d21-100	78	54-85 d3-100 d7-97 d14-82	98 d3-100 d7-99 d14-99				(Eichler, 1973)
50-75 mg/kg drench	97.8	100	100		99.3	100				(Baines and Bell, 1980)
112 mg/kg drench		99.3 EL4 - 68.3	100		100 EL4 -100					(Duncan *et al.*1979)

[1] Indicates 3-day-old larvae and percentage efficacy

[2] EL4 Early 4th stage larvae

TABLE 12.8 PERCENTAGE EFFICACY OF FENBENDAZOLE AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine			References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
3.5mg/kg drench	96.6-97.2 Imm-90.6[1]	97.1-100 Imm -58.7		97.4-98.1 Imm -0	99.9 Imm -97.6 *Capillaria* sp. 100 Imm-100	93.8 Imm-100		100 Imm-100	100 Imm -100		(Todd *et al.*1976; Tiefenbach, 1977)
5 mg/kg drench	88.7-100 Imm-95.6-100	97.1-100 d3-100[2] d10-100 Imm-62 - 99.2 EL4 -24- 99.2[3] Dvlp L4–75-99[4]	98.1-99.9 Imm-99-100	95.8-99.6 L4- 99.9 Imm-100	99.7-100 L4 -99.9 Imm-63-100 *Capillaria* sp. 100 Imm-100	99-100 Imm-100	99-100 Imm-100 *S. papillosus* 97.7	86-100 Imm-100	96.8-100 Imm-100	99-99.9 d6->99 d13->99 Imm-99.5	(Todd *et al.*1976; Duwel, 1974; Tiefenbach, 1977; Crowley *et al.*1977; Craig and Bell, 1978; Benz and Ernst, 1978a; Samizadeh-Yazd and Todd, 1978; Duwel, 1979; Williams *et al.*1981a; 1983; Duwel and Kirsch, 1981; Yazwinski *et al.*1985; Blagburn *et al.*1986)
5mg/kg paste			100								(Lyons *et al.*1989)
5 mg/kg feed premix	94.6-100	99.6-100	99.4-100		96.7-100	99.9-100	*S. papillosus* 100	100	96.7-100	99.9	(Benz and Ernst, 1978a; Blagburn *et al.*1986)
5 mg/kg 0.5% pellets	100	99.8-100			99.9-100		*S. papillosus* 96.7	100	100		(Blagburn *et al.*1986)

[1] Imm Immature stages
[2] Age of infection at time of treatment (days)
[3] EL4 Early 4th stage larvae
[4] Dvlp L4 Developing 4th stage larvae

TABLE 12.8 CTD. PERCENTAGE EFFICACY OF FENBENDAZOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
7.5mg/kg drench	99.8-100 L3-99.9[1] L4-99.9[2] Imm 98-100[3]	92-100 L3-95.1 L4-95.7 Imm 92.1-100 EL4-22-100[4] Dvlp L4-40-100[5]	90-100 Imm-100	95-100 Imm 98-100	99.4-100 L3-99.5 L4-99.9 Imm-97.6-100	93.8-100 Imm-0-100	99.3-100 L3-00 L4-100 Imm-100	99.6-100 Imm-100	99.9-100 L3-99.3 L4-99.7 Imm-100	98.9-100 Imm-99-100	(Anon, 1976; Tiefenbach, 1977; Todd *et al.*1976; Duncan *et al.* 1976; Anderson, 1977; Lancaster and Hong, 1977; Searson and Doughty, 1977; Craig and Bell, 1978; Duncan *et al.* 1978; Callinan and Cummins, 1979; Malan, 1979; Duwel and Kirsch, 1981; Williams *et al.* 1983)
7.5 mg/kg in feed										99.7 Imm-100	(Saad and Rubin, 1977)
8.5-9.3 mg/kg drench		EL4 - 0	100								(Elliott, 1977)
10 mg/kg drench	100 L4-100	99.2-100 EL4-97-97.5 Dvlp L4- 80-97	100		97.7-100 L4-98		100		100 L4-100 Imm-100	100	(Tiefenbach, 1977; Drudge *et al.* 1978; Williams *et al.*1979a; Williams, 1991)
12.5 mg/kg drench		100			100		100		100 Imm-100		(Tiefenbach, 1977)

[1] L3 3rd stage larvae
[2] L4 4th stage larvae
[3] Imm Immature stages
[4] EL4 Early 4th stage larvae
[5] Dvlp L4 Developing 4th stage larvae

TABLE 12.8 CTD. PERCENTAGE EFFICACY OF FENBENDAZOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
0.25 mg/kg feed 5d	95.6	100			99.9	99.9			100		(Crowley *et al.*1977)
0.35 mg/kg feed 5 d	93.7	100			99.9	99.9			100		(Crowley *et al.*1977)
0.5 mg/kg feed 5d	91	100			100	100			100		(Crowley *et al.*1977)
1 mg/kg feed 5 d	99.5-100	85.9-100 EL4-47.6[1] Dvlp L4-79[2]	100		94.5-100	99.1			100		(Crowley *et al.*1977; Williams *et al.*1981a)
1mg/kg/day Feedblock, 10 day access		99.9 -100 L3- 84.6[3] EL4 - 97.5 Dvlp L4- 100	100	100	99.7-100 L3 - 98.9 L4- 100					100 L3-100 L4-100	(McBeath *et al.*1977; McBeath, 1981)
2mg/kg Feedblock, 5 day access		100 L3-99.1 L4-99.9[4]			100 L3- 98.9 L4 -100					97.9 L3-100 L4-100	(McBeath, 1981)

[1] EL4 Early 4th stage larvae
[2] Dvlp L4 Developing 4th stage larvae
[3] L3 3rd stage larvae
[4] L4 4th stage larvae

TABLE 12.8 CTD. PERCENTAGE EFFICACY OF FENBENDAZOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
5mg/kg over 3d in Feedblock	100	99.8-100	100		99.9-100		100		100		(Blagburn *et al.*1987)
5mg/kg over 10d in Feedblock	100	99.8-100	100		99.9-100		100		100		(Blagburn *et al.*1987)
0.4-1 mg/kg in drinking water for 7d, 4 times every 2 weeks		99.5 EL4- 99.1[1]			95.3						(Downey and O'Shea, 1985)
0.2-0.4 mg/kg/day for 5 months Slow release bolus										98.7	(Jacobs *et al.*1996)
0.2-0.4 mg/kg/day for 5 months slow release bolus		79.5 EL4- 92.5			31 EL4- 86.8						(Bauer-C; *et al.*1997)

[1] EL4 Early 4th stage larvae

TABLE 12.9 PERCENTAGE EFFICACY OF FENBENDAZOLE AGAINST *OSTERTAGIA OSTERTAGI* IN CATTLE

	Ostertagia ostertagi			
Dose rate	Adults	Early 4th stage larvae (EL4)	Late 4th stage Larvae (Dvlp L4)	References
5 mg/kg drench	99	24	99	(Craig and Bell, 1978)
	96.9	82.9	74.7	(Williams *et al.*1981a)
	99.6	99.2	99.1	(Williams *et al.*1983)
	98.1	70	44.6	(Yazwinski *et al.*1985)
7.5 mg/kg drench		97.5		(Duncan *et al.*1976)
	98.8	97.3	92.8	(Lancaster and Hong, 1977)
	98.1	0	0	(Lancaster and Hong, 1977)
	98.7	86.7	92.8	(Searson and Doughty, 1977)
	99	72	100	(Craig and Bell, 1978)
	100	97		(Duncan *et al.*1978)
	94.2	89.3		(Duncan *et al.*1978)
	98.5	96.4-100	97-100	(Callinan and Cummins, 1979)
	99.2	99.2	98.9	(Williams *et al.*1983)
8.5 - 9.3 mg/kg drench		0		(Elliott, 1977)
10 mg/kg drench	100	97	80	(Williams *et al.*1979b)
	99.2	97.5	97.2	(Williams, 1991)
15 mg/kg drench	100	99	98	(Williams *et al.*1979b)

TABLE 12.10 PERCENTAGE EFFICACY OF OXFENDAZOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
0.625 mg/kg drench		69 EL4 - 67[1] Dvlp L4 - 64[2]									(Anderson and Lord, 1979)
1.25 mg/kg drench		87 EL4 - 57 Dvlp L4 - 66									(Anderson and Lord, 1979)
1.125 mg/kg intra-ruminal	100	96.4	99.9	69.9	99.4	100			100	100	(Yazwinski *et al.*1986)
2.5 mg/kg drench	100	91.8-100 EL4-76-90 Dvlp L4-68-88 L5-87-100[3]	99-100 EL4-100	93-100 EL4-65	99.5-100 EL4-96.2-100 Dvlp L4 -92-100 L5-99.7-100	94.6-100	100 *Chabertia* sp. 100	89-98.6 L4- 62-100[4] L5- 85-95	99.7-100	100 L5-100	(Downey, 1976; Armour *et al.* 1978; Baker *et al.* 1978; Chalmers, 1978; Todd and Mansfield, 1979; Anderson and Laby, 1979; Anderson and Lord, 1979; Chalmers, 1979)
2.5 mg/kg paste	99.9	99-100 EL4-87	99 EL4-100	99 EL4-21	99-99.9 EL4-100 Dvlp L4-100	100	100	86.7 *Chabertia* sp. 100	100	100	(Todd and Mansfield, 1979; Baker *et al.* 1978)

[1] EL4 Early 4th stage larvae

[2] Dvlp L4 Developing 4th stage larvae

[3] L5 5th stage larvae/immature adults

[4] L4 4th stage larvae

TABLE 12.10 CTD. PERCENTAGE EFFICACY OF OXFENDAZOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
2.5 mg/kg bolus	99.9	98-100 EL4-71[1]	99 EL4-100	99 EL4-23	99-99.9 EL4-100 Dvlp L4-100	100	100	98.3 *Chabertia* sp. 100	99-99.4	100	(Baker *et al.*1978; Todd and Mansfield, 1979)
2.5 mg/kg intra-ruminal	99.7-100	87.7-99.4 EL4 - 0 Dvlp L4-36[2]	99.9	77.4	96.5-99.7	98.7-99.7	100		100	100	(Yazwinski *et al.*1986; Miller *et al.*1988)
2.5 mg/kg feed crumbles	100	99.8			99.9	100	100	95	100	100	(Todd and Mansfield, 1979)
4.5-4.6 mg/kg drench		99.9-100 EL4 - 97 Dvlp L4 - 87	100		100 EL4-100 Dvlp L4 - 100				100	100	(Bairden *et al.* 1983; Duwel, 1979)
4.5 mg/kg intra-ruminal	99.7-100	94.6-99.8 EL4-33.5-97 Dvlp L4 -47-80	99.9	97.6	100 EL4-100 Dvlp L4 - 100	99.7			98.8-100	100	(Bairden *et al.* 1983; Yazwinski *et al.* 1986; Miller *et al.* 1988)

[1] EL4 Early 4th stage larvae

[2] Dvlp L4 Developing 4th stage larvae

TABLE 12.10 CTD. PERCENTAGE EFFICACY OF OXFENDAZOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
5 mg/kg drench	100	98-100 EL4-89-90.7[1] Dvlp L4 - 92[2] L5-100[3]	100	100	100 EL4-100 L4-100[4] L5-100			100		100 L5-100	(Downey, 1976; Armour *et al.*1978; Anderson and Lord, 1979)
6.25-6.75 mg/kg intra-ruminal	99.9	97.6-99.9 EL4 - 90 Dvlp L4 - 72	99.9	100	99.9-100	99.9-100	100		100	100	(Miller *et al.*1988; Yazwinski *et al.*1986)
0.48 mg/kg slow release bolus (5.5 d)		99 EL4 - 93 Dvlp L4 - 87									(Anderson and Laby, 1979)
0.29 mg/kg slow release bolus (8d)		68 EL4 - 84 Dvlp L4 - 57									(Anderson and Laby, 1979)

[1] EL4 Early 4th stage larvae
[2] Dvlp L4 Developing 4th stage larvae
[3] L5 5th stage larvae/immature adult
[4] L4 4th stage larvae

TABLE 12.11 PERCENTAGE EFFICACY OF OXFENDAZOLE AGAINST *OSTERTAGIA OSTERTAGI* IN CATTLE

	Ostertagia ostertagi			
Dose rate	Adults	Early 4th stage larvae (EL4)	Late 4th stage Larvae (Dvlp L4)	References
0.625 mg/kg drench	69	67	64	(Anderson and Lord, 1979)
1.25 mg/kg drench	87	57	66	(Anderson and Lord, 1979)
2.5 mg/kg drench	100	85.8		(Armour *et al.*1978)
	98	76		(Baker *et al.*1978)
	97.9-98.8	76-85.7	68-71	(Chalmers, 1978)
	91.8-94.8	85-90	75-88	(Anderson and Laby, 1979)
	95	85	88	(Anderson and Lord, 1979)
2.5 mg/kg paste	99	87		(Baker *et al.*1978)
2.5 mg/kg bolus	98	71		(Baker *et al.*1978)
2.5 mg/kg intra-ruminal	87.8	0	39	(Miller *et al.*1988)
4.5 mg/kg drench	100	97	87	(Bairden *et al.*1983)
4.5 mg/kg intra-ruminal	99.7	97	80	(Bairden *et al.*1983)
4.5 mg/kg intra-ruminal	94.6	33.5	7.2	(Miller *et al.*1988)
5 mg/kg drench	100	90.7		(Armour *et al.*1978)
	98	89	92	(Anderson and Lord, 1979)
6.25 mg/kg intra-ruminal	97.6	90	72	(Miller *et al.*1988)

TABLE 12.12 PERCENTAGE EFFICACY OF ALBENDAZOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*S. papillosus*	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
2.5 mg/kg drench	53-79 L4 -96[1]	97-99 L4- 99	99-99.3 L4-100	88	78-99 L4- 99	100 L4- 99	99	12 L4- 50	98.8		(Theodorides *et al.* 1976c; Benz and Ernst, 1977)
5mg/kg* drench	40-99.3 L4- 99	98.2-99 L4- 99	99-100 L4- 99	99	99.4-100 L4-100	99.7-100 L4-100	95-96.1	21-36 L4-25	99-100	99-100 Imm- 99-100	(Theodorides *et al.* 1976c; 1980; Benz and Ernst, 1977; Williams *et al.*1977b)
7.5 mg/kg drench	83.7-99	86.7-100 EL4- 0-99.3[3] Dvlp L4 -0-99.5[4]	96.3-100	*Nematodirus* spp. 100	99.6-100				99-100	96.7-100 Imm 99-100	(Benz and Ernst, 1977; 1978b Theodorides *et al.*1980; Williams *et al.* 1977a; Downey, 1978; Borgsteede, 1979; Todd and Mansfield, 1982; Courtney *et al.* 1986; Callinan and Riffkin, 1987)
7.5mg/kg paste	94.5 Imm-87.6[2]	93.5-95.9 EL4- 31-62 Dvlp L4-48-53	100 Imm-100								(Williams *et al.*1979a; 1981b)
7.5mg/kg feed premix	>99	>99 EL4 -55	>99		99						(Courtney *et al.*1986)

[1] L4 4th stage larvae
[2] Imm Immature stages
[3] EL4 Early 4th stage larvae
[4] Dvlp L4 Developing 4th stage larvae

TABLE 12.12 CTD. PERCENTAGE EFFICACY OF ALBENDAZOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*S. papillosus*	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
10mg/kg* drench	74-100 L4- 99-100[1]	99 EL4-84.9[3] Dvlp L4-95.3[4] L4- 99	99-100 L4-100	97	99.3-100 L4-100	100 L4-100	99	48 L4-83	100 L4-100	99-100 Imm -99-100	(Theodorides *et al.* 1976c; 1980; Herlich, 1977; Williams, 1991)
10mg/kg paste	100 Imm-54.6[2]	94.8 EL4-18.6 Dvlp L4-49.5	100 Imm-100								(Williams *et al.*1981b)
15 mg/kg paste		100 DvlpL4-93.2 EL4-84.9									(Williams *et al.*1979a)

[1] L4 4th stage larvae
[2] Imm Immature stages
[3] EL4 Early 4th stage larvae
[4] Dvlp L4 Developing 4th stage larvae

TABLE 12.13 PERCENTAGE EFFICACY OF FEBANTEL AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
2.5 mg/kg paste	99.4	91.5-95 EL4-94.9[4] Dvlp L4-76.6[5]	99.4		88-100 L4-98.5***				100		(Blagburn *et al.*1989)
5mg/kg drench	96.5 d8-96.1[1]	93.2 d8-78.1	74.8 d8-97.4		77.7 d8-98.9					100 d11-100	(Grelck *et al.*1978)
5mg/kg paste	66.7-100 L4- 64[2] Imm->99[3]	83.6-3-99 EL4- 0-95 Dvlp L4- 0-83 Imm -61-99	99.3-99.7 L4-100 Imm-100	97.1	99.3-100 L4- 99 Imm-100	100	99.4	68	100	90.6 Imm-97.1	(Ciordia *et al.* 1982; Courtney *et al.* 1988; Williams *et al.* 1988; Blagburn *et al.* 1989; Stuedemann *et al.*1990)
7.5mg/kg drench	100 d8-99.2	100 d8-97.6	100 d8-100		100 d8-100					100 d11-100	(Grelck *et al.*1978)
7.5mg/kg paste	100	98-99 EL4-80 Dvlp L4- 85.1 Imm -96.6	99.1-100	99.2	99.5-100 L4 -94.9				100		(Ciordia *et al.* 1982; Blagburn *et al.* 1989)
10 mg/kg paste	99.9-100	98.5-99.9 EL4-97.5 Dvlp L4-97.9 Imm-70	99.2-100	98.3	99.7-100 L4-98.3				100		(Ciordia *et al.* 1982; Blagburn *et al.* 1989)

[1] Indicates 8-day-old larvae and percentage efficacy
[2] L4 4th stage larvae
[3] Imm Immature stages
[4] EL4 Early 4th stage larvae
[5] Dvlp L4 Developing 4th stage larvae

TABLE 12.14 PERCENTAGE EFFICACY OF NETOBIMIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* sp.	*Oe. radiatum*	*D. viviparus*	
7.5mg/kg drench	95.1	67-96 EL4- 40-79[1] Dvlp L4 -31-83[2]	99.7 Imm-100[3]	100	88-97.7				100 Imm-100	98-100	(Williams *et al.* 1985; Duncan *et al.* 1986; Richards *et al.* 1987)
10mg/kg drench	100	98.7 EL4-74.7 Dvlp L4- 87	99.9 Imm-100		98.3				100 Imm-100		(Williams *et al.*1985)
12.5 mg/kg injectable*		91-92			64-65					98-99	(Duncan *et al.*1986)
20mg/kg drench	100	99.2 EL4- 63-87 Dvlp L4-40-91	99.8-100 Imm-100	99.9 L4- 99.4[4]	96.6-99.9 L4-100		100	90.8	98.9-100 Imm-100		(Williams *et al.*1985; Duncan *et al.*1986; Yazwinski *et al.*1992)

[1] EL4 Early 4th stage larvae
[2] Dvlp L4 Developing 4th stage larvae
[3] Imm Immature stages
[4] L4 4th stage larvae

13.0 PYRANTEL and MORANTEL

13.1 INTRODUCTION

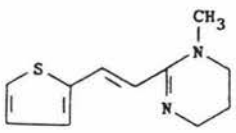
The 1960's saw a variety of new anthelmintic compounds released on to the market. These included the benzimdazoles, imidazoles and the tetrahydropyrimidines. The first information on pyrantel, a tetrahydropyrimidine, was presented in 1966 (Austin *et al.*1966). The tartrate salt of pyrantel exhibited a broad spectrum of activity against both mature and immature nematode infections of domestic animals. Doses of 25 mg/kg had a high level of activity against adult and immature *Haemonchus*, *Ostertagia*, and *Trichostrongylus* in the abomasum, and *Nematodirus*, *Cooperia* and *Trichostrongylus* in the small intestine of both sheep (Austin *et al.*1966; Cornwell, 1966a; 1966b; Cornwell *et al.*1966) and cattle (Austin *et al.*1966; Cornwell and Jones, 1970a). There was also activity against *Oesophagostomum* and *Chabertia* (Austin *et al.*1966). Critical tests and field trials with pyrantel tartrate in horses removed adult small strongyles (cyathostomes), *Oxyuris* and *Strongylus vulgaris* (Cornwell and Jones, 1968a; 1968b).

Further research on the tetrahydropyrimidine family developed the 3-methyl analogue of pyrantel, called morantel. This compound proved more potent than pyrantel in mice and dogs. Reports on its activity in sheep (Cornwell and Jones, 1970b; 1970c; 1970d), cattle (Cornwell *et al.*1972) and the horse (Cornwell *et al.*1973a; 1973b) confirmed the increased potency, while the compound retained the same broad spectrum against nematode gastrointestinal parasites (Austin *et al.*1966; Cornwell and Jones, 1970b).

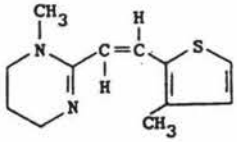
13.2 PHARMACOLOGY

Pyrantel is a thiophene-substituted tetrahydropyrimidine and morantel only differs by a 3-methyl substitution in the thiophene ring (Figure 13.1).

Figure 13.1 Structures of Pyrantel and Morantel



Pyrantel



Morantel

The water-soluble tartrate salt has mostly been used in ruminants. In sheep, after oral dosing of 25

mg/kg pyrantel tartrate, the drug is rapidly absorbed and excreted. Within 24 hours, 27% of the drug was excreted in the urine, and 60% in the faeces. Similar pharmacodynamcis occur in cattle in which after 48 hours, 78% of the drug has been excreted. Of this 17% is in the urine and 61% in the faeces (O'Brien, 1970). In both species, the drug present in the faeces is unchanged pyrantel.

Morantel tartrate is also very rapidly absorbed in sheep, peak blood levels occurring in 4-6 hours after dosing. Partial metabolism occurs in the liver and 17% is excreted as metabolites in the first 96 hours, with the remaining drug excreted in the faeces (O'Brien, 1970). However, in cattle, morantel tartrate could not be detected at all in plasma after a dose of 10 mg/kg (McKellar *et al.*1993). Concentrations could be measured in the rumen after oral dosing. Peak rumen concentration was reached at 2 hours, than followed by a peak in the abomasum at 8 hours. Levels remained around this peak for up to 36 hours. In both organs, concentrations had declined to barely detectable levels by 96 hours (Alvinerie and Fioramonti, 1986). The rumen appeared to be acting as a slow release depot, similar to that seen with some benzimidazoles.

Both pyrantel and morantel act as agonists at synaptic and extrasynaptic nicotinic acetylcholine receptors on nematode muscle cells. Binding of either anthelmintic to the receptor results in an increase in the membrane conductance and depolarisation of the membrane. This occurs by the opening of nonselective cation channels that are permeable to both sodium and potassium (Martin, 1997). Neuromuscular transmission is blocked, and the agonist effect results in contraction of muscle and spastic paralysis of the nematode (Aubry *et al.*1970; Eyre, 1970; O'Brien, 1970; Bamgbose *et al.*1973; Martin, 1997).

The salts of the tetrahydropyrimidines vary in water solubility. The tartrate salt is one of the most water soluble whereas the pamoate salt is one of the least. This offers the opportunity to control drug concentration along the gastrointestinal tract with different formulations. The more insoluble salts are less well absorbed resulting in higher concentrations in the caecum and large intestine and improving efficacy agiainst parasites in these locations. Pyrantel is not marketed for use in cattle, but was trialed as a drench. Morantel is used as a drench, suspension, and two formulations of slow release boluses. It was also tested as an in-feed premix and a bolus tablet.

13.3 EFFICACY

Only one series of trials was ever published on the efficacy of pyrantel. While it had reasonably good efficacy against most gastrointestinal nematodes, these only tended to be the lumen dwelling parasites (Table 13.1). Against adult *Ostertagia* spp., efficacy ranged from 85-100%, with no anthelmintic effect seen against larval stages, even at doses up to 33 mg/kg. Unusually, *Trichostrongylus* spp. were not quite as responsive at low doses as had been seen with the other

anthelmintic families. One trial using 50 mg/kg only removed 89% of *T. colubriformis* (Cornwell and Jones, 1970a). No efficacy was seen against *Dictyocaulus viviparus*.

Ironically, the first publication of the efficacy of morantel in cattle was when it was used in combination with diethylcarbamazine (DEC) (Table 13.2) (Cornwell *et al.*1972). Like pyrantel, morantel did not possess appreciable lungworm activity (Cornwell *et al.*1973c) and a search was on for an effective combination for treatment of gastrointestinal nematodes and lungworm. Whilst the potency of morantel was greater so that the dose (10 mg/kg) was half to a third that of pyrantel, efficacy against adult and immature *Ostertagia* was still the limiting factor (Cornwell *et al.*1972; 1973c; Conway *et al.*1973; Ciordia and McCampbell, 1973; Anderson and Marais, 1975). Following a drench with 10 mg/kg, up to 88% of 3rd stage *Ostertagia* larvae were removed prior to the larvae penetrating the abomasal mucosa. After this, there was no efficacy against the histotrophic phase (between days 7 and 10). There was some activity against the pre-emergent and emerging larvae on day 14 (43-84%) with an improvement to 91-100% for adult *Ostertagia*. Doubling the dose only marginally increased activity against 14-day-old worms to 32-52% in one trial (Cornwell *et al.*1973c) but markedly increased it in another (Cornwell *et al.*1972). Similar levels of anthelmintic activity were seen with in-feed (Conway *et al.*1973) and bolus formulations (Ciordia and McCampbell, 1973). The results of various trials are summarised in Table 13.3.

Because of the solubility of morantel tartrate, it can also be added to drinking water. Trials using a dose of 1.6 mg/kg for 6 weeks resulted in 97% and 76% reduction in excretion of *Dictyocaulus* larvae and *Ostertagia* eggs respectively. Total worm counts confirmed that low-dose morantel had drastically reduced the establishment of artificial infections of adult *Ostertagia* by 80% and adult *Cooperia* by 98.6%. Fewer lungworms were recovered from these calves than the controls, which implied that morantel must have killed the 3rd stage larvae before they left the gut as morantel is not active in the lungs (Downey and O'Shea, 1977). Another trial in the United Kingdom in which animals were dosed with 1 or 1.5 mg/kg in feed for 2 weeks prior to, and for up to 63 days during the first part of the grazing season, showed the same level of reduction in faecal egg count (Jones *et al.*1978). The conclusions drawn by both authors (Downey and O'Shea, 1977; Jones *et al.*1978) and confirmed by Pott *et al* (1979) was that this reduction in faecal egg output would result in a decrease in total pasture contamination. The major source of infection during the first grazing season is from overwintered larvae which when ingested propagate within the animal to produce high levels of contaminated pasture during the summer grazing period (Jones, 1981). By preventing this pasture contamination, it may reduce the need for further treatment while calves continued to graze the same pasture. However, many of these results are only applicable to the Northern Hemisphere where animals are housed over winter.

The high water solubility and potency of morantel tartrate against ingested larvae made it an ideal drug for low-level sustained release from a depot source. An intra-ruminal bolus designed to give

a continuous release of morantel tartrate over the first two months of the grazing season was feveloped and field trialed (Jones, 1981). The bolus consisted of a steel cylinder secured at each end by a polyethylene hydrogel impregnated membrane which contained 22.7 grammes of morantel tartrate. In contact with rumen fluid, morantel is released though the impregnated semipermeable membrane at a continuous level for a period of time.

Laboratory trials confirmed that rates as low as 0.26 mg/kg/day were highly efficacious in preventing the establishment and removing infections of *Ostertagia*, *Haemonchus*, *Cooperia* and *T. colubriformis* (Table 13.4). These same dose rates also prevented the establishment of *D. viviparus,* but were ineffective against established infections of this parasite (Jones, 1983). Good results were obtained in reducing *Ostertagia* and *Cooperia* numbers and reducing faecal egg output (Table 13.5) (Jones, 1981; 1983; Armour *et al.*1981; Brunsdon and Vlassoff, 1981; 1983; Bliss and Jones, 1983; Presson *et al.*1984b; Borgsteede *et al.*1988). However, some animals developed clinical parasitic bronchitis indicating that the epidemiological pattern differed between *Dictyocaulus* and *Ostertagia.* More research was needed to identify how the bolus could best control lungworm (Jones, 1981; Armour *et al.*1981). Other authors confirmed that use of the bolus at the beginning of the season rather than in autumn substantially reduced the level of pasture contamination with infective larvae (Armour *et al.*1981; Brunsdon and Vlassoff, 1981; 1983). The bolus prevented the establishment of ingested infective larvae and by removing adults, interrupted the life cycle of the parasites and reduced the buildup of infective larvae on pasture. This prevented the development of parasitic gastroenteritis in susceptible grazing animals (Bliss and Jones, 1983).

Morantel was also shown to have larvicidal properties. The presence of the drug in faeces was found to prevent the maturation of approximately 90% of *O. ostertagi* infective larvae between days 7 and 84 after administration of a bolus and 75% of larvae on day 91 (Rossiter *et al.*1988). This helped to explain why the level of pasture contamination was so low. Not only was the bolus actively reducing nematode egg output, but the larvae that hatched from these eggs were unable to develop though to the infective stage.

The design of this bolus was complicated and relied on the weight of the metal components to retain it in the rumen. Once all the morantel had been released, this resulted in redundant cylinders remaining in the reticulo-rumen. In some cases, the presence of these damaged machinery used in the processing of offal from slaughtered animals. A simpler bolus was developed which had no metal in it and was retained in the rumen due to its shape. This was composed of a trilaminate sheet consisting of a central lamina of a morantel tartrate/ethylene vinyl acetate matrix coated on both sides with a thin impermeable layer of ethylene vinyl acetate. A symmetrical pattern of circular perforations are made and when the device is in the rumen, morantel is released from the uncoated edges of the perforation and the perimeter of the device. The sizes of the perforations are adjusted so that the morantel is released at an approximate constant rate for up to 90 days after

administration. For administration, the device is provided rolled up and plugged at both ends like a cylinder, and held together with adhesive tape. Once in the rumen, the device unravels into the original configuration which prevents regurgitation. Animals slaughtered up to one year after dosing indicates that the depleted boluses disintegrate and are eliminated, most likely by regurgitation (Grimshaw *et al.*1989).

This new design was found to be as efficacious as the previous one. Burdens of *Ostertagia* and *Cooperia* were reduced by 88 and 97% respectively when compared to untreated controls (Table 13.5). There was also a decrease in pasture contamination of 73-8% for *Ostertagia* and 77-85% for *Cooperia* (Grimshaw *et al.*1989; Rickard *et al.*1989; Vercruysse *et al.*1992).

Concerns were raised about the potential problems of developing resistance to products based on controlled release technology. An analogy was made to the strong development of resistance associated with slow decaying insecticides. The two features of a controlled release device that, in theory, may minimise the risk of a rapid selection for resistance are, a high and constant release of anthelmintic, followed by a rapid decline to zero as the device becomes exhausted (Herd, 1984). However, trial work to test this theory did not show any decrease in faecal egg count reductions or total worm burden reductions when the device was used over consecutive seasons. Reductions of between 96-98% of *Ostertagia* and *Cooperia* infections were recorded (Table 13.6) (Newby *et al.*1985a; 1985b).

One of the other issues raised was whether the use of continuous slow release boluses would decrease the immunological response of calves and predispose them to increased nematode burdens once the bolus ran out (Yazwinski *et al.*1987; Vercruysse *et al.*1992; Fisher and Jacobs, 1995). In the calf, the immune response to *Ostertagia* is partially a product of the amount of daily exposure with a threshold level of exposure need for the host to become immune (Yazwinski *et al.*1987) and an effect of age (Fisher and Jacobs, 1995). Experiments with the metal cylinder slow-release bolus did not reveal any evidence of decreased immunity (Yazwinski *et al.*1987). While there appeared to have been partially impaired immunity to *Cooperia* in one trial with the trilaminate bolus (Vercruysse *et al.*1992), there was no evidence of this in another in which treated calves were challenged with *Ostertagia*, *Cooperia* and *Dictyocaulus* (Fisher and Jacobs, 1995). However, it was suggested that there may be more advantages to animals from using pulse-release boluses than continuous release boluses. The former have the advantage of allowing greater antigenic stimulation to induce immunity and also reducing selection pressure for anthelmintic resistance (Vercruysse *et al.*1992).

13.4 SIDE EFFECTS

The major side effect seen with both pyrantel and morantel resulted from the drug being inadvertently administered into the trachea instead of the oesophagus (O'Brien, 1970; Bamgbose *et al.*1973). Animals suffered respiratory embarrassment, muscle tremors and an immediate rise in blood pressure (Bamgbose *et al.*1973). Death resulted from cardiac depression and vasomotor collapse. Experimental intra-tracheal administration of pyrantel in sheep caused a biphasic rise in arterial blood pressure of approximately 100 mm Hg above the mean resting pressure and a tachycardia of 3-4 times the pretreatment value. Afer the initial rise, blood pressure fell to below 50 mm Hg with no sign of recovery (O'Brien, 1970). Administered orally, morantel has a minimum lethal dose of 300 mg/kg in sheep, and calves will tolerate 200 mg/kg, twenty times the therapeutic dose (Cornwell and Jones, 1970a). When used in combination with DEC (10 mg/kg morantel tartrate, 25.5 mg/kg DEC base), only up to eight times the recommended dose was tolerated without signs of depression and recumbency. It was found that if the combination was administered by stomach tube into the abomasum, the rapid absorption resulted in toxic signs at only three times the normal dose (Cornwell *et al.*1972).

No side effects were seen in pregnant cattle nor when animals were dosed at the same time with single or double therapeutic doses of pyrantel or morantel, and either anti-trematode anthelmintics such as hexachlorethane, oxyclozanide, nitroxynil, hexachlorophene,or topical ruelene (crufomate) (Cornwell and Jones, 1970a; Cornwell *et al.*1972; 1973c).

TABLE 13.1 PERCENTAGE EFFICACY OF PYRANTEL AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		References
Pyrantel salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*S. papillosus*	*Trichuris* spp.	*Oe. radiatum*	
tartrate	12.5 mg/kg drench		100	96	100	99			0		(Cornwell and Jones, 1970a)
tartrate	12.5 mg/kg drench at 2 week interval		86 L5-96.5[1]	98.9 L5-96.7		98.9	100	78.3 L5-92			(Cornwell and Jones, 1970a)
tartrate	25 mg/kg drench		85-100 d3-37[2] d7-0 d14-0 L5-91.6	99-100 L5-99.9	100	99-9.3	66 - 100 d7-53 d14-74	100 L5-100	0		(Cornwell and Jones, 1970a)
tartrate	25mg/kg drench at 2 week interval		97.4 L5-99.2	99.5 L5-92.1		100	100	87 L5-100			(Cornwell and Jones, 1970a)
tartrate	33 mg/kg drench		97 d2-73 d7-0 d14-11								(Cornwell and Jones, 1970a)
tartrate	50 mg/kg drench						89				(Cornwell and Jones, 1970a)

[1] L5 5th stage larvae/immature adults

[2] Indicates 3-day-old larvae and percentage efficacy

TABLE 13.2 PERCENTAGE EFFICACY OF MORANTEL AND DIETHYLCARBAMAZINE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
10mg/kg MT[1] 30 mg/kg DECC[2]		d14-45[3] d21-98								d14-30 d21-88	(Cornwell *et al.*1972)
10mg/kg MT 40 mg/kg DECC		d14-42 d21-99.9								d14-90 d21-60	(Cornwell *et al.*1972)
10 mg/kg MT 50 mg/kg DECC	d7-95 d14 -00 d21 -98	d2-88 d7-34-51 d10-0 d14-43-84 d18-91 d21-93-99.8 d24-99.7 d28-100			d7-60 d14-96 d21-99					d2-0 d7-0-42 d10-53 d14-98-99.9 d18-83 d21-68-100 d24-76 d28-19	(Cornwell *et al.*1972)
21 mg/kg MT 100 mg/kg DECC	d7 -99 d14 -100	d7-90 d14-93			d7-99 d14-100					d7-0 d21-92	(Cornwell *et al.*1972)

[1] MT Morantel tartrate

[2] DECC Diethylcarbamazine citrate

[3] Indicates 14-day-old larvae and percentage efficacy

TABLE 13.3 PERCENTAGE EFFICACY OF MORANTEL AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Morantel salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
tartrate	1.6mg/kg in water for 6 weeks		80 L4-0[1]			98.6						(Downey and O'Shea, 1977)
tartrate	5 mg/kg drench	97	68-92			98-99		99-100		100		(Cornwell *et al.*1973; Anderson and Marais, 1975c)
tartrate	7.5 mg/kg		97									(Cornwell *et al.*1973c)
tartrate	7.5-15 mg/kg in feed	100	91	94		100				100		(Conway *et al.*1973)
tartrate	9-10 mg/kg bolus	100	77-98.5	77		100				100		(Conway *et al.*1973)

[1] L4 4th stage larvae

TABLE 13.3 CTD. PERCENTAGE EFFICACY OF MORANTEL AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine			References
Morantel salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
tartrate	10 mg/kg drench	98 d7-12[1] d14-99	94-98 d3-0 d6-0 d7-10-19 d9-0 d14-0-26 d18-80			24-99 d3-30 d6-86 d7-24-99.7 d9-97 d14-12 d18-99					18-84 d3-30 d7-0-24 d14-12	(Cornwell *et al.* 1973c; McKellar *et al.*1993)c
tartrate	10 mg/kg in feed or crumbles	100	85-95 L3-87[2] L4-0[3]	95-99.3 L3-100		99.2-100	100			100		(Ciordia and McCampbell, 1973; Conway *et al.*1973)
tartrate	10.5-11.5 mg/kg bolus	100	89 L3-95 L4-0	99 L3-100		100	100					(Ciordia and McCampbell, 1973)
tartrate	14.5 mg/kg bolus		87.5	100								(Conway *et al.*1973)
tartrate	15 mg/kg in feed	100	88-96	99.3-99.8		100				100		(Conway *et al.*1973)
tartrate	20mg/kg drench	d14 - 99.6	d14-32-52			d14-98					18-34	(Cornwell *et al.*1973c)

[1] Indicates 7-day-old larvae and 12% efficacy

[2] L3 3rd stage larvae

[3] L4 4th stage larvae

TABLE 13.4 PERCENTAGE EFFICACY OF LOW-DOSE MORANTEL AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Morantel salt	Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
tartrate	0.46 mg/kg/day prevent[1]	100	96			97	98				100	(Jones, 1983)
tartrate	0.61-0.65 mg/kg/day prevent	100	88-91 L4-0[3] L5-23[4]			95-99 L4-95 L5-100	98				88 Imm-40[5]	(Jones, 1983)
tartrate	0.78 mg/kg/day prevent		96 L4-55 L5-65			99 L4-92 L5-92					99 Imm-100	(Jones, 1983)
tartrate	0.95-1.11 mg/kg/day prevent	100	81-100 L4-21 L5-16			100 L4-95 L5-100	100				100 Imm-100	(Jones, 1983)
tartrate	0.26 mg/kg/day treatment[2]	100	92			100	97				0	(Jones, 1983)
tartrate	0.51 mg/kg/day treatment	100	99			99	89				0	(Jones, 1983)
tartrate	1.04 mg/kg/day treatment	100	100			100	98				0	(Jones, 1983)

1 Dose used to prevent development of nematode infection
2 Dose used to treat existing parasite infection
3 L4 4th stage larvae
4 L5 5th stage larvae/immature adult
5 Imm Immature stages

TABLE 13.5 PERCENTAGE EFFICACY OF MORANTEL SLOW-RELEASE BOLUSES AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		References
Moratnel salt	Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oe. radiatum*	
tartrate	Slow release bolus 154 day trial	76-77 L4-93.8[1]	57-66 L4-87	91-93	L4-100	99.5-100 L4-100			49-70	100	(Presson *et al.*1984b)
tartrate	Slow release trilaminate bolus		88			97					(Grimshaw *et al.*1989)
	Slow release trilaminate bolus		66-70 L3-100[2] EL4-100[3] Dvlp L4-100[4]	100		87-100 EL4-99.2 Dvlp L4 - 98.1	89			100	(Rickard *et al.*1989)

1 L4 4th stage larvae
2 L3 3rd stage larvae
3 EL4 Early 4th stage larvae
4 Dvlp L4 Developing 4th stage larvae

TABLE 13.6 PERCENTAGE EFFICACY OF MORANTEL AGAINST GASTROINTESTINAL NEMATODES OF CATTLE PREVIOUSLY TREATED WITH A MORANTEL SLOW- RELEASE BOLUS

		Abomasum			Small Intestine			Large Intestine			References
Morantel Salt	Dose rate	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Trichuris* spp.	*Oe. radiatum*	*D. viviparus*	
tartrate[1]	10mg/kg drench (prev MRSB exposed)		96			97					(Newby *et al.*1985a)
tartrate[1]	10mg/kg drench (not prev exposed)		95			91					(Newby *et al.*1985a)
tartrate[2]	10mg/kg drench (prev MRSB tx pasture)		96 EL4-75[3] Dvlp L4-0[4]		99 L4-100[5]	97	64				(Newby *et al.*1985b)
tartrate[2]	10mg/kg drench (not rev tx pasture)		94 EL4-0 Dvlp L4-24		99 L4-100	99	98				(Newby *et al.*1985b)

[1] Calves artificially infected with larvae exposed or not exposed to a season of the morantel slow release bolus, and then dosed with 10mg/kg morantel tartrate

[2] Calves grazed on pasture infected with larvae exposed or not exposed to a seaosn of being gfrazed by calves dosed with the morantel slow release bolus and then dosed with 10 mg/kg morantel tartrate

[3] EL4 Early 4th stage larvae

[4] Dvlp L4 Developing 4th stage larvae

[5] L4 4th stage larvae

14.0 TETRAMISOLE AND LEVAMISOLE

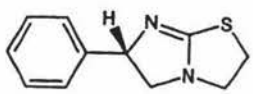
14.1 INTRODUCTION

One of the other major new anthelmintic action families to be discovered in 1966 were the imidazothiazoles. Anthelmintic activity was first seen in an aminothiazole derivative (thiazothienol), trialed against *Heterakis*, *Ascaridia* and *Capillaria* in chickens. The major metabolite was isolated from the faeces of treated birds, and identified as the imidazothiazole derivative. It too was active against a variety of gastronintestinal and pulmonary nematodes in sheep and poultry, at significantly lower levels than the aminothiazole derivative. An extensive series of chemically related compounds was synthesised, resulting in the emergence of tetramisole as the most promising substance. At very low doses when given orally or parenterally it was highly active against adult and mature gastronintestinal and pulmonary nematodes in many species, including cattle (Thienpont *et al.*1966).

14.2 PHARMACOLOGY

Tetramisole is a racemic mixture of two optically active compounds, the *laevo*-isomer and the *dextro*-isomer (Figure 14.1). The *laevo*-rotatory isomer (*l* -tetramisole), was found to be approximately twice as effective as *dl*-tetramisole, and several times more effective than *dextro*-tetramisole. It was also found that the toxicity was shared equally between isomers (Forsyth, 1968b).

Figure 14.1 Structure of Levamisole



Tetramisole and it salts are highly soluble in water. The salts most commonly used are the hydrochloride and phosphate. This high solubility has allowed a variety of formulations to be developed. As well as administration via drenching, drinking water or in feed, early work identified that the subcutaneous route was also effective (Thienpont *et al.*1966). For parenteral use, an aqueous formulation with the hydrochloride salt was more efficacious than levamisole base in propylene glycol. It was proposed that drug release from the propylene glycol and levels were below the threshold at which certain parasites are affected (Forsyth, 1968a). A topical levamisole formulation was developed in following years (Brooker and Goose, 1975) and was commercially released in 1976 (Quintana-da-Rosa *et al.*1976). There is also a slow release bolus available. The

bolus is oblong and covered with a coating that forces the drug to release through an uncoated central hole. The bolus contains 22.05g of levamisole that immediately after administration releases a therapeutic dose, with the remaining drug is released over three months.

After oral drenching or parenteral administration, levamisole is very rapidly absorbed, peak levels of between 1-1.5 mcg/ml being reached within 1-2 hours following dosing of 8mg/kg (Dorn and Federmann, 1976; Graziani and De Martin, 1977; Forsyth *et al.*1983; Nielsen *et al.*1983). Absorption after topical application can be more variable and requires a slightly higher dose rate to achieve similar peak concentrations that are reached 2-3 hours after treatment (Dorn and Federmann, 1976; Forsyth *et al.*1983). Possible reasons for the variability in serum levels associated with the rate of dermal absorption from topical application include, thickness of skin and length of the hair, age, breed, and climactic conditions such as season, and air temperature (Forsyth *et al.*1983). No differnce was seen wiht either fast or slolwy evaporating solvent bases (Curr, 1977). After absorption, excretion is very rapid, with little or no drug present within 24-48 hours (Graziani and De Martin, 1977; Forsyth *et al.*1983).

Tetramisole at low concentrations exerted a rapid paralysing effect on a variety of nematodes such as trichostrongylids, *Ascaridia*, *Chabertia* and *Bunostomum* (Thienpont *et al.*1966). This paralysis was associated with a sustained contraction of the parasites which was not blocked by curare or piperazine, indicating it was unlikely to be due to acetylcholine. There was also a reduction in intracellular resting potential from 34 to 10 mV (Aceves *et al.*1970). Van Nueten, (1972) concluded that tetramisole acts as a ganglion stimulant in both mammals and nematodes, resulting in neuromuscular inhibition of the depolarising type. This effect was blocked by the ganglion blockers mecamylamine and pempidine, which supported the nerve ganglion stimulant theory (Coles, 1974; 1977; Coles *et al.*1975).

Biochemical measurements were compatible with inhibition of fumarate reductase (Van den Bossche, 1972b). However, filarial worms not possessing this enzyme were also paralysed (Wang and Saz, 1974) which implied that this was not the primary mode of action. Also, because several helminths have been shown not to concentrate levamisole *in vitro*, it was unlikely that adequate concentrations would be reached to inhibit fumarate reductase *in vivo*. (Coles, 1977). More work on the changes in the resting potential of cells found that the same cation channels were being used for acetylcholine and levamisole (Harrow and Gration, 1985). The change in resting potential is due to levamisole acting as an agonist at synaptic and extrasynaptic nicotinic receptors resulting in the opening of non-selective cation channels permeable to sodium and potassium. This results in an influx of ions, depolarisation and stimulation of muscle contraction (Harrow and Gration, 1985; Martin, 1997). This mechanism of action is the same as that of pyrantel and morantel (Martin, 1997) which explains why resistance to the tetrahydropyrimidines also result in resistance to levamisole, and vice-versa.

Levamisole has been trialed in several formulations for use in cattle. These have included oral drenches, feed-premixes, those for administration to drinking water, and more recently a continuous release intra-ruminal bolus. Parenteral formulations have been restricted to those for subcutaneous injection, and there are also pour-on and spot-on topical preparations. Some innovative formulations have also been tried. Levamisole has been combined with clostridial vaccines to provide both an anthelmintic effect, and utilise the immunomodulatory effects of levamisole to improve the immune response (Hogarth-Scott *et al.*1980; Forsyth and Wynne-Jones, 1980). There was also a combination levamisole/oxyclozanide designed for the removal of liver fluke as well as nematodes (Walley, 1970)

14.3 EFFICACY

The first critical tests in sheep showed that tetramisole at doses of 5-20 mg/kg given orally, subcutaneously or intramuscularly was fully effective in a majority of animals against adult and immature forms of most gastronintestinal and pulmonary parasites. Similar results were found in cattle against all the gastronintestinal and pulmonary nematodes tested (*Ostertagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Trichuris* and *Dictyocaulus viviparus* (Thienpont *et al.*1966). These results were confirmed in sheep (Walley, 1966) but not in cattle (Tables 14.1 and 14.2). Doses that were effective against the majority of parasites in cattle (10 mg/kg subcutaneously), had low efficacy for adult *Ostertagia* spp. (80.1%) and *Trichostrongylus axei* (82.9%), and little if any efficacy for immature *Ostertagia* spp. (26.6%) (Forsyth, 1966). Increasing the oral dose rate of tetramisole up to 15-20 mg/kg did improve efficacy against immature *Ostertagia* (Forsyth, 1966), but in several trials it was still low (38-53%) (Ross, 1968). Other workers confirmed similar levels of efficacy in controlled trials when used at doses of 6-15 mg/kg orally or parenterally (Forsyth, 1968a; Ross, 1968; Smith and Archibald, 1969). These and higher doses (up to 20 mg/kg) provided good results in the field in outbreaks of parasitic bronchitis (McCulloch *et al.*1968) and Type II ostertagiasis (Reid *et al.*1968).

The discovery that anthelmintic activity resided in the laevo-isomer allowed the dose rate to be decreased by half while retaining the same level of efficacy (Forsyth, 1968b). *Haemonchus*, *Nematodirus*, *Cooperia*, *Oesophagostomum* and *Dictyocaulus* were 95 -100% removed with an oral drench of 5-7.5 mg/kg (Hart *et al.*1969; Broome and Lewis, 1974; Anderson, 1977; Lyons *et al.*1981; Forsyth *et al.*1983), but 8 mg/kg and above was required to remove 94-99% of adult *Ostertagia* and 60-100% of *T. axei.* (Rubin and Hibler, 1968; Alicata and Furumoto, 1969; Ciordia and Baird, 1969; Baker and Fisk, 1972a; Lyons *et al.*1972; Anderson, 1977) (Table 14.3). However, even at these higher doses, immature *Ostertagia* was still the dose limiting parasite (Rubin and Hibler, 1968; Alicata and Furumoto, 1969; Ciordia and Baird, 1969; Baker and Fisk, 1972a; Lyons *et al.*1972; Anderson, 1977). Occasionally there was no efficacy against early or developing 4th stage larvae

even at doses of 14.1 mg/kg (Anderson, 1977). There were no therapeutic advantages in increasing the oral dose above 8 mg/kg.

Levamisole has also been administered as a bolus (Ciordia and Baird, 1969; Lyons *et al.*1981; 1983; Berger *et al.*1984) and a gel (Berger *et al.*1984) (Table 14.3). Efficacy was comparable at the same dose rates as used for the drench, 8 mg/kg providing >94% efficacy against adult *Ostertagia* (Lyons *et al.*1983; Berger *et al.*1984). A similar response occurred when used as a medicated premix with doses of 8-12 mg/kg being the most effective (Rubin and Hibler, 1968; Presidente *et al.*1971; Ciordia and McCampbell, 1971b; Lyons *et al.*1975a).

When added to drinking water at 7.8-8 mg/kg, in one trial, the response was more variable against *Ostertagia* and *T. axei* than seen with a similar dose given by another route of administration (drench, subcutaneous, topical). This was associated with the volume of water (and drug) being consumed by individual animals being too low to result in anthelmintic efficacy against these two parasites (Baker and Fisk, 1972b). This is a common problem with ad-lib treatment systems. These doses however were highly effective (>99%) for small intestinal parasites and *Oesophagostomum* spp. (Lyons *et al.*1975a; Baker and Fisk, 1972b) (Emro J and Poeschel G; American Cyanamid Company, NJ, USA. Unpublished data, March 1, 1971 cited in Baker and Fisk, (1972b)).

Because of its water solubility, levamisole was also formulated for subcutaneous injection. The hydrochloride salt was the most commonly used, though some trials used the phosphate salts (Ronald *et al.*1977). Similar efficacy as oral administration was seen when administered at slightly lower doses (4-8 mg/kg subcutaneously) (Table 14.4). Again, adult and immature *Ostertagia* spp. and adult *Trichostrongylus axei* were the limiting species. However, clinically there was no difference between fenbendazole and levamisole used at 7.5 mg/kg SQ in a field outbreak of ostertagiasis (Forsyth and Shepherd, 1977).

Topical application of levamisole is another common route of administration. Two formulation types are used. A spot-on treatment which is applied to a small area, or a pour-on, applied along the back-line. Both are highly efficient at doses of 7.5 mg/kg and above (Table 14.5) (Brooker and Goose, 1975; Quintana-da-Rosa *et al.*1976; Curr, 1977; Rowlands and Berger, 1977; Guerrero *et al.*1984; Williams, 1991; Vanparijs and Quick, 1991). *Trichuris* are less susceptible than other gastrointestinal and pulmonary parasites even at doses as high as 12.5 mg/kg (Quintana-da-Rosa *et al.*1976; Guerrero *et al.*1984). It was shown that there could be a seasonal influence on efficacy, colder temperatures lowering the amount of active absorbed resulting in the removal of fewer worms (Table 14.6). However, another trial did not support these findings (Seibert *et al.*1986a).

A continuous release bolus has also been trialed to try and prevent calves developing infections with *Dictyocaulus*. The bolus did allow calves to develop a light infection, the advantage of which was

that the animals developed some immunity. The disadvantage was that once the infection became patent, the pasture was seeded with infective larvae (Borgsteede *et al.*1990).

Levamisole is very efficient at removing *Dictyocaulus viviparus*. Between 91-100% of adult, developing and inhibited larvae are removed following a single dose of 6-7.5 mg/kg (Forsyth, 1968b; Turton, 1969; Broome and Lewis, 1974; Ronald *et al.*1977; Oakley, 1980a; 1981; Forsyth *et al.*1983; Guerrero *et al.*1984; Yazwinski *et al.*1985). Up to 87.5% of worms are expelled within three hours after treatment. Fenbendazole and febantel both took up to 36 hours to remove 80-84% of parasites (Oakley, 1980b). This efficiency gave it considerable advantages over drugs such diethylcarbamazine which requires multiple doses at 24 hour intervals (Oakley, 1980a). However there were concerns that frequent dosing of calves with levamisole may result in animals developing lower levels of immunity, and on subsequent re-exposure to infective *Dictyocaulus* larvae, significant parasitic bronchitis would develop (Oakley, 1980a; Downey, 1980). Challenge trials did support some lowering in immunity, but this was still sufficient to provide between 66-99% protection in most calves when compared with untreated controls (Oakley, 1980a; 1982a; 1982b; Downey, 1980; Urquhart *et al.*1981). However, in some cases levels of protection were erratic (Oakley, 1980a). Because of this variability, vaccination was suggested as the only effective method of prophylaxis (Urquhart *et al.*1981). Comparing two or three treatments with 7.5 mg/kg levamisole at 14 day intervals provided a similar if not quite the same level of protection from larval challenge as vaccination (Urquhart *et al.*1981; Oakley, 1982a; 1982b). Some animals suffer acute respiratory distress and physical collapse following challenge infection. This is known as the "reinfection" or "post-patent syndrome" . It is associated with death of some migrating *Dictyocaulus* larvae in the lungs. This results in occlusion of bronchioles, loss of bronchial epithelium, proliferation of pneumocytes (epithelialisation) and a marked infiltrate of eosinophils and exudate. This condition may be fatal in some animals (Oakley, 1980a; Downey, 1980; Urquhart *et al.* 1981).

14.4 SIDE EFFECTS AND TOXICITY

The racemic tetramisole mixture had a rather low therapeutic index. Several calves treated with the therapeutic dose showed side effects immediately post drenching. these included lip licking, salivation, head shaking, skin tremours and increased excitability along with increased defaecation (Forsyth, 1966; 1968a; McCulloch *et al.*1968). Because the toxic side effects for the two isomers (*laevo* and *dextro*) and the racemic mixture are almost identical, it has been possible to reduce the dose and increase the safety factor two fold (Forsyth, 1968b; O'Brien, 1970). The side effects are the same as seen with tetramisole and these also include convulsions, pulmonary oedema, collapse and deaths. The excitability is due to arousal of the central nervous system (Hsu, 1980). The parenteral formulations tend to be associated with more side effects than oral use. Many of these signs resemble those seen in nicotine poisoning. As levamisole acts on the nicotinic receptors of

the ganglia this is not surprising (Hsu, 1980). These effects tend to peak around the time of peak plasma concentration, about 1-2 hours post dosing (Dorn and Federmann, 1976).

It was proposed that levamisole should not be used at the same time as organophosphates due to a potential depression of acetylcholinesterase. No synergistic decrease of this enzyme has been seen when these members of these two drug classes are used in combination (Ford and Evans, 1987).

One case of suspected levamisole resistance in cattle has been reported. However, while *Ostertagia ostertagi* larvae were present after dosing, no worm count was undertaken to support these findings (Geerts *et al.*1987).

TABLE 14.1 PERCENTAGE EFFICACY OF ORAL TETRAMISOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
12.5 mg/kg drench		93 Imm-52-66[1]		99.99	100						(Reid *et al.*1968; Smith and Archibald, 1969)
13.2 mg/lg drench	100	88.2-95.7 Imm-71-89	86.8-89.8 Imm-71-89	100	100				100	96.7	(Forsyth, 1966; Forsyth, 1968a)
15mg/kg drench		98 d4-38.8[2] d10-36.5			100 d4-99.8 d10-99.9					98.3 d4-89.3 d10-97.2	(Ross, 1968)
20 mg/kg drench		79.5 Imm-53									(Reid *et al.*1968)

[1] Imm Immature stages

[2] Indicates 4-day-old larvae and percentage efficacy

TABLE 14.2 PERCENTAGE EFFICACY OF PARENTERAL TETRAMISOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine				Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
6 mg/kg SQ[1]	100	83.2	77.6		100					86.5	(Forsyth, 1968a)
6.6mg/kg SQ	100	82.1	86		100		99.6		100		(Forsyth, 1968a; 1968b)
8.5 mg/kg SQ	100	92.3	78.4		100					88.2	(Forsyth, 1968b)
8.9 mg/kg SQ	100 d10-100[2] d24-100	83.8-91.3 d10-9.6-39.8 d24-91.3	57.8-89.8 d10-65-76 d24-58-76		100 d10-98.5-99.5 d24-100		99.6 d10-84.7		100 d10-84.7		(Forsyth, 1968a; 1968b)
10mg/kg SQ	100	80.1-88.7 Imm-27-86.3[3]	82.9- 92.4 Imm-27-86.3	100	99.9-100				100	96.1	(Forsyth, 1966; Forsyth, 1968a)

[1] SQ Subcutaneous injection

[2] Indicates 10-day old larvae

[3] Imm Immature stages

TABLE 14.3 PERCENTAGE EFFICACY OF ORAL LEVAMISOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
HCl[1]	4mg/kg drench		99	27		100						(Rubin and Hibler, 1968)
HCl	5 mg/kg drench	100	85	83	100	99				99	88	(Hart *et al.*1969)
HCl	6 mg/kg drench	100	45-93.3 L4-0[2] Imm-16.1[3]	91.2- 95 L4-86		99.5	96.6				95	(Lyons *et al.* 1981b; Forsyth *et al.* 1983)
HCl	7 mg/kg drench		42-68 EL4-0-29[4] Dvlp L4-0[5]	92.2-100								(Anderson, 1977)
HCl	7.5 mg/kg drench	100	84	97	100	100				100	95.7- 100 d3-93.5[6] d9-94.8 d16-86.6	(Hart *et al.*1969; Broome and Lewis, 1974)

[1] HCL Hydrochloride salt
[2] L4 4th stage larvae
[3] Imm Immature stages
[4] EL4 Early 4th stage larvae
[5] Dvlp L4 Developing 4th stage larvae
[6] Inidcates 3-day-old larvae and percentage efficacy

TABLE 14.3 CTD. PERCENTAGE EFFICACY OF ORAL LEVAMISOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
HCl[1]	8 mg/kg drench	96-100 L4-53[2]	94-99 L4-33 Imm-86[3]	60-99	99-100	99-100 L4-100	99-100	100	99	97	98-100 Imm-98	(Rubin and Hibler, 1968; Alicata and Furumoto, 1969; Ciordia and Baird, 1969; Baker and Fisk, 1972a; Lyons *et al.* 1972)
HCl	10.6 mg/kg drench		46-87.3 EL4-0-43.3[4] Dvlp L4-0[5]	96.8-100								(Anderson, 1977)
HCl	12 mg/kg drench		96 Imm-81	99	99	99	100				98	(Baker and Fisk, 1972a)
HCl	14.1 mg/kg drench		74-88.3 EL4-0-73 Dvlp L4-0	97.9-99.3								(Anderson, 1977)

[1] HCl Hydrochloride salt
[2] L4 4th stage larvae
[3] Imm Immature stages
[4] EL4 Early 4th stage larvae
[5] Dvlp L4 Developing 4th stage larvae

TABLE 14.3 CTD **PERCENTAGE EFFICACY OF ORAL LEVAMISOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE**

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
HCl[1]	5.4 mg/kg bolus	100	94	81		100	100	100		99		(Ciordia and Baird, 1969)
HCl	5.4-10.2 mg/kg bolus		23 L4-0[2]	85							100	(Lyons *et al.*1981b)
HCl	8 mg/kg bolus		94-98 Imm-66-100[3]									(Lyons *et al.*1983; Berger *et al.*1984)
HCl	8mg/kg Gel		99.6									(Berger *et al.*1984)
HCl	7.8 mg/kg in drinking water		78-99 Imm-10-100	76-99 Imm-78-100	99-100	99			40-76	100		(Baker and Fisk, 1972b)
HCl	8 mg/kg in drinking water	99-100	77-90 L4-64	85-92		99-100 L4-100	100			99-100		Emro, J., & Poeschel,G.; American Cyanamid Company, NJ, USA. Unpublished data, March 1, 1971 cited in (Baker and Fisk, 1972a), (Lyons *et al.* 1975a)

[1] HCl Hydrochloride salt
[2] L4 4th stage larvae
[3] Imm Immature stages

TABLE 14.3 CTD. PERCENTAGE EFFICACY OF ORAL LEVAMISOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
HCl[1]	4mg/kg medicated feed		99	36		100						(Rubin and Hibler, 1968)
HCl	8mg/kg medicated feed	99	80-100 L4-0[2] Imm-18-71[3]	52-99	99	67-100 L4-100	96.7- 100	94.5- 100	12-38	100		(Rubin and Hibler, 1968; Presidente *et al.* 1971; Ciordia and McCampbell, 1971b; Lyons *et al.*1975a)
HCl	12mg/kg medicated pellets		86 Imm-28	94		100		100	100	100		(Presidente *et al.*1971)

[1] HCl Hydrochloride salt
[2] L4 4th stage larvae
[3] Imm Immature stages

TABLE 14.4 PERCENTAGE EFFICACY OF PARENTERAL LEVAMISOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
HCl[1]	1.5 mg/kg SQ	100	97	1	100	100	93			99		(Hart *et al.*1969)
HCl	3-3.75 mg/kg SQ	100	78.9-98	49-86.6	100	95.6- 100	97	100		100		(Hart *et al.* 1969; Quintana-da-Rosa *et al.* 1976; Forsyth, 1968b)
PO4[2]	4 mg/kg SQ	98.3 Imm-91.8[3]	98 Dvlp L4-96.3[5]	100		99.5				100		(Ronald *et al.*1977)
HCl	4.45 mg/kg SQ	100	90.5 d10-0	89.3 d10-55		100 d10-98.7		100		100 d10-100		(Forsyth, 1968b)
PO4	6 mg/kg SQ	98.5- 100 Imm-94	81.3-95.7 EL4-42.7[6] Dvlp L4-0-87.6 L4-14.8[7] Imm-0-41.5	72.3-100	100 L4-99.6	98.9-100 L4-0-99.2	99.1-100	91.1		100	91.2-100	(Ronald *et al.* 1977; Forsyth *et al.* 1983; Guerrero *et al.* 1984; Yazwinski *et al.* 1985)
PO4	6.6 mg/kg SQ	100 d1-100[4]	88.7-95.1 d10-15-57.7	40.5-93.8 d10-44-83.6		100 d10-98.5-99		100		100 d10-100		(Forsyth, 1968b)

[1] HCL Hydrochloride salt [2] PO4 Phosphate salt [3] Imm Immature stages [4] Inidcates 1-day-old larvae and percentage efficacy
[5] Dvlp L4 Developing 4th stage larvae [6] EL4 Early 4th stage larvae [7] L4 4th stage larvae

TABLE 14.4 CTD. PERCENTAGE EFFICACY OF PARENTERAL LEVAMISOLE AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
HCl[1]	7.5 mg/kg SQ		97.7 L4-47.3[3] L5-30.1[4]	d8-88 d15-98		99.9 L4-99.7 L5-99.2					95.9-98.1 d3-81.2-93 d9-91-98.8 d16-98.2-99.9 Inhib-93.5-97.7[7]	(Turton, 1969; Broome and Lewis, 1974; Oakley, 1980a; Oakley, 1981)
HCl	8 mg/kg SQ	99.9 Imm-91[2]	93-97.9 Dvlp L4-93.8[5] L4-23	99-99.1		99.8-100 L4-100	100			100		(Lyons *et al.* 1975a; Ronald *et al.* 1977)
HCl	8.9 mg/kg SQ		d10-31.5[6]	d10-88.4		d10-99.5				d10-94.3		(Forsyth, 1968b)
HCl	10 mg/kg SQ		99.3 L4-58.6 L5-55.2			99.7 L4-99.6 L5-99.4				d7-96.8	96.4	(Turton, 1969; Pouplard *et al.* 1986)

[1] HCl Hydrochloride salt
[2] Imm Immature stages
[3] L4 4th stage larvae
[4] L5 5th stage larvae/immature adult
[5] Dvlp L4 Developing 4th stage larvae
[6] Inidcates 3-day-old larvae and percentage efficacy
[7] Inhib Inhibited larvae

TABLE 14.5 PERCENTAGE EFFICACY OF LEVAMISOLE POUR-ON AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
HCl[1]	5mg/kg	100	93.7	77.4		96.5	100			99.8	98	(Quintana-da-Rosa *et al.*1976; Vanparijs and Quick, 1991)
HCl	6.5 mg/kg	100	84.4			98.1	92.5	*S. papillosus* 100	36.4	100		(Quintana-da-Rosa *et al.*1976)
HCl	7.5 mg/kg	100	86.6-91.6	93.5	92.5	99.9-100	99.3	97.9	50.9	98.4-99.3		(Quintana-da-Rosa *et al.*1976; Guerrero *et al.*1984)
Levamisole base. Rapid evaporating solvent	8 mg/kg	97.1-100 Imm-100[2]	88.5-100 Imm-40-100	89.2-100		100 Imm-96.3-100				98.7-100	100	(Curr, 1977)
Levamisole base. Slowly evaporating solvent	8mg/kg	100	98.6 Imm-0	95.1		100 Imm-98.2				100		(Curr, 1977)

[1] HCl Hydrochloride salt
[2] Imm Immature stages

TABLE 14.5 CTD. PERCENTAGE EFFICACY OF LEVAMISOLE POUR-ON AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
HCl[1]	9.75 mg/kg		100			100	100					(Brooker and Goose, 1975)
HCl	10 mg/kg	98.9-100 L3-72-99.3[2] L4-100[3]	60.1-98.3 L3-85.5 L4-38.1-65.8 EL4-32.1[4] Dvlp L4-21.8[5] Imm-0-100[6]	54-97.8	96.3-97.2 L4-42	94.6-100 L3-98.9 L4-89.6-100 Imm-97.8	79-100	98.5-100 L3-83 L4-100	77	97.9- 100 L3-47.4 L4-94.9	93.8-100 L3-79.3 (d3) L4-94.1 L5-90.9[7]	(Curr, 1977; Rowlands and Berger, 1977; Guerrero *et al.* 1984; Williams, 1991; Vanparijs and Quick, 1991)
Levamisole base. Rapid evaporating solvent 1977	12mg/kg	100	100 Imm-100	100		100 Imm-100					84.1	(Curr, 1977)
HCl	12.5 mg/kg		95.5	90.6	94	100		97.9	47.9	99.3		(Guerrero *et al.*1984)

[1] HCl Hydrochloride salt
[2] L3 3rd stage larvae
[3] L4 4th stage larvae
[4] EL4 Early 4th stage larvae
[5] Dvlp L4 Developing 4th stage larvae
[6] Imm Immature stages
[7] L5 5th stage larvae/immature adult

TABLE 14.6 PERCENTAGE EFFICACY OF LEVAMISOLE POUR-ON AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE WHEN APPLIED IN DIFFERENT SEASONS

		Abomasum			Small Intestine				Large Intestine		Lungs	References
Salt	Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia* spp.	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
Trial 1 Winter 3-12C	10mg/lg	98.9-100	60.1-72.1 Imm-23.5-36.5[1]	54-71.8		99.7	79-98.6			100	50.3-85.6	(Forsyth *et al.*1983)
Trial 1 Summer 13-24C	10mg/kg	100	81.7-98.9 Imm-0-41.2	83.9-97.4		99.2-99.9	96.6-100				97.1	(Forsyth *et al.*1983)
Trial 2 Summer 26 C	10 mg/kg	97.7	89.6	83.3	94.1	99.9		100		97.3		(Seibert *et al.*1986a)
Trial 2 Winter 2 C	10 mg/kg	98.7	98.1	89.2	93.8	99.9		100		98.3		(Seibert *et al.*1986a)

[1] Imm Immature parasites

15.0 AVERMECTINS AND MILBEMYCINS

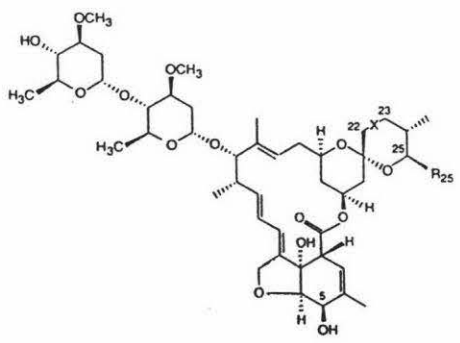
15.1 INTRODUCTION

The avermectin/milbemycin family of anthelmintics was the last new broad spectrum action anthelmintic family to have been discovered. These compounds have extraordinary broad spectrum anthelmintic and ectoparasiticide activity at microgram/kilogram doses. Because of this combination of endo- and ectoparasiticide activity, the term endectocide was coined to describe them.

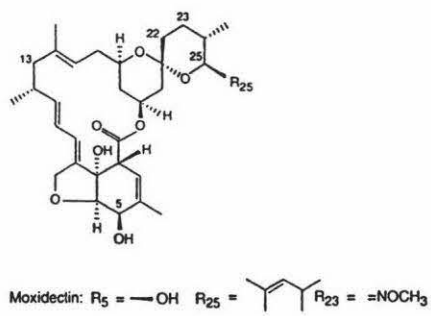
Since their first introduction, considerable research has been carried out on these molecules in an attempt to maximise endectocide activity and safety while retaining potency. Along with the benzimidazole and imidazole classes of anthelmintics, the avermectins/milbemycins have proved the mainstay of intensive agricultural production over the last 25 years. While it is likely that with research and development, more molecules will be produced, it may be some time before another action family as efficient as these compounds is seen.

The avermectins and milbemycins are closely related 16-membered macrocyclic lactones (Figure 15.1). They are produced through fermentation by soil dwelling acting actinomycetes of the genus *Streptomyces* (Burg *et al.*1979; Campbell, 1985; Doscher *et al.*1989; Goudie *et al.*1993; Shoop *et al.*1996a). Ivermectin or 22, 23-dihydro avermectin B1a, was the first avermectin to be commercially released as an anthelmintic in 1981. This was followed by another avermectin, abamectin (avermectin B1a) which was registered in 1985 for use in cattle in Australia (Benz and Cox, 1989). Since then, many drug companies have pursued the development of their own endectocide molecules. The first milbemycin for commercial use in cattle (moxidectin) was released in Argentina in 1990 (McKellar and Benchaoui, 1996). Following this, further modifications were made to the avermectin B1a molecule resulting in doramectin (Goudie *et al.*1993) and more recently eprinomectin (Shoop *et al.*1996a).

Figure 15.1 Structures of the Avermectin and Milbemycin anthelmintics



Ivermectin: X = $-CH_2CH_2-$ R_{25} = $CH(CH_3)CH_2CH_3$ and $CH(CH_3)_2$
Abamectin: X = $-CH=CH-$ R_{25} = $CH(CH_3)CH_2CH_3$ and $CH(CH_3)_2$
Doramectin: X = $-CH=CH-$ R_{25} = Cyclohexyl



Moxidectin: R_5 = —OH R_{25} = R_{23} = $=NOCH_3$

15.2 PHARMACOLOGY

The avermectins are a series of macrocyclic lactone derivatives that, in contrast to the macrolide or polyene antibiotics, lack significant antibacterial or antifungal activity, but have extraordinary anthelmintic activity (Burg *et al.*1979). They were identified within a fermentation broth of an actinomycete isolated from soil samples from the Kitasato Institute in Japan (Bowen, 1981). The actinomycete was a previously unidentified organism and was later named *Streptomyces avermitilis*. Ultraviolet irradiation of the original culture resulted in a mutant that grew more rapidly and produced avermectins at a higher rate for much longer (Campbell *et al.*1983). The resultant avermectin complex was composed of four major compounds, designated A1a, A2a, B1a and B2a, and four minor compounds, A1b, A2b, B1b and B2b (Bowen, 1981). Compound B1 was found to have the greatest anthelmintic activity and developed further as an anthelmintic. Because these are fermentation products, purifying the compounds is not possible, and so the anthelmintics while given a single name, are a mixture of avermectins.

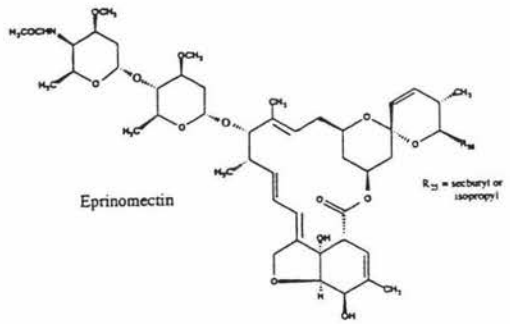
A mixture containing not more than 80% of B1a and less than 20% of B1b was given the generic name abamectin. It had very good efficacy against gastrointestinal and pulmonary nematodes of sheep and cattle at doses as low as 25 micrograms/kilogram (Egerton *et al.*1979). To improve the efficacy seen with the B1a and B1b components, structural modification via selective hydrogenation resulted in 22, 23 dihydro avermectin B1 (Campbell *et al.*1983; Shoop *et al.*1995). The generic name attributed to this mixture of compounds was ivermectin. It maintained the excellent potency and spectrum of activity seen with abamectin, but had a greater safety margin (Shoop *et al.*1995). This was the compound that was commercialised worldwide. The natural fermentation product abamectin was initially registered for arthropod crop control, but was only registered as a cattle anthelmintic in Australia in 1985 (Benz and Cox, 1989).

The milbemycins had been discovered in 1973 as acaricidal and insecticidal compounds but the full potential of these compounds was not realised until the acaricidal, insecticidal and nematocidal activities of the avermectins were (Shoop *et al.*1995). In 1989, nemadectin was produced from the actinomycete *Streptomyces cyanogriseus* subsp. *noncyanogenus* (Doscher *et al.*1989; Asato and France, 1990). Structural modification of this compound by the insertion of a tri-substituted double bond resulted in moxidectin. This differs from ivermectin by having no sugar moiety at C-13, and a saturated side chain at C-25 (Zimmerman *et al.*1992; Zulalian *et al.*1994). The lack of the sugar moiety at C13 on the macrocycle ring is what distinguished the milbemycins from the avermectins (Zulalian *et al.*1994).

A further series of novel avermectins was prepared by mutational biosynthesis and the best of these was doramectin. The only difference between this compound and abamectin is the cyclo-hexyl attachment to the C-25 position of the avermectin ring (Goudie *et al.*1993).

More recently, further structural changes to avermectin B1 have shown that the 4-epi-amino-subclasses have potent broad spectrum activity against endoparasites including *Cooperia*, and a low plasma-milk partitioning complex. The low milk partitioning would allow the compound to be used in lactating dairy cattle, a group of animals in which there were major constraints on anthelmintic use. The member of the 4"-epi-amino family that possessed the best combination of efficacy and low milk partitioning is eprinomectin (Figure 15.2) (Shoop *et al.*1996a). It is also a mixture of two homologues, 4-epi-acetyloamino-4-deoxy avermectins B1a and B1b in a ratio of 90:10 (Gogolewski *et al.*1997b). It was first released commercially in New Zealand, in 1997.

Figure 15.2 Structure of Eprinomectin



The pharmacokinetics of these chemicals vary depending on the route of administration and formulation. They are all systemically absorbed following administration by the oral, parenteral or topical routes (Campbell, 1985). However, measurable concentrations of ivermectin were not present in ruminal or abomasal fluids even when up to ten times the therapeutic subcutaneous dose was given (Bogan and McKellar, 1988). Higher doses are used for topical administration (500 mcg/kg) than for parenteral use (200 mcg/kg) because of the variable absorption kinetics of the bovine skin. Topical administration of ivermectin results in a plateau of peak plasma concentration on day 2-3 followed by a slow decline until day 28 (Herd *et al.*1996). This compares with a subcutaneous injection of ivermectin that peaks with a 25% higher plasma concentration on days 3-5 and declines with a half life of 3-7 days to negligible levels on day 35 (Hsu *et al.*1989; Herd *et al.*1996). It was found that there was much more inter-animal variation in the time to the start of the peak concentration with the use of the topical formulation (Herd *et al.*1996), but no therapeutic differences have been seen. A similar dose response has been seen with moxidectin (Zulalian *et al.*1994; Woodward, 1996) and doramectin (Goudie *et al.*1993; Nowakowski *et al.*1995).

Following absorption, and some metabolism in the liver (primarily by hydroxylation), more than 95% of the avermectin/milbemycin is excreted into the faeces via the bile, with less than 2-3% excreted in the urine (Campbell, 1985; Bogan and McKellar, 1988; Hsu *et al.*1989; Zulalian *et al.*1994).

Avermectins induce rapid, non-spastic paralysis in nematodes. One common feature is the modulation of trans-membrane chloride ion channel activity in nematode nerve cells. The chloride channels may be gated by a variety of neurotransmitter receptors including GABA, glutamate and acetylcholine. Activation of the chloride channels by avermectins lead to an increase in chloride

conductance that results in a changed membrane potential, and this causes inhibition of electrical activity in the target nerve. The motor neuron remains negatively charged and so the signals (inhibitory and excitatory) are not registered by the recipient cell. The muscle cells do not function, and this results in non-spastic paralysis (Bowen, 1981; Campbell, 1981; 1985; Campbell *et al.*1983; Turner and Schaeffer, 1989; Conder *et al.*1993; Zulalian *et al.*1994; Shoop *et al.*1995; Roberts, 1996). Besides the GABA-gated channels, there are also avermectin sensitive glutamate chloride channels. The effects of avermectins at low concentrations are to potentiate the effect of glutamate on these channels, and at higher concentration, the avermectins open the glutamate channel directly (Martin, 1997).

There has been debate about whether the avermectins and milbemycins have the same mode of action. An *in vitro* trial using GABA-innervated muscle fibred of the crustacean *Pachygrapsus crassipes* (shore crab) showed that both ivermectin and moxidectin produce quantitatively similar responses. Both anthelmintics were reversed by picrotoxin, a potent blocker of chloride channels. The rapid onset of changes in conductance after exposure to ivermectin or moxidectin are characteristic of chloride channel agonists on exposed target membranes (Conder *et al.*1993). These similar responses would suggest that the mode of action is the same. Doramectin is a structural analogue of avermectin B1 and would also be expected to have the same mode of action.

In cattle, the avermectins were initially trialed as an oral formulation. However, subcutaneous injection fitted better with cattle management practices and this became the standard method of administration. All the current avermectins and milbemycins used in cattle, except eprinomectin, are available in formulations for form of administration. Technology changes have also seen the introduction of topical application and intra-ruminal slow-release boluses. Ivermectin, moxidectin and doramectin are all currently registered in topical preparations. In recognition of market demand for ease of cattle treatment, eprinomectin was only released as a topical preparation. Currently only ivermectin is available in a slow release bolus, but it will not be too long before the other compounds are also available in this formulation also.

15.3 EFFICACY

The currently registered avermectins (ivermectin, abamectin, doramectin and eprinomectin) and milbemycin (moxidectin) all have a very broad spectrum of activity against adult, developing and hypobiotic stages of most gastrointestinal nematodes. They are also very effective against the lungworm *Dictyocaulus viviparus* at very low doses. A summary of the published literature on their efficacy is provided in Tables 15.1-15.18.

The dose limiting parasites for ivermectin, abamectin and doramectin are *Cooperia* spp. and

Nematodirus spp. (Egerton *et al.*1979; Armour *et al.*1980; Alva-Valdes *et al.*1986; Heinze-Mutz *et al.*1993; Kaplan *et al.*1994; Goudie *et al.*1993; Jones *et al.*1993). Some trials with ivermectin also showed that *Trichostrongylus colubriformis* may also be less sensitive than other nematodes (Benz and Ernst, 1981; Egerton *et al.*1981; Alva-Valdes *et al.*1986). *Cooperia* also seems to be the limiting species for moxidectin, a small number of viable eggs being passed after treatment at the standard dose-rate, but not to the same extent as for ivermectin (Scholl *et al.*1992; Williams *et al.*1992c; Taylor *et al.*1993).

The 200 mcg/kg dose-rate is the minimum effective oral or subcutaneous dose required to remove most gastrointestinal and pulmonary nematodes (Tables 15.1, 15.2, 15.7, 15.8, 15.10, 15.11, 15.15). Topical preparations are not as efficacious at this dose level, and the lowest effective dose for the dose-limiting species is 500 mcg/kg (Tables 15.4, 15.13, 15.17, 15.18). An intra-ruminal bolus that releases 8 mg/day ivermectin over 120 days is also very efficacious (>99%) against most gastrointestinal and pulmonary nematodes, including *Cooperia* spp. (Table 15.6) (Egerton *et al.*1986; Soll *et al.*1988; Alva-Valdes *et al.*1988; Zimmerman *et al.*1991). In this case it was *Trichuris* spp. that were the dose-limiting species (Soll *et al.*1988; Alva-Valdes *et al.*1988).

Because of the very high efficacy of these compounds, considerable attention has been paid to their ability to limit pasture contamination. Many trials with these compounds have shown that a single strategic drench of young animals in the spring will result in a decrease of between 94-99% in faecal egg and larval counts for up to 5-6 weeks (Armour *et al.*1987; Vercruysse *et al.*1993; Fisher *et al.*1995; Vercruysse *et al.*1995; Eysker *et al.*1996). By not requiring a further drench until the end of this 5-8 week period, the number of treatments can be decreased compared with the use of other anthelmintics (Brunsdon *et al.*1989), while still resulting in a considerable lowering in late summer and autumn pasture larval numbers (Armour *et al.*1987; Eysker *et al.*1996; Vercruysse *et al.*1993; 1995; Fisher *et al.*1995).

Not only are these compounds effective in suppressing faecal egg output, they also have a persistent lethal activity against ingested larvae (Tables 15.3, 15.5, 15.9, 15.12, 15.14, 15.16) (Barth, 1983; Armour *et al.*1985). For all of these compounds, *Cooperia* spp. were the limiting species, with a protective period against infective larvae of between 7-14 days and in occasional instances up to 21 days (Barth, 1983; Armour *et al.*1985; Weatherley *et al.*1993; Yazwinski *et al.*1994d; Eysker and Eilers, 1995; Entrocasso *et al.*1996; Rolfe *et al.*1997). *Dictyocaulus* sp. were the most sensitive species, with >99% protection against infective larvae occurring for up to 28 -35 days, (McKenna, 1989; Weatherley *et al.*1993; Hong *et al.*1995; Eysker and Eilers, 1995; Barth *et al.*1997; Vercruysse *et al.*1997) and in some cases up to 90% protection for 42 days (Hubert *et al.*1995). Activity against ingested *Ostertagia* larvae was intermediate, ranging between 14-28 days for ivermectin and doramectin (Swan and Harvey, 1983; Armour *et al.*1985; McKenna, 1989; Weatherley *et al.*1993; Hong *et al.*1995), but moxidectin appeared to have a protective effect of

>99% for up to 35 days (Hubert *et al.*1995; Eysker and Eilers, 1995; Eysker *et al.*1996).

Combining the suppressive effect on FEC and the persistent activity against ingested larvae has provided an opportunity to protect calves and limit pasture protection for up to 6-8 weeks. The persistent activity also allows animals to be returned to infective pasture after treatment before being moved onto new pasture. The high degree of efficacy will also limit the level of pasture contamination subsequently (Armour *et al.*1985; Yazwinski *et al.*1994d). However, there is concern that such a suppressive regime could impair the immune status of treated animals (Armour *et al.*1988; Yazwinski *et al.*1994d; Vercruysse *et al.*1995). Trial work did indicate that there may be some decrease in immunity, but in most cases it was not of of clinical significance (Armour *et al.1988;* Vercruysse *et al.*1995).

Nevertheless, besides the advantages of extending the treatment interval, being able to graze treated animals on infective pastures and lowering pasture contamination, there are also some potential disadvantages. The persistent nature of these compounds has resulted in extended withdrawal periods because of residues, and there may be the potential for the preferential selection of nematode species naturally resistant/tolerant to the avermectins (Yazwinski *et al.*1994d). It is possible that the use of slow release boluses may accentuate the potential problems of delayed immunity and selection of resistant/tolerant nematodes. However, neither of these has been seen with the morantel slow release bolus, which has been marketed in this formulation for several years (Newby *et al.*1985a; 1985b; Yazwinski *et al.*1987; Fisher and Jacobs, 1995).

15.4 SIDE EFFECTS AND TOXICITY

The avermectins/milbemycins when used at therapeutic dose rates are generally very safe. An important difference between invertebrates and mammals is that in mammals, GABA-mediated nerves only occur in the central nervous system, whereas in many invertebrates they regulate peripheral muscles. Because of the blood-brain barrier, these compounds should have a wide safety margin in mammals (Campbell *et al.*1983).

Abamectin has the lowest therapeutic index of the avermectins/milbemycins licensed for cattle. Doses of 1 mg/kg (five times the therapeutic dose of 200 mcg/kg) have been found to be the maximum tolerated dose with animals showing depression and ataxia. At doses of 2-8 m/kg animals showed greater signs of toxicity with some dying (Pulliam and Preston, 1989). One instance has been reported where a herd of Murray Grey cattle showed acute neurological signs after treatment with the therapeutic dose. Post mortem showed that CNS abamectin levels were ten times those of the control animals suggesting that these animals had characteristics that allowed the abamectin to cross the blood brain barrier (Seaman *et al.*1987).

Animals have been treated subcutaneously with up to 6 mg/kg (thirty times the recommended dose of 200 mcg/kg) with ivermectin and shown no signs of toxicity (Campbell *et al.*1983). Others have recorded CNS depression, ataxia and in some cases death at only twenty times the use level (4 mg/kg), but these signs were also seen when the vehicle formulations alone were administered (Pulliam and Preston, 1989). No reproductive abnormalities have been seen in male or female cattle treated at twice the recommended dose at any stage of the reproductive cycle, or during embryo and foetal development (Campbell *et al.*1983; Campbell and Benz, 1984; Pulliam and Preston, 1989).

Moxidectin has also been shown to have no effect on cow reproductive performance during the oestrus cycle at three times the recommended dose (Coles *et al.*1994; Woodward, 1996). When given at up to a ten-fold dose (2 mg/kg) by subcutaneous injection mild depression, and ataxia were seen which soon resolved. Dermal doses of 12.5 mg/kg (25 times the therapeutic dose) were not toxic to cattle or calves (Woodward, 1996).

No safety studies on doramectin have been reported, but it would be expected to have similar side effects to ivermectin or abamectin.

A major point of contention with this class of compounds is the effect they are reputed to have on pasture ecosystems associated with cattle dung. Several insects have evolved to exist uniquely in association with cattle dung. However, some of these are flies responsible for flystrike of sheep. Australia is in a unique situation in that the native dung beetle cannot breakdown the dung of introduced cattle and horses. Species of African Dung Beetle (*Ornithophagus gazella*, *Onitis alexis*) were introduced to help break down this dung more rapidly and so help limit the breeding sites for the flies (Wall and Strong, 1987). Some trials have shown that use of ivermectin does not affect the breakdown of dung pats by these beetles and other flora (McKeand *et al.*1988; Wratten *et al.*1994; Forbes, 1996) whereas others reached opposite conclusions (Holter *et al.*1994; Herd, 1995; Herd *et al.*1996). Faecal levels of ivermectin do remain high for a period depending on the formulation used. Following topical or injectable administration, peak levels in faeces occur on days 2-3 with a gradual decline until day 35 (Herd *et al.*1996). It has been suggested that these kinetics of drug excretion have less effect on the degradation of the dung as they will still allow insects to breed once levels have decreased to a non-toxic level (Houlding *et al.*1991). However, the slow release bolus is the formulation that causes the most concern because of the constant faecal levels of ivermectin (Herd, 1995; Herd *et al.*1996). This is an ongoing debate that will only become more intense as the environmental effects of this class of drugs are investigated further.

TABLE 15.1 PERCENTAGE EFFICACY OF ORAL IVERMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
25 mcg/kg	94	97 EL4-53[2]	80		25 EL4-37	17	41			100	100	(Egerton *et al.*1981)
50 mcg/kg	99 d8-97[1] d15-99	99 EL4-71	99 d8-95 d15-85		41 EL4-93 d8-96 d15-43	82 d8-90 d15-88	52 d8-65 d15-37			100 d8-99 d15-100	100 d8-100 d15-100	(Egerton *et al.*1981)
100 mcg/kg	99 d8-99 d15-99	99-100 EL4-93-99.9 Dvlp L4-100[3]	99-99.4 d8-99 d15-99	63.5	98-100 EL4-98-100 d8-99 d15-74	99 d8-99 d15-94	90 d8-99 d15-83			100 d8-100 d15-100	100 d8-100 d15-100	(Egerton *et al.*1981; Armour *et al.*1980)

[1] Indicates 8-day-old larvae and percentage efficacy

[2] EL4 Early 4th stage larvae

[3] Dvlp L4 Developing 4th stage larvae

TABLE 15.1 CTD. PERCENTAGE EFFICACY OF ORAL IVERMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
200 mcg/kg	99->99 L3-99[1] L4-99-100[2] d5-99[3] d8-99 d12-99 d15-99	99->99 L3-99 L4-99	>99 L4->99 d5-99 d8-99 d12-99 d15-99	97 L4-98	>99 L4-100 d5-99 d8-99 d12-99 d15-99	>99 L4->99 d5-99 d8-99 d12-99 d15-99	100 L4->99 d5-99 d8-99 d12-89 d15-99	99->99 L3-99 L4-98.9-100	85-100	99-100 L3-89 L4-98.6-100 d5-53 d8-99 d12-95 d15-100	100 L4->99 d5-100 d8-100 d12-100 d15-100	(Egerton *et al.*1981; Swan *et al.*1985; Benz *et al.*1989)
200 mcg/kg					*Cooperia* spp. 97.4 L3-99 L4-99							(Swan *et al.*1985)
200 mcg/kg Paste	d18-100	99.9 d17-99.8 d18-100	99 d17-99.3 d18-100	d18-99	97.1 d17-99.5 d18-99	92.1 d17-98.1	92.5-95 d18-100			99.1 d17-99.1 d18-100	d18-100	(Benz *et al.*1983; Alva-Valdes *et al.*1984)

[1] L3 3rd stage larvae
[2] L4 4th stage larvae
[3] Indicates 8-day-old larvae and percentage efficacy

TABLE 15.2 PERCENTAGE EFFICACY OF PARENTERAL IVERMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
50 mcg/kg	99-99.2 d8-97[1] d14-99	97-100 EL4-97-99.5[4] Dvlp L4-100[5]	95-99.7 d8-95 d14-85	34.4	15-79.8 EL4-91.6 d8-96 d14-43	52-88.6 d8-90 d14-88	0-66 d8-65 d14-37			100 d8-99 d14-100	100 d8-100 d14-100	(Armour *et al.*1980; Benz and Ernst, 1981; Egerton *et al.*1981)
100 mcg/kg	99-100 d8-99 d14-99	99 -100 EL4-99-99.9 Dvlp L4-100	99-100 d8-99 d14-99	0	42.3-98.3 EL4-92.4 d8-99 d14-74	81-95 81 d8-99 d14-94	27-100 d8-99 d14-83			100 d8-100 d14-100	92.8-100 d8-100 d14-100	(Wescott *et al.*1980; Armour *et al.*1980; Benz and Ernst, 1981; Egerton *et al.* 1981)
200 mcg/kg B1a	98-100 L3-99[2] L4- 96-99[3] d8-99 d14-99	99-100 EL4-99-100 Dvlp L4-99-100 L4-99.6-100	99-100 L4-98 d8-99 d14-99	82.3-99	80.1-99.8 L3-99 EL4-95.8 L4-99-100 d8-99 d14-99	95-99.6 L4 -95-100 d8-99 d14-99	90-100 L4-97 d8-99 d14-98	99->99 L3-99 L4-98.9-99 *S.papillosis* >99	77.5-100	96.7-100 L3-95-99 L4-98.6-100 d14-100	100 L4->99 Inhib L5-100[6] d8-100 d13-100 d14-100	(Wescott *et al.* 1980; Armour *et al.* 1980; Yazwinski *et al.* 1981; Lyons *et al.* 1981c; Benz and Ernst, 1981; Williams *et al.* 1981c; Egerton *et al.* 1981; Benz *et al.* 1984; 1989; Swan *et al.* 1985; Barth and Preston, 1987)

[1] Indicates 8-day-old larvae and percentage efficacy

[2] L3 3rd stage larvae

[3] L4 4th stage larvae

[4] EL4 4th stage larvae

[5] Dvlp L4 Developing 4th stage larvae

[6] Inhib L5 Inhibited 5th stage larvae

TABLE 15.3 PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF PARENTERAL IVERMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum							Large Intestine			References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
200 mcg/kg 5d pre-infection	63.5-99.6	100		*Cooperia* spp 99.9-100			100		97.6-99.9		(Swan and Harvey, 1983; Bremner *et al.*1983)
200 mcg/kg 7d pre-infection	84.9-98	99-100		*Cooperia* spp 99-100 L4-100[2]	99-100 L4-100		95.5		94.4-95.8	99-100	(Barth, 1983; Swan and Harvey, 1983; Bremner *et al.*1983; Armour *et al.* 1985; Williams and Broussard, 1995)
200 mcg/kg 9-10d pre-infection	37-56.9	95.8-99		*Cooperia* spp 61.3- 87.8			97		58.4-73.3	100	(Bremner *et al.*1983; Swan and Harvey, 1983; Armour *et al.*1985)
200 mcg/kg 14d pre-infection	77-99.3	45-99 EL4-39[1]	99.3	0-99.8 L4-92.2	39.4-99 L4-92.2	86.7			98	98-100	(Barth, 1983; Armour *et al.*1985; Yazwinski *et al.* 1994b; Williams and Broussard, 1995)
200 mcg/kg 21d pre-infection	95.1	75-99.7	58.9	45-71.3	39	41.5			100	99-100	(Barth, 1983; Armour *et al.* 1985; Yazwinski *et al.*1994b)
200 mcg/kg 28d pre-infection		94.8								96.7	(Yazwinski *et al.*1994b)

[1] EL4 Early 4th stage larvae

[2] L4 4th stage larvae

TABLE 15.3 CTD. PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF PARENTERAL IVERMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine			References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
200 mcg/kg 14d trickle infection	99		99		100	99						(Barth *et al.* 1997)
200 mcg/kg 15d trickle infection	99		99		100	100						(Barth *et al.* 1997)
200 mcg/kg 21d trickle infection		99								99		(Barth *et al.* 1997)
200 mcg/kg 22d trickle infection		100								99		(Barth *et al.* 1997)
200 mcg/kg 28d trickle infection											100	(Barth *et al.* 1997)

TABLE 15.4 PERCENTAGE EFFICACY OF TOPICAL IVERMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
200 mcg/kg	100	99	90	71	96	86	85			100	100	(Alva-Valdes *et al.*1986)
500 mcg/kg	>99-100 L4-93[1]	>99-100 EL4-95.5->99[2] Dvlp L4-95.5-96[3]	95.1-96 L4-99	100	*Cooperia* spp. 23.1-96 El4-93.7 Dvlp L4-52.2-96	100	80-99 L4-84	*S.papillosis* 97	78.7-87	>99-100 L4-100	100 L4->99	(Alva-Valdes *et al.*1986; Benz *et al.* 1989; Bisset *et al.* 1990;)
1000 mcg/kg	100	100	99	100	99	99	100			100	100	(Alva-Valdes *et al.* 1986)

[1] L4 4th stage larvae
[2] EL4 4th stage larvae
[3] Dvlp L4 Developing 4th stage larvae

TABLE 15.5 PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF TOPICAL IVERMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
500 mcg/kg 7 days pre-infection	77.7	100 L4-100[1]			100 L4-100	100 L4-100						(Williams and Broussard, 1995)
500 mcg/kg 14 days pre-infection	84.5-96.6	99-100 L4-100	98.9-99		89.1-97.2 L4-100	93.6 L4-100	98.4			98	99-100	(McKenna, 1989; Yazwinski *et al.* 1994b; Hong *et al.* 1995; Williams and Broussard, 1995)
500 mcg/kg 21d pre-infection	24.5	96.8	69.4		60.4		31			98	96.7	(Yazwinski *et al.* 1994b)
500 mcg/kg 28d pre-infection		72.7-99	87								90-100	(McKenna, 1989; Yazwinski *et al.* 1994b; Hong *et al.* 1995;)

[1] L4 4th stage larvae

TABLE 15.5 CTD. PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF TOPICAL IVERMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
200 mcg/kg 14d trickle infection	100		99		100	99						(Barth *et al.* 1997)
200 mcg/kg 15d trickle infection	99		99		100	99						(Barth *et al.* 1997)
200 mcg/kg 21d trickle infection		99								99		(Barth *et al.* 1997)
200 mcg/kg 22d trickle infection		100								100		(Barth *et al.* 1997)
200 mcg/kg 28d trickle infection											100	(Barth *et al.* 1997)

TABLE 15.5 CTD. PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF TOPICAL IVERMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
500 mcg/kg 0-7d grazing after treatment	94.9	100 EL4-100[1] Dvlp L4-100[2]			100	100				100		(Williams and Broussard, 1995)
500 mcg/kg 10-17d grazing after treatment	84.9	95.6 EL4-93.6 Dvlp L4-95.1			72.6	72.3				100		(Williams and Broussard, 1995)
500 mcg/kg 20-27d grazing after treatment	80.7	84.5 EL4-66.4 Dvlp L4-0			0	0				100		(Williams and Broussard, 1995)

[1] EL4 Early 4th stage larvae
[2] Dvlp L4 Developing 4th stage larvae

TABLE 15.6 PERCENTAGE EFFICACY OF AN IVERMECTIN SUSTAINED RELEASE BOLUS AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
2.5 mcg/kg/day	99	94	82	0	38	88	0			100	10	(Egerton *et al.* 1986)
5 mcg/kg/day	93	99	99	66	95	98	97			100	100	(Egerton *et al.* 1986)
10 mcg/kg/day	99	99	99	92	99	99	99			100	100	(Egerton *et al.* 1986)
20 mcg/kg/day	99	99	99	90	99	99	99			100	100	(Egerton *et al.* 1986)
40 mcg/kg/day	99	99	99	99	99	99	99			100	100	(Egerton *et al.* 1986)
8mg/day for 120 days	100 L4-100[1]	99.9-100 L4-100 EL4-100[2] Dvlp L4-100[3]	>99-100 L4-100	L4-100	*Cooperia* spp 100 L4-100	100 L4-100	100	100	92.2-94	100	100	(Soll *et al.*1988; Alva-Valdes *et al.* 1988; Zimmerman *et al.* 1991; Williams and Plue, 1992d)

[1] L4 4th stage larvae
[2] EL4 Early 4th stage larvae
[3] Dvlp L4 Developing 4th stage larvae

TABLE 15.7 PERCENTAGE EFFICACY OF ORAL ABAMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
11 mcg/kg	d8->99[1] d15 ->99	95-99 d8-59 d15-0	d8-98 d15-99		d8-92 d15-18	d8-81 d15-72	d8-95 d15-86			d8->99 d15-100	d8-100 d15-100	(Egerton *et al.* 1979)
22 mcg/kg	d8 ->99 d15 - >99	94-97 d8-98 d15-78	d8->99 d15->99		d8-99 d15-71	d8-99 d15-99	d8->99 d15-99			d8->99 d15-100	d8-100 d15-100	(Egerton *et al.* 1979)
25 mcg/kg drench	97	>99 EL4-83[2]	>99		0 EL4->94		49			100	100	(Egerton *et al.* 1979)
50 mcg/kg	89.2- 98	>99-99.8 EL4-77	>99-100		69 EL4->94	53.8-99.4	96-100			100	100	(Egerton *et al.* 1979; Benz and Ernst, 1979; Wescott *et al.* 1980)
100 mcg/kg	89.2->99	>99-99.8 EL4-97	>99-100		95.1-97 EL4->94	96.6-98.9	98.5-100			100	100	(Egerton *et al.* 1979; Benz and Ernst, 1979; Wescott *et al.*1980)
200 mcg/kg	89.2	98.4-98.9	99.3-100		95.8	98.1- 99.3	96.7-100			88- 100	100	(Benz and Ernst, 1979; Wescott *et al.*1980)

[1] Indicates 8-day old larvae and percentage efficacy

[2] EL4 Early 4th stage larvae

TABLE 15.8 PERCENTAGE EFFICACY OF PARENTERAL ABAMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
.25 mcg/kg	>98	>99 EL4-89[2]	>99		3 EL4-63		28			100	>99	(Egerton *et al.* 1979)
50 mg/kg	61.7->98	78.4->99 EL4->99	54.4->99		0-47 EL4-91		72.3-96			100	100	(Egerton *et al.* 1979; Wescott *et al.*1980)
100 mcg/kg	>98	>99 EL4->99	>99		75 EL4-89		98			100	100	(Egerton *et al.* 1979)
200 mcg/kg	100 L4-100[1]	>99-100 EL4->99-99.6 Dvlp L4-99-100[3]	>99-100 L4-100	89.9-99 L4-94.3-100	*Cooperia* spp. >99 L4-99-100	100 L4-99		100 L4-100	100 *Chaberti a ovina* 100	>99-100 L4-100	100 L4-100 L5-94[4]	(Benz and Cox, 1989; Williams *et al.*1992b; Heinze-Mutz *et al.* 1993; Kaplan *et al.* 1994)

[1] L4 4th stage larvae
[2] EL4 Early 4th stage larvae
[3] Dvlp L4 Developing 4th stage larvae
[4] L5 5th stage larvae/immature adults

TABLE 15.9 PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF PARENTERAL ABAMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
200 mcg/kg 7d pre-infection	99-100	99-100	100		*Cooperia* spp. 99-100	100				99-100		(Eagleson *et al.* 1992; Heinze-Mutz *et al.* 1993)
200 mcg/kg 10d pre-infection	98.3	99			*Cooperia* spp. 99					99		(Eagleson *et al.* 1992)
200 mcg/kg 14 pre-infection	20.9-100	88.8-99.4	99-99.5		*Cooperia* spp. 63.1-100	99.7				26.3-100	96.4–100	(Eagleson *et al.* 1992; Yazwinski *et al.* 1994d; Heinze-Mutz *et al.*1993; Meeus *et al.*1997)
200 mcg/kg 21d pre-infection	71	93	71		*Cooperia* spp. 14						100	(Yazwinski *et al.*1994d; Rolfe *et al.*1997)
200 mcg/kg 28d pre-infection	87	54										(Rolfe *et al.*1997)

TABLE 15.9 CTD. PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF ABAMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
200 mcg/kg 14d trickle infection	100		100		100	99						(Barth *et al.*1997)
200 mcg/kg 15d trickle infection	100		100		100	100						(Barth *et al.*1997)
200 mcg/kg 21d trickle infection		99								100		(Barth *et al.*1997)
200 mcg/kg 22d trickle infection		99								100		(Barth *et al.*1997)
200 mcg/kg 28d trickle infection											99	(Barth *et al.*1997)

TABLE 15.10 PERCENTAGE EFFICACY OF ORAL MOXIDECTIN AGAINST GASTROINTESTINAL NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	Capillaria sp	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
0.2 mg/kg		99.9 EL4-99.8[1] Dvlp L4-99.9[2]	99.9	100 EL4-100 Dvlp L4-100	*Cooperia* spp. 99.6 EL4-100 Dvlp L4-100			100	100	100		(Zimmerman *et al.*1992)
0.4 mg/kg		99.9 EL4-99.8 Dvlp L4-99.8	99.9	100 EL4-100 Dvlp L4-100	*Cooperia* spp. 100 EL4-100 Dvlp L4-100			100	100	100		(Zimmerman *et al.*1992)

[1]EL4 Early 4th stage larvae
[2] Dvlp L4 Developing 4th stage larvae

TABLE 15.11 PERCENTAGE EFFICACY OF PARENTERAL MOXIDECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
0.2 mg/kg	100 L4-100[1]	99.9 EL4-99.9-100[2] Dvlp L4-99.9-100[3]	99.6-100	98.5 L4-98.7	*Cooperia* spp. 99.4-100 L4-100	99.7-98.4 L4-100	100	100 L4-100	100	100	100 L5-100[4] d8-100[5]	(Williams *et al.* 1992a; 1992c; Samson *et al.* 1992; Ranjan *et al.* 1992; Eysker and Boersma, 1992)
0.3 mg/kg	100 L4-100	99.5-99.9 EL4-99.9 Dvlp L4-07.9-99.9	99.6-100	99.7 L4-100	*Cooperia* spp. 99.8-100 L4-100	98.4-100 L4-100	100		100	100	100	(Williams *et al.* 1992a; Ranjan *et al.* 1992)

[1] L4 4th stage larvae
[2] EL4 Early 4th stage larvae
[3] Dvlp L4 Developing 4th stage larvae
[4] L5 5th stage larvae/immature adults
[5] Indicates 8-day old larvae and percentage efficacy

TABLE 15.12 PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF PARENTERAL MOXIDECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
200 mcg/kg SQ 7d pre-infection		100									100	(Hong *et al.* 1995)
200 mcg/kg SQ 14d pre-infection		100									100	(Hong *et al.* 1995)
200 mcg/kg SQ 15d pre-infection		99.7									100	(Hubert *et al.* 1995a)
200 mcg/kg SQ 21d pre-infection		99.9									100	(Hubert *et al.* 1995a)
200 mcg/kg SQ 28d pre-infection		86									86	(Hong *et al.* 1995)
200 mcg/kg SQ 28d pre-infection		99.8									100	(Hubert *et al.* 1995a)
200 mcg/kg SQ 35d pre-infection		99.9									100	(Hubert *et al.* 1995a)
200 mcg/kg SQ 42d pre-infection		79.2									94.6	(Hubert *et al.* 1995a)

TABLE 15.12 CTD. PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF PARENTERAL MOXIDECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
0.5 mg/kg SQ 0-21d pre-infection		99.9									100	(Vercruysse *et al.* 1997)
0.5 mg/kg SQ 14-21d pre-infection											100	(Vercruysse *et al.* 1997)
0.5 mg/kg SQ 21-28d pre-infection		Dvlp L4-99.6[1]									100	(Vercruysse *et al.* 1997)
0.5 mg/kg SQ 28-35d pre-infection		99.8 L4-85.4[2]									L5-99.8[4]	(Vercruysse *et al.* 1997)
0.5 mg/kg SQ 32-35d pre-infection		L3-44.8[3]										(Vercruysse *et al.* 1997)
0.5 mg/kg SQ 35-42d pre-infection		L4-53.7 Dvlp L4-92.1									L4-99.1	(Vercruysse *et al.* 1997)

[1] Dvlp L4 Developing 4th stage larvae
[2] L4 4th stage larvae
[3] L3 3rd stage larvae
[4] L5 5th stage larvae/immature adults

TABLE 15.13 PERCENTAGE EFFICACY OF TOPICAL MOXIDECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
0.25mg/kg	100	100 L4-100[1]	100 L4-100	100	99.9	99.9	100	100	100	100		(Morin *et al.* 1996)
0.35 mg/kg		100 L4-100	100	100 L4-99.4	*Cooperia* spp. 99.4 L4-99.4						100	(Hubert *et al.* 1995)
0.5 mg/kg	100 L4-100	99.9-100 L4-100	100 L4-100	100 L4-100	*Cooperia* spp. 98.8-100 L4-92.4-97.6	99.9	100	100	100	100	100	(Eysker and Boersma, 1992; Hubert *et al.* 1995b; Morin *et al.*1996; Williams *et al.*1996;)

[1] L4 4th stage larvae

TABLE 15.14 PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF TOPICAL MOXIDECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph* .spp.	*D. viviparus*	
0.5 mg/kg 14d pre-infection		99.9									100	(Hubert *et al.* 1995a)
0.5 mg/kg 21d pre-infection		96									99.2	(Hubert *et al.* 1995a)
0.5 mg/kg 28d pre-infection		90.3									98.3	(Hubert *et al.* 1995a)
0.5 mg/kg 35d pre-infection		99									98.3	(Hubert *et al.* 1995a)
0.5 mg/kg 42d pre-infection		67.2									90	(Hubert *et al.* 1995a)

TABLE 15.14 CTD. PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF TOPICAL MOXIDECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
0.5 mg/kg 0-21d pre-infection		99.9									100	(Vercruysse *et al.* 1997)
0.5 mg/kg 14-21d pre-infection											L5-99.7	(Vercruysse *et al.* 1997)
0.5 mg/kg 21-28d pre-infection		100 Dvlp L4-97.3[1]									100 L4-96.6	(Vercruysse *et al.* 1997)
0.5 mg/kg 28-35d pre-infection		100 L4-97.3[2]									L5-100[4]	(Vercruysse *et al.* 1997)
0.5 mg/kg 32-35d pre-infection		L3-35.5[3]										(Vercruysse *et al.* 1997)
0.5 mg/kg 35-42d pre-infection		Dvlp L4-99.7									L4-100	(Vercruysse *et al.* 1997)
0.5 mg/kg 42-45d pre-infection		L4- 95-100										(Vercruysse *et al.* 1997)

1 Dvlp L4 Developing 4th stage larvae
2 L4 4th stage larvae
3 L3 3rd stage larvae
4 L5 5th stage larvae/immature adults

TABLE 15.15 PERCENTAGE EFFICACY OF PARENTERAL DORAMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
50 mcg/kg		L4-100			37						100	(Goudie *et al.*1993)
100 mcg/kg		L4-100			80						100	(Goudie *et al.*1993)
200 mcg/kg	99.9-100 L4-99.9-100[1]	99.9-100 EL4-99.9-100[2] Dvlp L4-99.9-100[3] d6-99.9[4]	99.9-100 L4-99.9-100 d6-100	56.5- 97.9 L4-0-92.3	97-100 EL4-100 Dvlp L4-99.9	99.9-100 L4-99.9	93- 99.9 L4-99.9	100 S. papillosus 100	92.3- 94.6	99.9-100 L4-99.9	99.6-100 L4-100 L5-100[5]	(Markus and Sherma, 1992; Eddi.C. *et al.*1993; Goudie *et al.* 1993; Jones *et al.* 1993; Yazwinski *et al.* 1994a; 1994c; Barton *et al.* 1995; Whelan *et al.*1995; Watson *et al.*1995)

[1] L4 4th stage larvae
[2] EL4 Early 4th stage larvae
[3] Dvlp L4 Developing 4th stage larvae
[4] Indicated 6-day old larvae and percentage efficacy
[5] L5 5th stage larvae/immature adults

TABLE 15.16 PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF PARENTERAL DORAMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
200 mcg/kg 14d pre-infection			99		*Cooperia* spp. 99						100	(Barton *et al.* 1995; Rolfe *et al.*1997)
200 mcg/kg 21d pre-infection	87	99	87		*Cooperia* spp. 61						99.5	(Barton *et al.* 1995; Rolfe *et al.*1997)
200 mcg/kg SQ 28d pre-infection	19	74									94.1	(Barton *et al.* 1995; Rolfe *et al.*1997)
400 mcg/kg 4d pre-infection		100			100							(Goudie *et al.* 1993)
400 mcg/kg 8d pre-infection		100			99.9							(Goudie *et al.* 1993)
400 mcg/kg 12d pre-infection		100			95.1							(Goudie *et al.* 1993)

TABLE 15.16 CTD. PERSISTENT ACTIVITY (PERCENTAGE EFFICACY) OF PARENTERAL DORAMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph* .spp.	*D. viviparus*	
200 mcg/kg 14d trickle infection		99.9			99.2							(Weatherley *et al.* 1993)
200 mcg/kg 21d trickle infection		99.9			90.7						100	(Weatherley *et al.* 1993)
200 mcg/kg 28d trickle infection		93.7									99.9	(Weatherley *et al.* 1993)

TABLE 15.17 PERCENTAGE EFFICACY OF TOPICAL EPRINOMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph.* spp.	*D. viviparus*	
80 mcg/kg	>99 L4-42[1]	>99 L4-97	99 L4-99	>99 L4-66	88 L4-99	99 L4-98	87 L4-99			100 L4-100	100	(Shoop *et al.* 1996b)
125 mcg/kg	>99	>99-100 EL4-79.5-94.9[2]	85.9-92.8		*Cooperia* spp. 98->99 L4-96.8-100		0	95.2		97.9 L4-100	100	(Williams *et al.* 1997)
200 mcg/kg	>99 L4-98	>99 L4-99	99 L4->99	>99 L4->99	99 L4->99	99 L4->99	98 L4->99			100 L4-100	100	(Shoop *et al.* 1996b)
250 mcg/kg	100	>99-100 EL4->99	>99- 100		*Cooperia* spp. >99-100 L4-100		89.5	100		L4-100	100	(Williams *et al.* 1997)
500 mcg/kg	>99-100 L4-98	>99-100 EL4->99-100 Dvlp L4-100[3]	>99-100 L4->99-100	>99-100 L4->99-100	99-100 L4->99-100	99-100 L4->99	85-100 L4->99	100	97.8-100 L4-84.3	100 L4-100	100	(Shoop *et al.* 1996b; Gogolewski *et al.* 1997a; Yazwinski *et al.* 1997; Williams *et al.* 1997)

[1] L4 4th stage larvae
[2] EL4 Early 4th stage larvae
[3] Dvlp L4 Developing 4th stage larvae

TABLE 15.18 PERCENTAGE EFFICACY OF TOPICAL EPRINOMECTIN AGAINST GASTROINTESTINAL AND PULMONARY NEMATODES OF CATTLE UNDER VARIOUS MANAGEMENT CONDITIONS

	Abomasum			Small Intestine					Large Intestine		Lungs	References
Dose	*Haemonchus* spp.	*Ostertagia* spp.	*Trich. axei*	*Nematodirus* spp.	*Cooperia oncophora*	*Cooperia punctata*	*Trich.* spp.	*Bunostomum phlebotomum*	*Trichuris* spp.	*Oesoph* .spp.	*D. viviparus*	
500 mcg/kg Rain 1 hr before, 1, 3, 6 hr after	100	99.9-100	100									(Gogolewski *et al.* 1997b)
500 mcg/kg short hair		100	100		99.7						100	(Gogolewski *et al.* 1997b)
500 mcg/kg long hair		>99.9	100		99.5						100	(Gogolewski *et al.* 1997b)
500 mcg/kg animals housed inside	100 L4-100[1]	99.9-100 L4-100	99.8-100 L4-100	100 L4-100	*Cooperia* spp. 99.8-100 L4-100					100		(Gogolewski *et al.* 1997b)
500 mcg/kg animals remain outside	100	99.9-100 L4-97.8-100	99.8-100 L4-100	100 L4-99.6	*Cooperia* spp. 99.5-100 L4-100					100		(Gogolewski *et al.* 1997b)

[1] L4 4th stage larvae

16.0 CONCLUSION

The last 35 years have seen a major revolution in parasite control. However, we are now starting to pay for the price of our excesses. Resistance has become a major issue in sheep parasites and there are reports of it occurring in cattle (Eagleson and Bowie, 1986; Jackson *et al.*1987; McKenna, 1991; 1996; West *et al.*1994; Hosking *et al.*1996; Vermunt *et al.*1996; Waller, 1997a). It has been 18 years since the last new action family was discovered. The more recent anthelmintic products being launched are only chemical modifications of the avermectin/milbemycin family or the reformulation of existing compounds. Because the cost of developing anthelmintic is continuing to increase, it is unlikely that a new action family will be released onto the market in the next 5-10 years. Therefore, to maximise the use of the anthelmintic resources, we will have to look at other strategies to control parasitic nematodes (Williams, 1997).

One area in which there can be further development is improving the delivery of the current anthelmintics ie. modifying the formulation or delivery mechanism to increase the activity of anthelmintic compounds (Hennessy, 1997). There is considerable knowledge about the epidemiology of the major nematode parasites, and there is still a need for more strategic use of these compounds to maximise the effect on the nematode life-cycle (Barger, 1997). The opportunities for biological control are continually being developed. These include not only manipulation of the environment using dung beetles and earth worms to help limit pasture contamination, but also control of the parasites using parasite antagonists such nematode-destroying fungi and bacteria (Waller, 1997b; Larsen *et al.*1997). Grazing animals on pasture that has a "natural" anthelmintic effect eg. tannin containing plants, is still in its infancy but show some promise. While there is a vaccine available for lungworm, it has proved more difficult to produce similar vaccines to stimulate the more rapid onset of immunity for the trichostrongyles (Klei, 1997). Selection of animals that are more resistant to internal nematodes is possible, but will take a considerable period of time (McEwan *et al.*1997). Current research on genetic selection has focussed heavily on sheep, mainly because this is where the major resistance problems are, and there is little information on cattle.

Anthelmintics are a very important resource for livestock farming throughout the world. Unless they are used wisely, they will result in the farming community returning to the low stocking and production management practices of earlier in the century. The days of every farmer using anthelmintics haphazardly are gone. Both the farming and pharmaceutical communities need to take stock of the current dilemma we face and plan to ensure anthelmintics are still working in the next 20-30 years.

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PART 2.

Efficacy of two formulations of moxidectin pour-on against gastrointestinal nematodes and the effects of treatment on serum pepsinogen and gastrin levels and tissue gastrin in cattle

1.0 INTRODUCTION

Gastrointestinal parasitism in young cattle may be due to several nematode species, but the abomasal parasite *Ostertagia ostertagi* is undoubtedly one of the most pathogenic nematode species affecting cattle both in New Zealand (Brunsdon, 1969; Brunsdon, 1972a; 1972b; Bisset, 1994) and overseas (Armour, 1970; 1974). Many of the clinical changes seen in infected animals have been associated with specific stages in the life cycle of *O. ostertagi*. Anderson *et al.*(1965) classified the disease caused by this parasite into three syndromes, two of which are clinically apparent. Type 1 corresponds to the classical parasitic gastroenteritis in which calves show loss of weight and diarrhoea associated with the ingestion and maturation of large numbers of infective *Ostertagia* larvae. There is an intermediate condition called Pre-Type 2 where clinical disease is not apparent, but where large numbers of ingested infective larvae become inhibited in the early 4th stage. Type 2 disease results from the maturation, up to six months later, of these inhibited larvae subsequently causing weight loss and diarrhoea (Anderson *et al.*1965).

Traditionally the diagnosis of gastrointestinal parasitism has relied heavily on the demonstration of parasites, eggs or larvae in the faeces of the infected individual. However, the presence of these only provides an approximate estimate of the level of infection, and cannot give an accurate indication of the level of damage to the gastrointestinal tract (Schillhorn-van-Veen, 1988). The diagnosis of ostertagiosis has presented particular problems because of the poor correlation of faecal egg counts with worm burdens. Because of this, there has been a search for serum biochemical changes that are more reliable diagnostically. Anderson *et al.*(1965) first tried to correlate changes in the levels of gastrointestinal enzymes (pepsinogen) in the blood to pathological changes in the abomasum. Many others have since measured pepsinogen, gastrin or antibody levels in the search for a diagnostic tool for bovine ostertagiosis (Jennings *et al.*1966; Armour *et al.*1967; Allen *et al.*1970; Brunsdon, 1971; Henriksen *et al.*1976; Ford, 1976; Michel *et al.*1978; Chiejina, 1978; Snider *et al.*1981; Chalmers, 1983; Entrocasso *et al.*1986; Eckersall *et al.*1987; Fox *et al.*1987; Xiao *et al.*1992; Berghen *et al.*1993).

Pepsinogen is the proenzyme of pepsin produced principally by the chief cells of the fundus of the abomasum and it is converted to pepsin under acidic conditions in the abomasum (Jennings *et al.*1966). Because pepsinogen is released into the abomasum directly, it was assumed that an increase in blood levels was an indicator of tissue damage with the proenzyme escaping through broken cell junctional complexes (Anderson *et al.*1965; Jennings *et al.*1966; Berghen *et al.*1993). There has been considerable debate about the diagnostic use of pepsinogen levels because under field conditions, there is considerably greater variation between animals in the relationship between pepsinogen levels and parasites burdens, than has been seen with experimental infections (Hotson, 1967; Ross *et al.*1968; Mylrea and Hotson, 1969). Furthermore a variety of assay methods has

been used by different laboratories (Anderson *et al.*1965; Ross *et al.*1967; Mylrea and Hotson, 1969; Jensen, 1977; Snider *et al.*1981; Turner and Shanks, 1982; Harvey-White *et al.*1983; Chalmers, 1983; Berghen *et al.*1987; Pomroy and Charleston, 1989). However, several authors have shown a moderate correlation between worm burden and pepsinogen levels (Anderson *et al.*1965; Anderson *et al.*1966; Allen *et al.*1970; Murray *et al.*1970; Brunsdon, 1971; Ford, 1976; Entrocasso *et al.*1986; Xiao *et al.*1991; Yang *et al.*1993) and it has remained as one of the diagnostic tools available to clinicians. A potential complicating factor in its routine use in the field is that pepsinogen levels can remain elevated for a considerable period (Jennings *et al.*1966; Brunsdon, 1969). Although other authors have shown they can decrease rapidly following anthelmintic treatment (Ross *et al.*1968; Armour *et al.*1967; McSporran, 1986; Wiggin and Gibbs, 1987; 1990; Simpson *et al.*1997).

Gastrin is a polypeptide hormone principally produced by G-cells in the pyloric mucosa. It has activity on parietal cells, and its two major functions are stimulation of parietal cell multiplication and release of HCl (Snider *et al.*1988). Gastrin levels are also elevated in ostertagiasis. This is associated with achlorhydria from damage to the acid-secreting parietal cells as the parasite matures, and subsequent replacement of these cells with mucous or undifferentiated epithelial cells (Anderson *et al.*1966; Jennings *et al.*1966; Murray *et al.*1970). This results in a marked rise in abomasal pH, stimulating secretion of gastrin from G-cells in the pyloric antrum (Anderson *et al.*1976; Titchen and Anderson, 1977; Anderson *et al.*1981; Fox *et al.*1987). It has also been proposed that in sheep, that *Ostertagia* actually stimulate the secretion of gastrin directly; (Anderson, Hansky and Titchen, unpublished data, cited in Titchen and Anderson, 1977), (Anderson *et al.*1976), but Fox *et al.*(1993) was unable to confirm this in calves. Xiao *et al.*(1991) showed that the resulting increase in gastrin was more related to the level of abomasal damage and overall pathological processes whereas pepsinogen reflected the abomasal worm burden. Pitt et al (1988), (Pitt, SR, Gerrelli, D., Fox, MT, and Jacobs, DE unpublished data cited in Pitt *et al.*1988) have also shown that gastrin levels increase in the presence of worms, show no apparent 'animal age effect' unlike that of pepsinogen, and that blood levels decrease more rapidly than pepsinogen after loss of adult worms. Other authors have shown that abomasal pH levels and serum gastrin levels decrease quite rapidly (2-14 days) following anthelmintic treatment of ovine (Anderson *et al.*1976; 1981), or bovine ostertagiosis (Armour *et al.*1967; Fox *et al.*1989). This encouraged the use of this hormone as a diagnostic tool for bovine ostertagiosis.

Anthelmintics play an important role in the management of gastrointestinal parasitism once disease has been diagnosed, as well as prophylactically. There are three families of broad spectrum anthelmintics available for control of gastrointestinal nematodes. These are the benzimidazoles, levamisole/morantel and the avermectin/milbemycins. Recent years has seen the development and commercial release of four avermectins and one milbemycin as cattle anthelmintics.

Moxidectin is a macrocyclic lactone produced by chemical modification of nemadectin (CL287,088) a natural fermentation product of the actinomycete *Streptomyces cyaneogriseus* subspecies *noncyanogenus*. Moxidectin is a milbemycin endectocide with a broad spectrum of activity against internal and external parasites of cattle. The efficacy of injectable and pour-on formulations against gastrointestinal and pulmonary nematodes in cattle has been demonstrated (Zimmerman *et al.*1992; Williams *et al.*1992; Eysker and Boersma, 1992; Samson *et al.*1992; Ranjan *et al.*1992; Williams *et al.*1992; Hubert *et al.*1995a; 1995b; Williams *et al.*1996; Vercruysse *et al.*1997).

The purpose of this investigation was twofold. One aim was to compare the efficacy of two pour-on formulations of 0.5% moxidectin at a dose rate of 500 mcg per kilogram bodyweight against gastrointestinal nematodes of grazing cattle in New Zealand. The other purpose was to compare serial changes in blood pepsinogen and gastrin levels as indicators of abomasal cellular regeneration following removal of abomasal nematodes by treatment with these formulations of moxidectin. Measurements of tissue gastrin were also made to see if any serological changes in gastrin levels were associated with a change in tissue concentration.

2.0 MATERIALS AND METHODS

2.1 *Animal management and selection*

Twenty-nine, 9-10 month old Friesian bull calves were chosen from a herd of 108 that was being managed under normal New Zealand farm management conditions on the Jennersmead Research Farm, Massey University. The twenty-nine animals were grazed as a group on known infective pasture, and were not drenched for eight weeks before moving onto a cattle holding pad.

All animals were regularly faecal sampled while grazing to confirm a high level of parasitism, using larval cultures to provide an indication of the genera and species present in the calves. In addition, one very weak animal from the group of 29 was euthanased and a total worm count, including a saline abomasal digest, undertaken.

The remaining 28 calves were removed from grass and transported to a cattle holding pad to prevent any further parasitic infection. The cattle holding pad was 20 metres x 11 metres with a four metre wide concrete strip down one side allowing access to hay nets, feed bins and a water trough. The remaining area of the pad was a seven-metre wide strip of soil covered with a thick layer of sawdust. All grassed areas around the pad had been previously sprayed with a herbicide to remove pasture.

The calves were double ear-tagged and maintained on the holding pad on a diet of calf meal (Top Calf Cereal, Harvey Farms), ad-lib aged meadow hay and had unlimited access to water for 28 days.

The twenty-eight calves moved to the cattle pad included four additional to those actually required for the trial animals. These were included to ensure that group numbers could be maintained in the event any trial animal succumbed to parasitism while on the pad. None of the four additional calves was required and all animals survived the 28-day period. However at different stages after the start of the trial, three animals became severely ill with clinical parasitism (No.12 and No 29 on Day 2, No.16 on Day 7) and were moved to the Large Animal Hospital in the Veterinary Clinical Sciences Department. Here they were treated with antibiotics and oral electrolytes and housed on straw in separate concrete pens for the remainder of the trial. They were fed the same calf meal and aged meadow hay as the other calves. Two of the calves belonged to the group treated with moxidectin Formulation 2, (No's 12 and 29) and the third was a control animal (No 16). None of the treatments was believed to prejudice the continued inclusion of the three animals in the trial.

2.2 *Anthelmintic Treatment*

All calves were weighed and faecal sampled on days -2 and -1 pre-treatment and restrictively randomized to produce three groups with similar mean faecal egg counts and bodyweights. (Appendix 1.1). The three groups were then randomly allocated to one of the treatments. The three groups were untreated controls, calves treated with 500 mcg/kg (1ml/10kg) of Formulation 1 (Batch Numbers AC7215/26/(13) and (19)), and calves treated with 500 mcg/kg (1ml/10kg) of Formulation 2 (Batch Numbers AC7215/34/A56 and A63). The treatment was applied to the midline of the back between the shoulders and tail-base using a calibrated syringe. Immediately after treatment, the groups of calves were separated to prevent contact, and remained separated for the remainder of the trial.

2.3 *Blood Collection and Analysis*

Samples to measure the pre-treatment baseline were collected 4 and 2 days before treatment, and then daily for the remainder of the trial. Jugular or caudal tail vein samples were collected from each calf between 1-3 pm to reduce any fluctuations associated with feeding (7-8 am) (Yasuda *et al.*1986). Blood was collected into plain evacuated tubes (Vacutainer), allowed to clot at room temperature and serum was removed with a Pasteur pipette. Serum samples were labelled and frozen at -20° C until analysis.

2.4 *Experimental schedule*

The following outlines the chronological order of events with Day 0 being the day of treatment.

Day -74	Last regular anthelmintic treatment with oral 4.5 mg/kg oxfendazole (Systamex, Coopers Animal Health)
Day -36	Selection of animals for the trial, faecal egg counts (FEC) and larval cultures
Day -21	Faecal egg counts and larval cultures
Day -20	Calf (Y8) sacrificed and a total worm count and saline abomasal digest performed
Day -14	All animals moved to cattle pad ("safe" grazing)
Day -4	Blood sample taken for pretreatment pepsinogen and gastrin levels
Day -2	Faecal egg counts and body weights Blood samples taken for pretreatment pepsinogen and gastrin levels
Day -1	Faecal egg counts
Day 0	Animals grouped on the basis of Day -2 and Day -1 faecal egg counts and bodyweights. Groups assigned randomly to treatments. Moxidectin Formulation 1 and 2 administered to treatment groups.
Days +1-+13	Twice daily observations and collection of faeces for faecal egg counts. Daily blood sample (1-2pm) for pepsinogen and gastrin measurements
Days +14-+16	Animals sacrificed and gastrointestinal tract removed for total worm counts and saline abomasal digests. Faecal egg counts.

Hay was removed for 48 hours and calf meal for 36 hours before slaughter, but animals had free access to water. Animals were slaughtered over three days starting on day +14, with approximately equal numbers from all three groups being slaughtered daily. Each animal was stunned with a captive bolt gun, exsanguinated, and immediately opened to give access to the gastrointestinal tract.

2.5 *Parasitology procedures*

Nematode eggs in faeces were counted using a modified McMaster's technique in which one egg counted represented 50 eggs/gramme of faeces (epg) (Stafford *et al.*1994).

Immediately after slaughter, the abomasa, small and large intestines were ligated and separated for processing according to WAAVP guidelines (Powers *et al.*1982). The specific procedures used for preparing the aliquots for worm counting and speciation are described below:

The abomasum was tied off and separated from the small intestine, opened and washed thoroughly with warm water. The contents and washings were collected and made up to 10 litres. Two 5% aliquots (500 ml) were removed and retained in labelled jars and formalinised. Only one aliquot was

counted. The washed abomasum was placed in 10 litres of warm 0.85% saline and maintained at 37°C for eight hours and then placed in a chiller (4-6 C) before processing the next day. The mucosa was washed and rubbed vigorously in water over a 53µm sieve. The 10 litres of saline were sieved through a 53µm sieve and the sievings made up to six litres. Two 5% aliquots (300 ml) were removed and formalinised. Only one aliquot was counted.

The small intestine was tied off and separated from the large intestine. All the mesentery was removed and the intestine was opened along its entire length into a bucket. The mucosa was washed under warm water and the contents and washings were made up to 10 litres. Two 5% aliquots were removed and stored, and one aliquot was counted as before.

The large intestine was opened along its entire length and washed through a 750µm sieve. All sievings were collected and made up to 10 litres. Two 10% aliquots were collected and stored as above. Both aliquots were counted later.

All samples were washed through a 53 µm sieve before counting.

2.6 *Counting and identification of nematodes:*

For the abomasal and small intestinal samples one 5% aliquot was counted. All nematodes in the aliquot were identified to genus, stage of development and sex (if adult). Males of each genus were picked out as encountered in the counting process and identified to species level up to a maximum of 100 per genus (Anon., 1986). Both 10% aliquots from the large intestine were counted; only adult nematodes were counted and identified to species level (Anon., 1986).

2.7 *Pepsinogen Assays*

Serum pepsinogen concentrations were estimated using a modification (Pomroy and Charleston, 1989) of the method described by Uete *et al.*(1969) and which is described in detail in Appendix 2.1. Briefly, hydrochloric acid was added to the serum sample resulting in the activation of pepsinogen to pepsin. Using bovine serum albumin as a substrate, the pepsin released tyrosine which was then measured colorimetrically, after oxidation by Folin-Ciocalteau reagent under alkaline conditions. The difference between incubated samples and unincubated controls determined the pepsinogen concentration, expressed as IU tyrosine/litre. The amount of variation between samples was calculated to be 13%.

2.8 *Gastrin Assays*

Gastrin concentrations were measured by radioimmunoassay using a modification (Simpson *et al.*1993) of the method of Hansky and Cain (1969) and which is described in detail in Appendix 3.1. All samples were assayed in triplicate. Radio-labelled gastrin and normal rabbit serum was added to the serum sample along with antibody to gastrin. After incubation to allow an equilibrium reaction between antibody and the gastrin, a second antibody (sheep anti-rabbit gamma globulin) was added. This precipitated out all the antibody bound to the gastrin. After removing supernatant, the radioactivity associated with the precipitate was counted in a gamma counter. Gastrin concentrations were determined from a standard curve and expressed in picomoles/litre (pM). In this laboratory the mean sensitivity and between assay variability has been determined to be 5 pM and 10% respectively. Concentrations above 100 fMol were diluted by a factor of two or four and re-assayed. Circulating levels of gastrin are expressed as picomoles per litre of serum.

2.9 *Tissue Gastrin*

Tissue samples were collected from both the abomasum and proximal duodenum (duodenal bulb) before processing for parasites. Three samples were collected from the lesser curvature of the abomasum near the pylorus and combined for analysis. A single sample was collected from the duodenum because the G-cells are less evenly distributed than in the abomasum. Samples were dried with a tissue, placed in a microfuge tube and immediately snap frozen in liquid nitrogen. These were stored at -20° C until extraction of tissue gastrin (Reynolds *et al.*1984).

Briefly the frozen tissue was weighed and the gastrin extracted by boiling in 10ml deionised water in a boiling water bath for 30 minutes. The tissue fragment was removed, the tube was centrifuged at 1500 rpm for 20 minutes and the supernatant decanted and frozen at -20° C until analysis by radioimmunoassay as described in Appendix 3.1. Abomasal samples were diluted 1/3000 and proximal small intestinal samples were diluted between 1/100 -1/500 before assay.

The concentrations of immunoreactive gastrin in gastrointestinal tissue is expressed as picomoles per gramme of mucosa.

2.10 *Statistical analyses*

The software package STATISTIX 4.0 (Analytical Software, Minnesota/St. Paul, MN, USA) was used for all statistical calculations.

The total numbers of nematodes in abomasal, small intestinal and large intestinal contents were calculated from aliquot counts. The number of nematodes of each species was calculated by multiplying the total nematode count by the percentage of nematodes of that species in an aliquot. The calculation of anthelmintic efficiency was determined as the relative reduction in the geometric mean count between the control and treated groups. Statistical comparisons between treated and nontreated groups were made for each parasite species and stage by Analysis of Variance (ANOVA) on log-transformed data (count + 1).

A Wilk-Shapiro/Rankit plot was determined for all samples for serum pepsinogen, serum gastrin and tissue gastrin. All samples were compared by one-way ANOVA.

3.0 RESULTS

3.1 *Anthelmintic efficacy*

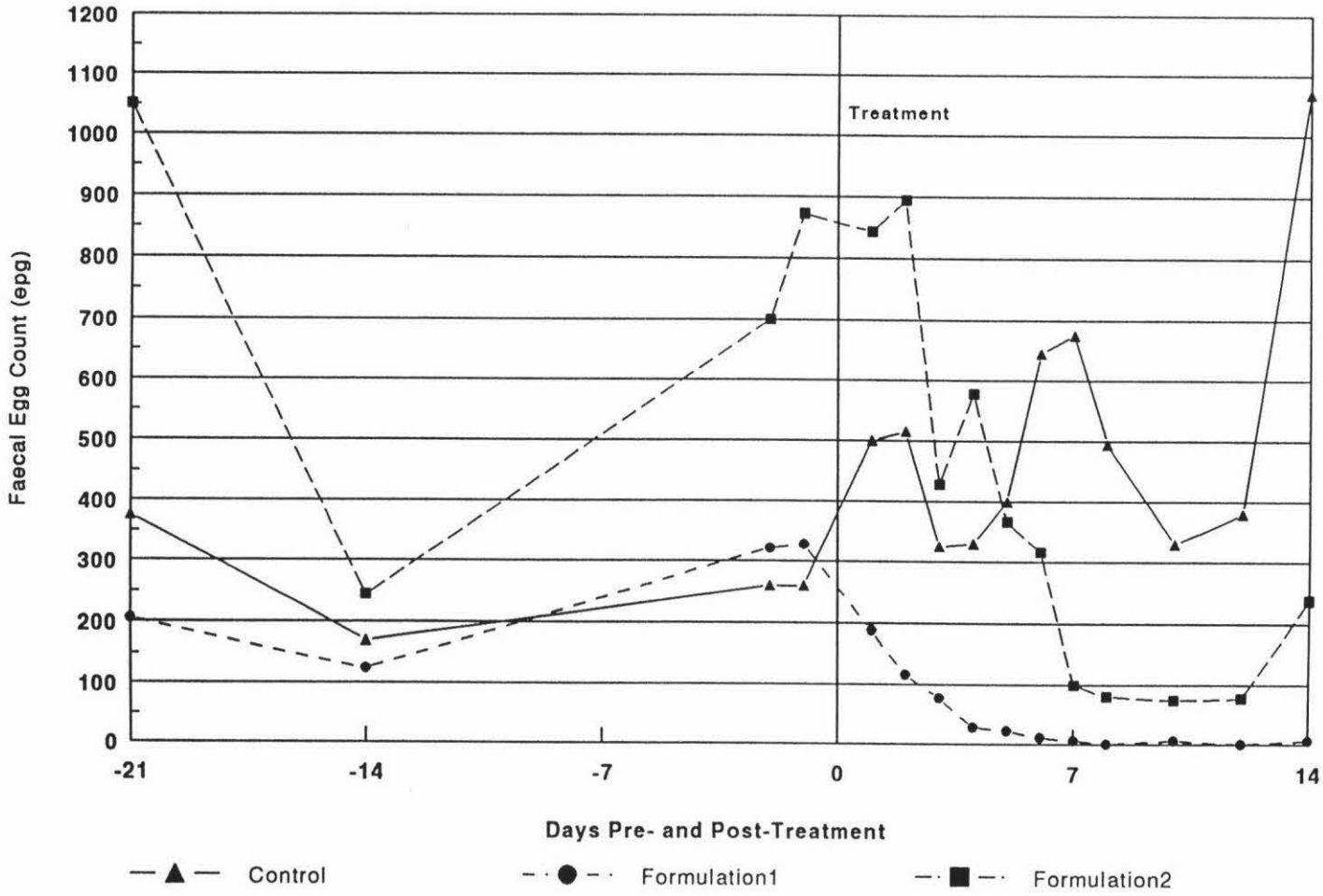
The weak calf sacrificed before the removal of the 28 animals to safe grazing was heavily parasitised and the results of the total worm count are presented in Table 1. These demonstrated a moderate burden of inhibited *Ostertagia* larvae (24,000) and confirmed that sufficient worms were present to expect a valid result in the trial.

No adverse reactions or toxicosis due to moxidectin treatment were observed in any of the treated calves over the 14 days following treatment.

3.1.1 *Faecal Egg Counts*

Examination of faecal egg counts pretreatment indicated consistency of strongyle egg counts in all calves at all sampling dates. Pretreatment larval culture on day -35 showed a mixed infection of *Ostertagia* spp., *Trichostrongylus* spp. and *Cooperia* spp. that changed to a predominately *Cooperia* infection two weeks before treatment (Table 2). After treatment, calves treated with Formulation 1 had a more rapid and statistically significant reduction in mean FEC compared with the controls and Formulation 2 (Figure 1) (Table 3). There were only three intervals (days +7, +10 and +14-16) when there was a significant difference in FEC between the control group and the calves treated with Formulation 2. Individual animal FEC's for all groups are given in Appendices 1.2.1-1.2.3.

TABLE 1 CHANGES IN FAECAL EGG COUNTS PRE- AND POST-TREATMENT IN UNTREATED CONTROL CALVES AND CALVES TREATED WITH 0.5% MOXIDECTIN



3.1.2 *Worm burdens*

Five nematode genera (*Ostertagia*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum* and *Trichuris*), involving eight species were recovered at postmortem from the control group. The mean numbers of *Oesophagostomum* and *Trichuris* were too small for statistical comparison of the effect of treatment in the two treated groups.

The efficacy of both formulations of moxidectin in removing the individual species is included in the tables. Geometric means were used throughout for the efficacy calculations and are provided in Table 4. Table 5 provides the arithmetic means of the total counts of the adult worms and larvae in the abomasum, small intestine and large intestine for both treated groups and the controls. Individual animal worm counts are provided in Appendices 1.3.1-1.3.9. Formulation 1 was >99.9% efficacious ($P<0.01$) for all abomasal nematodes including adult and immature *Ostertagia* spp. and adult *Trichostrongylus axei*. Against small intestinal nematodes, efficacy was 100% for *Trichostrongylus* spp. and 96.25% for adult *Cooperia* spp.. The early 4th stage larvae of *Cooperia* were more susceptible (97.3%) than the later 4th stage larvae (91.07%), but low parasite numbers meant these were not significantly different from the controls.

Formulation 2 was also highly efficacious against adult and immature abomasal nematodes (adult and immature *Ostertagia* spp. >99.9% ($P<0.01$) and adult *Trichostrongylus axei* >99.8% ($P<0.01$). There was no statistically significant effect against adult or immature stages of *Cooperia* spp.

The predominant species of *Ostertagia* was *Ostertagia ostertagi*, making up 94.5%. The other species identified was *Ostertagia lyrata* (Table 6). The proportion of *Cooperia oncophora* was approximately the same in the control (86%) and Formulation 2 treated groups (86.5%). Treatment with Formulation 1 increased the proportion of *Cooperia oncophora* remaining to 93.5%. No *Cooperia punctata* were recovered from either of the treated groups. The other *Cooperia* spp. recovered from all groups was *Cooperia mcmasteri*. Individual animal results are given in Appendices 1.3.10-1.3.15.

3.2 *Pepsinogen and Gastrin results*

All pre- and post-treatment serum pepsinogen and gastrin, and tissue gastrin samples analysed by Wilk-Shapiro/Rankit plot had values approaching 1.0 (0.9336-0.9657). This suggested that data collected for each parameter was normally distributed and the use of parametric statistical methods (Analysis of Variance) was valid.

3.2.1 *Serum pepsinogen*

Serum pepsinogen levels were determined before calves were treated. Mean values on day-2 were 2.35 IU tyrosine/litre (+/- standard deviation (sd) 0.86), 1.81 (+/-sd 0.54) and 2.39 (+/-sd 1.23) for the controls and calves to be treated with Formulations 1 and 2 respectively (Table 7). Following treatment on day 0, pepsinogen values decreased uniformly in all three groups (Figure 2). Except for day +7 in calves treated with Formulation 1, there was no significant difference between any of the groups at any sampling interval. As the first samples were taken after the animals had been on the feed pad and away from infective grazing for 10 days, it was not clear if this decrease was a continuation of a natural decline or associated with anthelmintic treatment. The fact that all groups decreased at the same rate would suggest that it was unrelated to administration of the moxidectin. By day +14 serum pepsinogen levels in all calves were 25-40% (0.56-0.72 IU tyrosine/l) those on day -4. These were well within the normal range for New Zealand cattle (<2 IU/l) (McSporran, 1986; S Bisset; WAG Charleston (1998) pers. comm.). Means and standard deviations for the groups and individual animals are provided in Appendices 2.2.1-2.2.3.

3.2.2 *Serum gastrin*

Likewise, serum gastrin levels decreased over the 18 days of sampling (Table 8). In the control group there was an initial increase between days -4 and day +3 before decreasing linearly to 71 pM/litre on day +14 (Figure 3). Calves treated with Formulation 1 showed a significant difference from the untreated calves from day +6, but there was no statistical difference between the two treated groups. By day +8 both treatment groups had gastrin concentrations 50-55% lower than the control group. Individual animal and group means and standard deviations are presented in Appendices 3.2.1-3.2.3.

Comparisons of serum pepsinogen and gastrin levels within each of the three groups are shown in Figures 4, 5 and 6 for the Control, Formulation 1 and Formulation 2 treated calves respectively.

3.2.2 *Tissue gastrin*

The mean and standard deviation of concentrations of immunoreactive gastrin are shown in Figure 7 and Table 9. Individual animal results are presented in Appendix 3.3. Antral concentrations (1148-1323 picomoles/gramme) were between 25-35 times that found in the proximal duodenum (32.3 - 50.9 picomoles/gramme) for all three groups. The most variation was seen in the samples analysed from calves treated with Formulation 2. There was no significant difference between the control and treatment groups for any of the gastrin concentrations in tissues.

TABLE 2 CHANGES IN MEAN SERUM PEPSINOGEN CONCENTRATIONS PRE- AND POST-TREATMENT IN UNTREATED CONTROL CALVES AND CALVES TREATED WITH ONE OF TWO FORMULATIONS OF 0.5% MOXIDECTIN POUR-ON

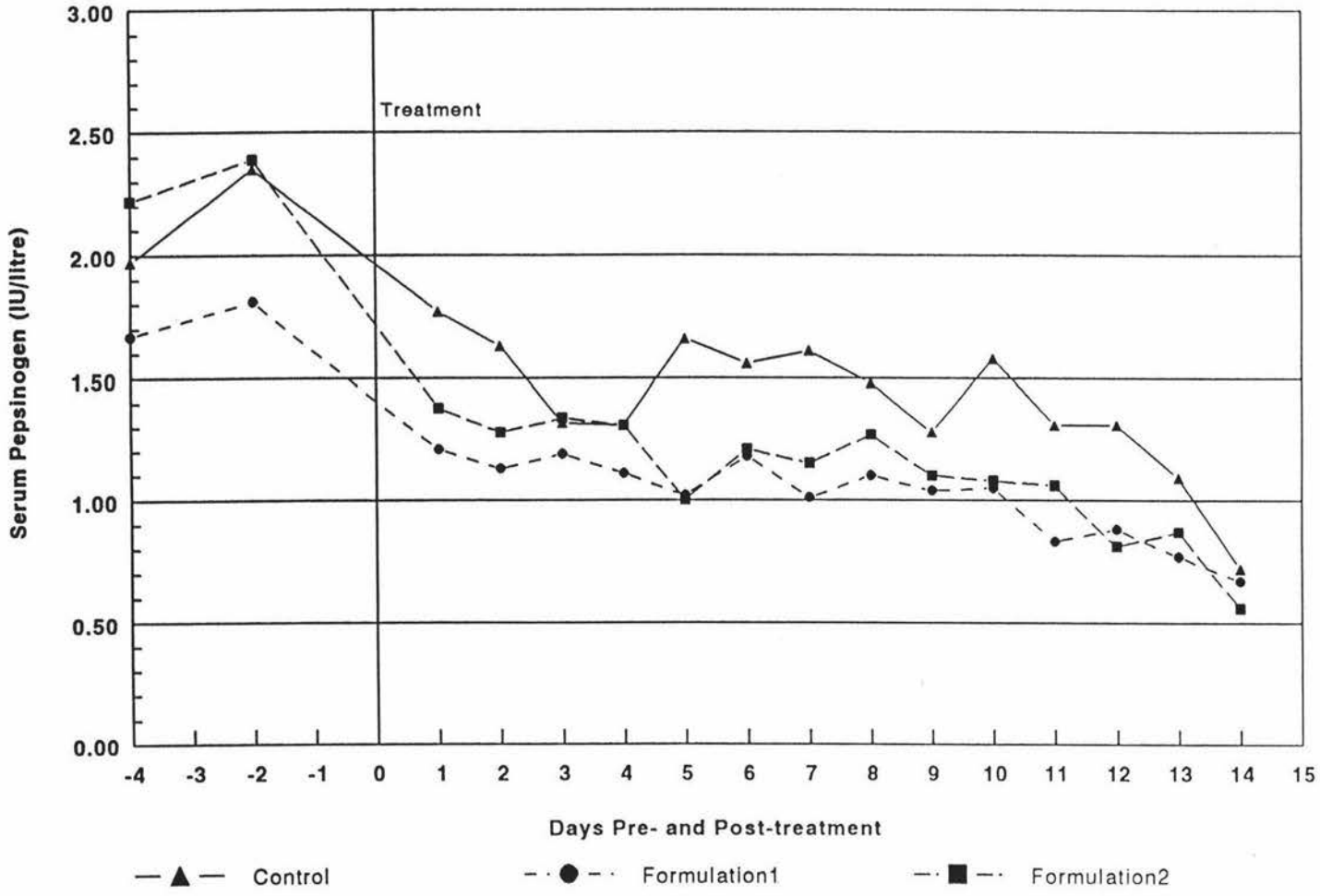


TABLE 3 CHANGES IN MEAN SERUM GASTRIN CONCENTRATIONS PRE- AND POST-TREATMENT IN UNTREATED CONTROL CALVES AND CALVES TREATED WITH ONE OF TWO FORMULATIONS OF 0.5% MOXIDECTIN POUR-ON

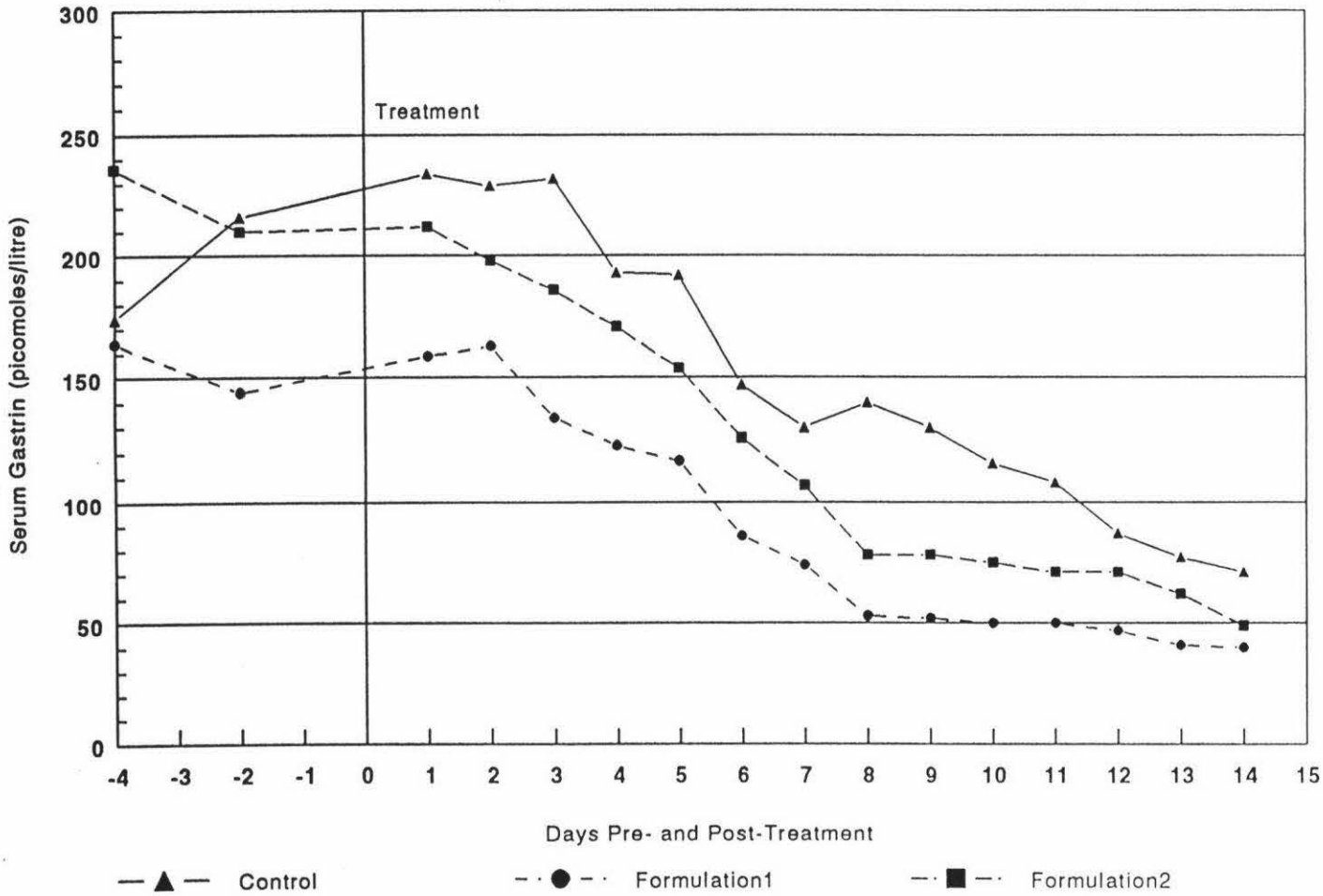


TABLE 4 CHANGES IN MEAN SERUM PEPSINOGEN AND GASTRIN CONCENTRATIONS IN UNTREATED CONTROL CALVES KEPT ON A FEED-PAD FOR 28 DAYS

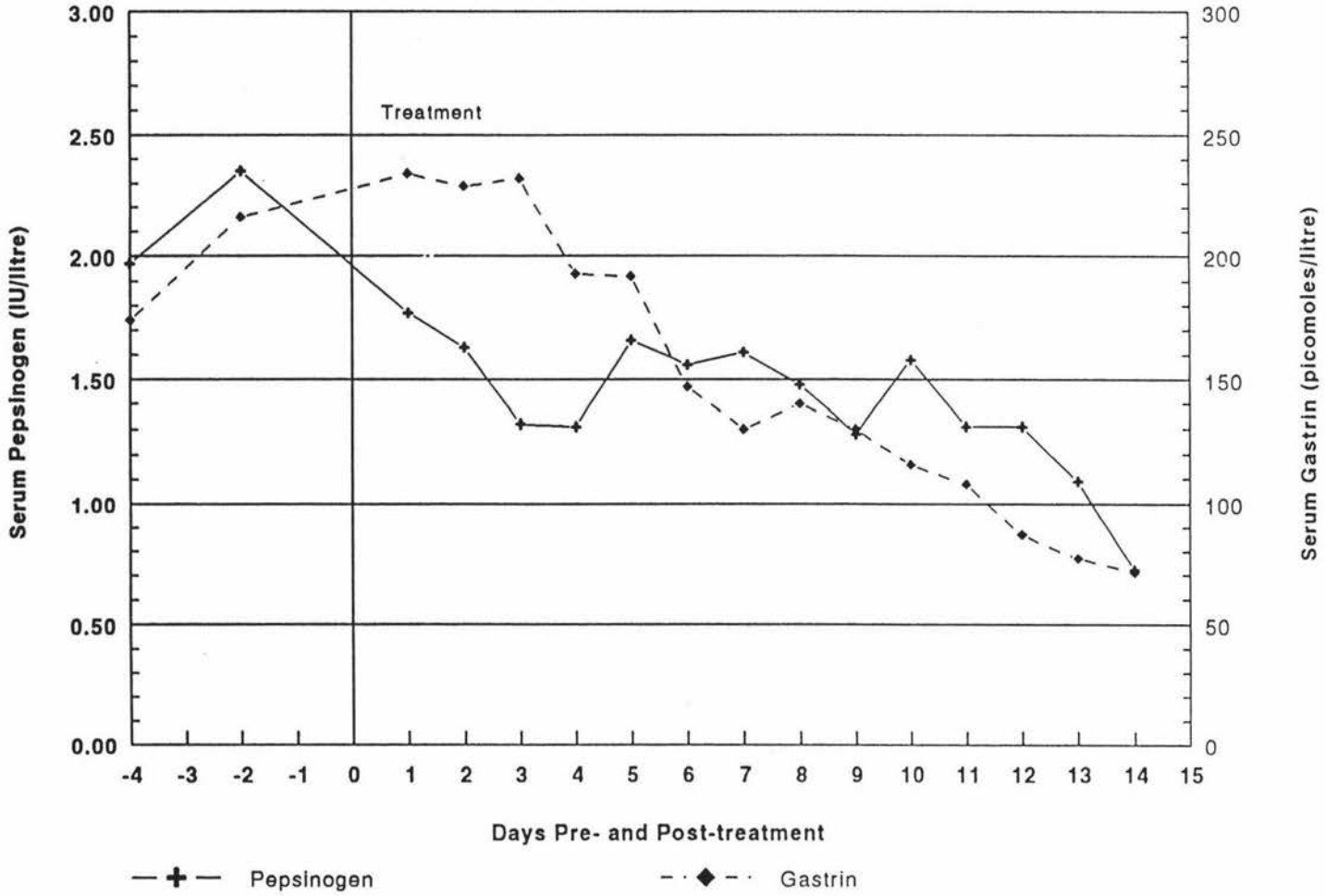


TABLE 5 **CHANGES IN MEAN SERUM PEPSINOGEN AND GASTRIN CONCENTRATIONS IN CALVES TREATED WITH 0.5% MOXIDECTIN POUR-ON (FORMULATION 1)**

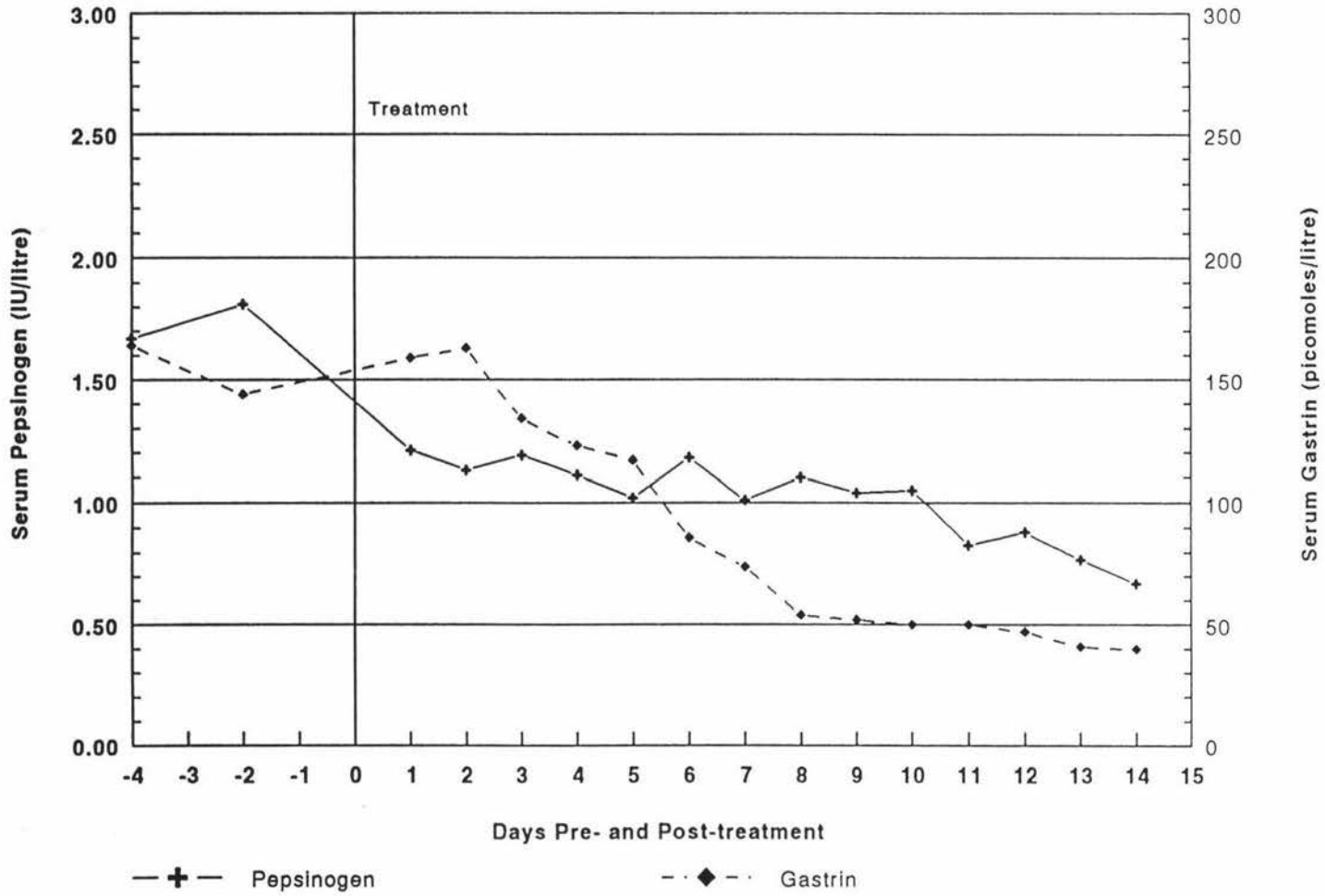


TABLE 6 **CHANGES IN MEAN SERUM PEPSINOGEN AND GASTRIN CONCENTRATIONS IN CALVES TREATED WITH 0.5% MOXIDECTIN POUR-ON (FORMULATION 2)**

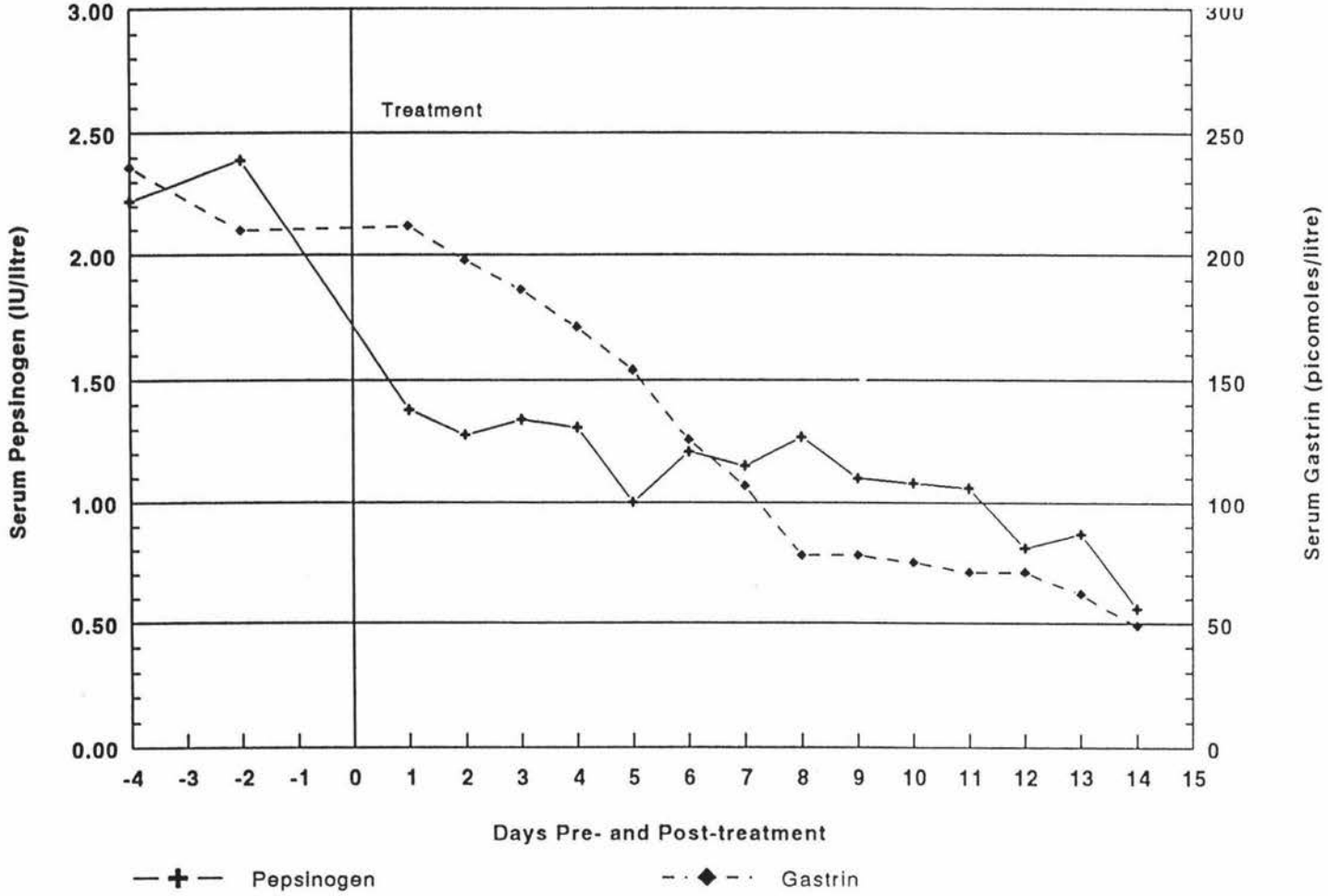
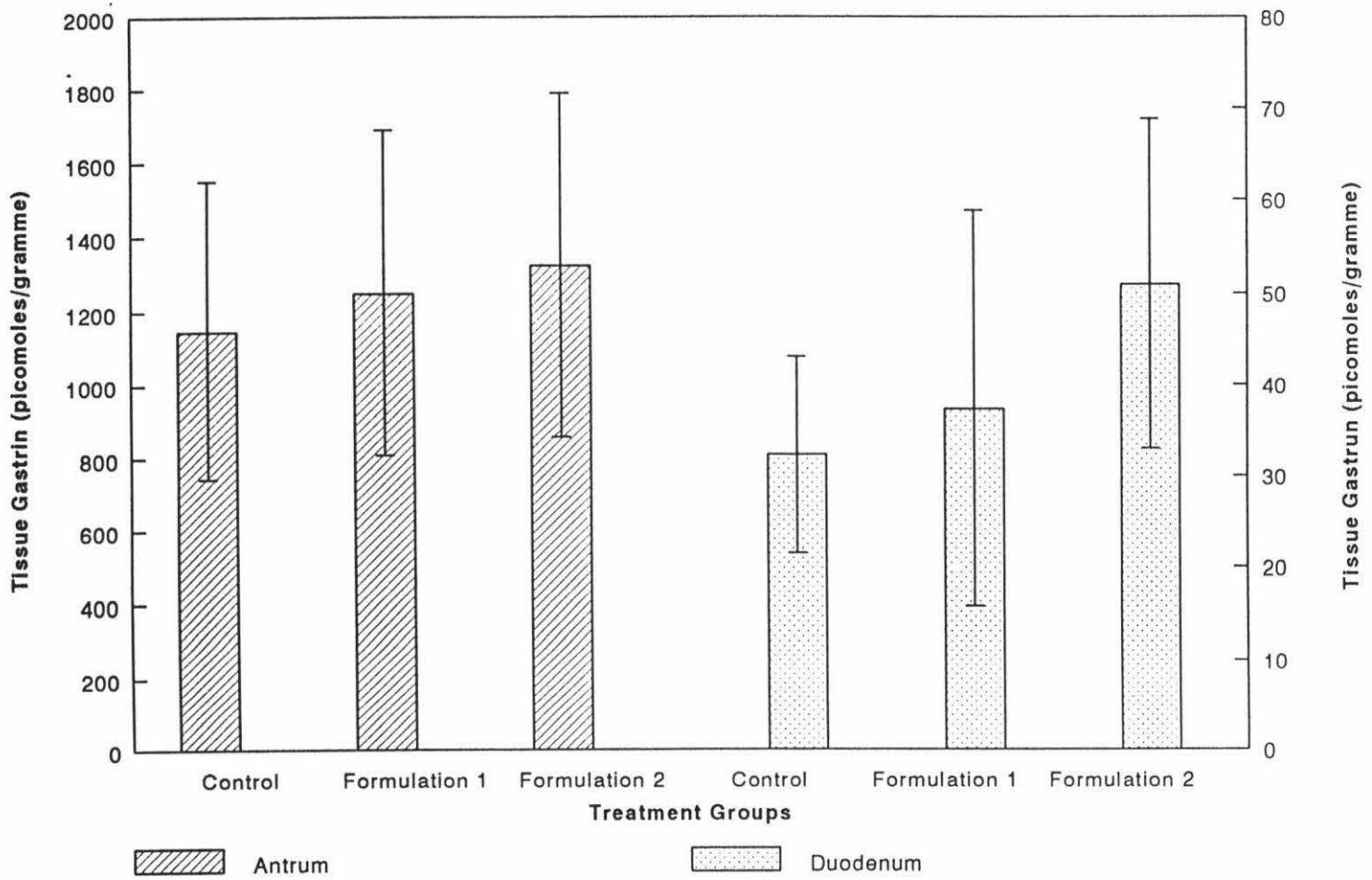


TABLE 7 ANTRAL AND PROXIMAL DUODENAL TISSUE GASTRIN CONCENTRATIONS FROM CALVES TREATED WITH ONE OF TWO FORMULATIONS OF 0.5% MOXIDECTIN POUR-ON AND UNTREATED CONTROL CALVES



4.0 DISCUSSION

4.1 *Anthelmintic efficacy*

The cattle used in this trial to compare the efficacy of two pour-on formulations of moxidectin were rising yearlings with moderate to heavy naturally acquired parasitic burdens. The predominant parasites present (*Ostertagia*, *Trichostrongylus* and *Cooperia*) typified the species most likely to be found in New Zealand.

In this study, both pour-on formulations at a dose rate of 500 mcg per kilogram bodyweight showed a high level of anthelmintic efficacy (>99.9%) against the abomasal parasites. *Cooperia* was the dose-limiting species for both formulations. These results against *Cooperia* are similar to other efficacy trials with moxidectin pour-on (Whang *et al.*1994; Hubert *et al.*1995b; Williams *et al.*1996; Morin *et al.*1996) and injection (Samson *et al.*1992; Ranjan *et al.*1992; Williams *et al.*1992; Whang *et al.*1994). Formulation 2 appeared to have minimal efficacy against any of the *Cooperia* species and could not be recommended for commercial release.

Cooperia spp., particularly *Cooperia oncophora*, appears to be the dose-limiting species for other members of the avermectin/milbemycin endectocide family, namely ivermectin (Wescott *et al.*1980; Swan *et al.*1985; Bisset *et al.*1990), abamectin (Benz and Ernst, 1979; Wescott *et al.*1980; Egerton *et al.*1979) and doramectin (Goudie *et al.*1993). Eprinomectin was designed to improve efficacy against this particular species (Shoop *et al.*1996a; 1996b).

In New Zealand, there have been recent reports about the lack of efficacy of moxidectin against *Cooperia* when used under field conditions against infections that did not respond to ivermectin (Vermunt *et al.*1996). It was unclear though whether this was anthelmintic resistance as such (Vermunt *et al.*1996), or anthelmintic tolerance (McKenna, 1995). *Cooperia* spp. are a common parasite of the small intestine of young cattle but are not regarded as being highly pathogenic when compared with *Ostertagia ostertagi* (Bisset, 1994; West *et al.*1994; Vermunt *et al.*1996). However, the repeated use of endectocides that allow the continued excretion of *Cooperia* eggs may result in a disproportionate contamination of pasture with *Cooperia* spp. (West *et al.*1994). The continued exposure of these worms to the avermectin/milbemycin family may in turn encourage the development of true resistance (McKenna, 1995). While the increased exposure to cattle of *Cooperia* larvae has not yet been reported to result in production losses under New Zealand conditions, it is an area that should be studied.

It was concluded that both pour-on formulations of moxidectin in this trial had a high level of efficacy against *Ostertagia* spp. and *Trichostrongylus axei*. The level of efficacy against *Cooperia* spp. was

lower in comparison, and Formulation 1 was distinguished from Formulation 2 by having a greater efficacy against this nematode species.

4.2 *Pepsinogen and Gastrin*

The pathophysiological changes seen in ostertagiosis are a combination of changes induced either directly or indirectly by the parasite.

Following ingestion, infective larvae pass from the rumen into the abomasum, enter the gastric glands on day 2 and moult to become the fourth larval stage. The parasite continues to develop in the gastric glands, moults to become the immature adult, matures to the adult stage and then emerge to lie on the mucosal surface (Armour, 1970). The principal pathophysiologic changes of ostertagiosis in the abomasum are associated with the emergence of the adult worm from the gastric gland on days 16-21 post-ingestion (Ritchie *et al.*1966). These changes include reduced abomasal acidity, raised blood pepsinogen and gastrin levels, marked tissue hyperplasia and loss of abomasal cellular differentiation (Anderson *et al.*1965; Ritchie *et al.*1966). Prior to emergence, cellular changes are confined to the parasitised glands and significant changes do not occur in the biochemical values of either the abomasal fluid or blood (Anderson *et al.*1965; Armour, 1970).

4.2.1 *Serum pepsinogen*

Recently it has been established that there are several types of pepsinogen (Eckersall *et al.*1987; McKellar *et al.*1988; Mostofa *et al.*1990) though none have been correlated with specific types of ostertagiosis (McKellar *et al.*1988; Mostofa *et al.*1990). Because pepsinogen is released into the abomasum directly, it was initially assumed that an increase in blood levels were an indicator of tissue damage, and the proenzyme was escaping between damaged cell junctional complexes (Anderson *et al.*1965; Jennings *et al.*1966; Berghen *et al.*1993). In the case of grazing cattle, this damage was most likely due to adult and immature *Ostertagia* (Ritchie *et al.*1966; Al Saqur *et al.*1982), though other parasites have been shown in laboratory trials to also cause an increase in serum pepsinogen (Ross *et al.*1968; Snider *et al.*1985; Shoo and Wiseman, 1986; Fox *et al.*1988). However, Stringfellow and Madden, (1979) proposed that pepsinogen secreted by chief cells was released directly into the circulation rather than taken up from the gastric contents as had been suggested (Armour, 1974; Ford, 1976). More recent work has shown that a concentration gradient between the mucosal and blood along with capillary permeability and lymphatic uptake could also account for the higher than normal blood concentrations (Baker *et al.*1993). Experimentally transferred adult *Ostertagia* spp. have also been shown to cause a rapid increase (within 24-48

hours) of serum pepsinogen and gastrin levels when transplanted into worm-free sheep (Anderson *et al.*1985) and cattle (McKellar *et al.*1986; McKellar *et al.*1987). This may be due to either a direct effect by a parasympathomimetic secretagogue (McKellar *et al.*1990), or indirect stimulation of zymogen cells, both of which could result in an increase in production and secretion of pepsinogen and gastrin (McKellar *et al.*1986; McKellar *et al.*1987). This also suggests that leakage through the mucosa may not be the mechanism for the increase in serum pepsinogen levels.

While a good correlation has been found between increasing levels of ingested infective larvae and pepsinogen concentration (Anderson *et al.*1966; Mylrea and Hotson, 1969; Armour *et al.*1979; Snider *et al.*1981), correlation to worm numbers varies. Worm burdens of 30,000 and above were associated with a plasma pepsinogen level greater than 2.6 IU tyrosine/litre (Brunsdon, 1971). Allen *et al.*(1970) and others have also been able to correlate the numbers of active *Ostertagia* with pepsinogen levels (Entrocasso *et al.*1986; McKellar *et al.*1986). However, no such correlation was found between serum pepsinogen and *Ostertagia* numbers in this trial. This is in line with the results of other authors (Mylrea and Hotson, 1969; Michel *et al.*1978; Chalmers, 1983). The size of the standard deviations may well have overshadowed a true difference, but because of the small number of animals this could not be detected.

Considerable debate has occurred about the most appropriate serum pepsinogen concentration for diagnostic purposes (Chiejina, 1977; Selman *et al.*1977; Richardson, 1977; Entrocasso *et al.*1986). A contributing factor to this has been the level of variation seen between laboratories using a variety of methods, and substrates used for release of tyrosine (H Simpson (1998), pers. comm.). Clinical signs of disease have been seen with blood concentrations as low as 2.9 IU/litre after artificial infections of 100,000 *O. ostertagi* larvae, but levels as high as 5- 7 IU/l have also been seen in animals with no obvious clinical signs (Michel *et al.*1978; Hilderson *et al.*1989). In some UK diagnostic laboratories levels <1 IU/l are regarded as normal, with 2 - 2.9 IU/l being regarded as indicative of significant abomasal damage associated with high levels of *Ostertagia* in calves (Chiejina and Clegg, 1978). Other authors have preferred to use up to 2.5 IU/l for clinically normal grazing cattle (Chiejina, 1978) and >3 IU/l as indicative of severe clinical disease (Armour, 1970; 1974; Entrocasso *et al.*1986). In Belgium it was proposed that the diagnostic threshold for clinical ostertagiasis be >5 IU/l (Hilderson *et al.*1989). Under New Zealand conditions, levels above 2 IU/l in yearling cattle are regarded as being associated with clinical disease and production loss (McSporran, 1986; S. Bisset (1998). pers.comm.). Normal levels in older cattle in New Zealand range between 0.2-2.5 IU/litre (Chalmers, 1983).

Other factors that need to be taken into account when interpreting serum pepsinogen concentrations are: the normal increase with age (Brunsdon, 1971), the variable length of time for raised levels to decrease to normal (4-9 weeks) following treatment (Anderson *et al.*1965; Brunsdon, 1972; Michel *et al.*1978; Jorgensen *et al.*1978; Armour *et al.*1979; Snider *et al.*1981) and apparent seasonal

fluctuations (Chiejina and Clegg, 1978; Chiejina, 1978; Armour *et al.*1979). However, McSporran, (1986) believed that the apparent long half-life of pepsinogen was actually due to the continuous effects of pasture larval challenge in grazing animals. Other authors have shown that it can decrease rapidly following anthelmintic treatment (Ross *et al.*1968; Armour *et al.*1967; Wiggin and Gibbs, 1987; 1990; McSporran, 1986; Simpson *et al.*1997), which as above, suggest it is directly associated with the presence of *Ostertagia* or its secretions/excretions rather than damage to pepsinogen secreting cells.

It was suggested by (Armour *et al.*1979) that some of the "seasonal" increase in pepsinogen levels may in fact be due to an allergic reaction of the abomasal mucosa. Further work added credence to the theory that increases in serum pepsinogen levels were due to a combination of two factors. One was the direct pathologic effects of infecting larvae and developing worms. The second factor being a hypersensitivity component resulting from the effects of larval antigens on immune cells of the gut mucosa in the sensitised host, when re-exposed to *Ostertagia* larvae (Jacobs *et al.*1987; Wiggin and Gibbs, 1987; 1989; 1990).

Results from this trial showed a 60-75% decrease in serum pepsinogen levels to within normal levels in both treated and untreated groups of calves over 2 weeks. This is consistent with the removal of calves from larval challenge. But these animals would have also been naturally losing abomasal parasites (Michel, 1969; Murray *et al.*1970), and the numbers of developing larvae and remaining adults in the abomasum may have insufficient to stimulate a hyperpepsinogenaemia. It would have been interesting to measure serum pepsinogen levels in the same cattle following their return to infective pasture, to see what level of response occurred.

4.2.2 *Serum gastrin*

Consequential to the emergence of the adult parasites from the gastric glands, the surrounding acid secreting parietal cells are also damaged. These damaged cells are subsequently replaced with rapidly dividing undifferentiated non-acid secreting cells (Anderson *et al.*1965; Ritchie *et al.*1966) which results in an elevation of abomasal pH (from 2 up to 5-7) (Anderson *et al.*1965; Armour *et al.*1967; 1974; Jennings *et al.*1966; Ross *et al.*1968). The effect of this increase in pH is to inhibit the conversion of pepsinogen to pepsin. This impairs protein digestion, and can allow an increase in abomasal bacterial numbers (Jennings *et al.*1966). The decrease in abomasal pH initiates a positive feedback loop for gastrin secretion ie. the increased pH stimulates the release of gastrin, which in turn activates parietal cells to produce more hydrochloric acid (Fox *et al.*1987; Yasuda *et al.*1988; Fox *et al.*1993). If sufficient parietal cells are damaged, insufficient acid is able to be produced to lower the abomasal pH and a hypergastrinaemia results (McKellar, 1993). An additional mechanism for gastrin secretion has been shown in both sheep (Anderson *et al.*1981; Lawton *et*

*al.*1996) and cattle (McKellar *et al.*1987), whereby transplanted adult *Ostertagia* stimulated the secretion of gastrin in previously worm-free animals. Because this is not associated with any tissue damage, it would appear that the presence of the parasites may be the primary stimulus for increases in gastrin secretion, not the loss of parietal cells.

Compared to other trials where serum gastrin levels were measured after removing cattle from infective pasture, initial levels in this trial (164-236 pM) were comparatively low (Entrocasso *et al.*1986; Berghen *et al.*1993; Ploeger *et al.*1994) and further decreased to quite low concentrations (40-71 pM). Parasite-naive calves have serum gastrin concentrations of 93 -106 pg/ml (42-50 pM) which increased up to 350 - 2000 pg/ml following dosing with various levels of infective *Ostertagia* larvae (Entrocasso *et al.*1986; McKellar *et al.*1987; Schillhorn-van-Veen, 1988; Xiao *et al.*1992; Fox *et al.*1993). Using group means, levels of 400 pg/ml are considered to be indicative of subclinical parasitic disease, while levels of 1000 pg/ml or greater are representative of clinical Type I ostertagiosis (Berghen *et al.*1993).

Based on this, calves in this trial had levels which would be indicative of abomasal damage, but not diagnostic for Type I ostertagiosis. Because samples were not taken when animals first came onto the feed pad, it is unknown if higher levels had been present and subsequently decreased. The fourteen days on the feed pad before treatment would have allowed immature *Ostertagia* larval stages (other than the inhibited ones) to complete their life cycle. Following on from this, a rise in pepsinogen and gastrin levels would have been expected in all groups by the day of treatment, and this rise to continue for the control group with the continued emergence of adult *Ostertagia* from the gastric glands. The results of a pre-treatment total worm count in one animal (288,600 adult *Ostertagia*) had indicated that very high *Ostertagia* numbers would be present, but even though animals were being grazed on known infective pasture at a time when under New Zealand conditions *Ostertagia* and *Cooperia* pasture larval numbers would be increasing (Brunsdon, 1972a; 1972b; Bisset and Marshall, 1987), no rise in gastrin or pepsinogen levels were seen. This implied a low intake of infective larvae by calves, and the small numbers of adult *Ostertagia* (Arithmetic mean 10,597.5) in the control group at the end of the trial supported this.

Serum gastrin levels differed significantly between the control group and calves treated with Formulation 1, which could imply a secretagogue effect due to *Ostertagia*. However, both Formulations removed >99.8% of all abomasal parasites, yet serum gastrin concentrations of calves treated with Formulation 2 were not significantly different from the other two groups. As with the differences between groups for serum pepsinogen, the small number of animals used and the size of the standard deviation may have over shadowed a true difference between groups. However, the significant decrease in Formulation 1 treated calves does correspond with work done in sheep artificially infected with adult *Haemonchus contortus* and drenched 4 days later. Gastrin levels decreased within 3-4 days along with abomasal pH (Simpson *et al.*1997). This did imply that in this

trial, removal of the parasites by the anthelmintic also removed any secretagogue effect. The case may be argued that serum gastrin levels in the control calves also were decreasing. But the counter argument could be that these animals were naturally losing abomasal parasites (Michel, 1969; Murray *et al.*1970), and the numbers of developing larvae and remaining adults in the abomasum were insufficient to stimulate an increase in serum gastrin. Unlike work by other authors (Entrocasso *et al.*1986; McKellar *et al.*1987; Xiao *et al.*1991; 1992), there was no correlation between serum gastrin levels and numbers of *Ostertagia* spp in the abomasum. Similar results were seen by (Ploeger *et al.*1994).

No histopathology or immunohistochemistry (Fox *et al.*1993) were performed, so it is unknown whether there was any changes in the number of G-cells or parietal cells as has been seen in sheep (H Simpson, (1998) unpublished observations). Abomasal pH was not measured in this trial so the physiologic effects of gastrin were unable to be quantified.

Besides stimulating abomasal acid secretion, gastrin also has a negative effect on abomasal motility and gastric emptying (Bell *et al.*1977; Onapito *et al.*1978), and feed intake (Fox *et al.*1989a; 1989b). It was not practical to specifically measure levels of faecal consistency or feed intake in this trial, as animals were kept in treatment groups.

Because many factors such as measuring methods, animal age and immunity, and grazing management can affect serum pepsinogen and gastrin concentrations, the use of both these parameters for predicting clinically apparent ostertagiosis is limited and should be considered in conjunction with other findings (Xiao *et al.*1992). The benefit of gastrin appears to be that unlike pepsinogen, it is not influenced by hypersensitivity reactions due to incoming larvae. However, the increased levels of pepsinogen maybe an indicator of levels of pasture larval ingestion (Ploeger *et al.*1994). Both parameters are best used on a herd basis to provide an overall picture of the level of ostertagiosis, as interpreting data from individual animal's can potentially be misleading. The following situation that occurred during the trial bears out these comments.

On day-2 before treatment, the treatment groups had mean serum pepsinogen concentrations ranging from 1.81-2.35 IU/litre. However two days post-treatment, the two lightest animals in the trial (No. 12 and 29) had to be moved because of clinical signs of parasitism. Neither had had pre-or post-treatment serum pepsinogen levels >2 IU/litre and serum gastrin concentrations were also similar to other members of their group (Formulation 2) (216.2 and 157.8 pm respectively). It was possible that these clinical signs were due to *Cooperia* spp., as both calves had moderate levels of *Cooperia* on post-mortem. Calf No.12 had the highest *Cooperia* adult (10,800) and early L4 count (77,000) of any of the treated calves. But Calf No. 29 only had 280 adult *Cooperia* and 4800 early L4. Alternatively, the clinical signs seen may have been due to animals still harbouring significant numbers of *Ostertagia* spp. or *T. axei* due to pour-on formulations taking 2-3 days to reach

maximum serum concentrations (Herd *et al.*1996; Shoop *et al.*1996). Based on the literature and clinical condition of the animals, significantly higher serum concentrations of pepsinogen and gastrin would have been expected.

4.2.3 *Tissue gastrin*

High levels of gastrin have been found in the antrum of the abomasum in cattle (Fox *et al.*1993; Purewal *et al.*1997) and sheep (Reynolds *et al.*1979; Reynolds *et al.*1984). Tissue concentrations in this trial were comparable to those found by (Purewal *et al.*1997), but antral concentrations were up to five times those found by (Fox *et al.*1993). This difference may have been due to natural variation and/or the different level of natural larval exposure of these trial calves. However, unlike (Purewal *et al.*1997) no differences were found between the controls and treatment groups. This could have been due to insufficient gastrin containing tissue samples being taken, though the very similar tissue concentrations in both trials tended not to support this. The other possibility is that oedema of the tissue from control calves may have resulted in underestimation of true levels. Pyloric mucosal weight was not measured as these animals were being used for an anthelmintic efficacy trial, and abomasal tissue was required for saline digests.

Antral levels of gastrin in cattle appear to be three to four times less than those reported in sheep (Reynolds *et al.*1979; Reynolds *et al.*1984). It is unclear why this is so. One possibility is that due to the difference in organ size between the species, sheep may have a higher concentration of gastrin producing tissues or alternatively both sets of result may be from animals at opposite ends of a normal distribution. In this trial, there was no histology or immunohistochemistry performed on antral or duodenal samples to look for any evidence of hypertrophy or hyperplasia.

While no difference in tissue or serum gastrin between control and treated animals was seen in this trial, (Purewal *et al.*1997) has proposed four mechanisms that might contribute to the hypergastrinaemaia seen in *Ostertagia* infected calves. These are an increase in gastrin synthesis, direct or indirect stimulation of gastrin secretion by the autocrine peptide pancreastatin, release of previously stored reserves of gastrin and/or a reduction in the metabolism of gastrin.

Whilst there is a considerable amount of literature on the actions and effects of pepsinogen, the information on the effects of nematodes on hormones such as gastrin is only starting be published. It appears that nematodes such as *Ostertagia* can have a considerable effect on the hormonal physiology of the abomasum. The use of anthelmintics does result in rapid removal of the parasites, but it appears that a period of time is still required before the abomasal physiology returns to normal. A possibility for future research in this area is measuring the effects on abomasal physiology of long acting anthelmintics such as the avermectin/milbemycins.

TABLE 1 PRE-TREATMENT TOTAL WORM COUNT[1]

Abomasum				Small Intestine			
Ostertagia spp.			*Trich. axei.*	*Cooperia* spp.			*Oesophagostomum* spp.
Adult	Early L4	Late L4	Adult	Adult	Early L4	Late L4	L4
288600	24100	48000	12100	82100	222300	900	40

[1] Based on a 1% aliquot

TABLE 2 PRE-TREATMENT BULK FAECAL LARVAL CULTURES

Nematode genus	Day -36	Day -21
Ostertagia spp.	14/100	5/100
Trichostrongylus spp.	24/100	-
Cooperia spp.	62/100	94/100
Chabertia spp.	-	1/100

TABLE 3 MEAN FAECAL EGG COUNTS FOR THE THREE GROUPS (EGGS PER GRAM OF FAECES)

	Day of sampling															
Treatment	-36	-21	-14	-2	-1	+1	+2	+3	+4	+5	+6	+7	+8	+10	+12	+14-+16
Control	6	444	169	294	269	381	488	381	400	500	816	844	619	406	469	1069
Formulation 1	6	219	136	331	344	206	125	88	25	25	13	6	0	19	0	6
Statistical Significance[1]	NS[2]	NS	NS	NS	NS	0.1	0	0	0	0	0	0	0	0	0	0
Formulation 2	13	771	264	494	388	842	213	213	513	331	281	63	71	75	125	238
Statistical Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0	NS	0	NS	0.088

[1] Kruskal-Wallis non-parametric one-way analysis of variance
[2] NS Not significant

TABLE 4 GEOMETRIC MEAN WORM BURDENS AT POSTMORTEM FOR CALVES TREATED WITH 0.5% MOXIDECTIN POUR-ON AND PERCENT EFFICACY FOR EACH FORMULATION

	Control (n=8)	Formulation 1 (n=8) 500mcg moxidectin per kg bodyweight			Formulation 2 (n=8) 500mcg moxidectin per kg bodyweight		
Nematodes species and stage[1]	**Mean**	**Mean**	**Efficacy %**	**Statistical significance[2]**	**Mean**	**Efficacy**	**Statistical significance[**]**
Abomasum							
Ostertagia spp.	4973.1	0.0	100	<0.01	0.0	100	<0.01
Ostertagia spp. Early 4th stage larvae	4481.8	0.8	99.99	<0.01	2.9	99.94	<0.01
Ostertagia spp. Late 4th stage larvae	723.8	0.0	100	<0.01	0.5	99.93	<0.01
Trichostrongylus axei	2229.5	1.7	99.92	<0.01	4.4	99.81	<0.01
Small Intestine							
Cooperia spp.	2272.3	85.2	96.25	0.05	649.6	71.41	NS
Cooperia spp. Early 4th stage larvae	463.1	12.5	97.30	NS[3]	152.2	67.13	NS
Cooperia spp. Late 4th stage larvae	22.4	2.0	91.07	NS	8.0	64.29	NS
Trichostrongylus spp.	3.7	0.0	100	0.05	0.0	100	0.05
Large Intestine							
Oesophagostomum radiatum	4.4	0.0	-	ND[4]	0.0	-	ND
Trichuris ovis	2.5	0.0	-	ND	0.6	-	ND

[1] Adult nematodes unless otherwise indicated
[2] One-way analysis of variance between control and treatment group
[3] NS Not significant at P<0.05
[4] ND Too few infected animals for statistical comparison

TABLE 5 ARITHMETIC MEAN WORM BURDENS AT POSTMORTEM FOR CALVES TREATED WITH 0.5% MOXIDECTIN POUR-ON AND PERCENT EFFICACY FOR EACH FORMULATION

	Control (n=8)	Formulation 1 (n=8) 500 mcg moxidectin per kg bodyweight		Formulation 2 (n=8) 500mcg moxidectin per kg bodyweight	
Nematodes species and stage[1]	**Mean**	**Mean**	**Efficacy %**	**Mean**	**Efficacy**
Abomasum					
Ostertagia spp.	10597.5	0.0	100.00	0.0	100.00
Ostertagia spp. Early 4th stage larvae	11285.0	12.5	99.89	20.0	99.82
Ostertagia spp. Late 4th stage larvae	2145.0	0.0	100.00	2.5	99.88
Trichostrongylus axei	4025.0	12.5	99.69	50.0	98.76
Small Intestine					
Cooperia spp.	8117.5	1032.5	87.28	3687.5	54.57
Cooperia spp. Early 4th stage larvae	4997.5	685.0	86.29	12035.0	75.89
Cooperia spp. Late 4th stage larvae	254.0	37.5	98.52	172.5	93.21
Trichostrongylus spp.	32.5	0.0	ND[2]	0.0	ND
Large Intestine					
Oesophagostomum radiatum	4.4	0.0	ND	0.0	ND
Trichuris ovis	2.5	0.0	ND	0.6	ND

[1] Adult nematodes unless otherwise indicated
[2] ND Too few infected animals for statistical comparison

TABLE 6 SPECIATION OF MALE ABOMASAL AND SMALL INTESTINAL WORMS EXAMINED

	Control		Formulation 1		Formulation 2	
Abomasum	**No. examined**	**Percent**	**No. examined**	**Percent**	**No. examined**	**Percent**
Ostertagia ostertagi	573/606	94.5	0	0	0	0
Ostertagia lyrata	33/606	5.5	0	0	0	0
Trichostrongylus axei	517/517	100	2/2	100	10/10	100
Small Intestine						
Cooperia mcmasteri	70/570	12.5	5/74	6.5	41/301	13.5
Cooperia oncophora	492/570	86	69/74	93.5	260/301	86.5
Cooperia punctata	8/570	1.5	0/74	0	0/301	0

TABLE 7 MEAN SERUM PEPSINOGEN CONCENTRATIONS (IU TYROSINE/LITRE) IN CONTROL AND MOXIDECTIN TREATED CALVES

	Day of sampling															
Treatment	**-4**	**-2**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**	**+14**
Control	1.97	2.35	1.77	1.63	1.32	1.31	1.66	1.56	1.61[a]	1.48	1.28	1.58	1.31	1.31	1.09	0.72
Formulation 1	1.67	1.81	1.21	1.13	1.19	1.11	1.02	1.18	1.01[b]	1.10	1.04	1.05	0.83	0.88	0.77	0.67
Formulation 2	2.22	2.39	1.38	1.28	1.34	1.31	1.00	1.21	1.15[ab]	1.27	1.10	1.08	1.06	0.81	0.87	0.56
Statistical Significance[1]	NS	NS	NS	NS	NS	NS	NS	NS	0.05	NS	NS	NS	NS	NS	NS	NS

[1] NS Not significant at $P<0.05$
Means with different superscript letters are significantly different from each other

TABLE 8 MEAN SERUM GASTRIN CONCENTRATIONS (PICOMOLES/LITRE) IN CONTROL AND MOXIDECTIN TREATED CALVES

	Day of sampling															
Treatment	**-4**	**-2**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**	**+14**
Control	174	216	234	229	232	193	192	147^{a}	130^{a}	140^{a}	130^{a}	116^{a}	108^{a}	87^{a}	77^{a}	71^{a}
Formulation 1	164	144	159	163	134	123	117	86^{b}	74^{b}	54^{b}	52^{b}	50^{b}	50^{b}	47^{b}	41^{b}	40^{b}
Formulation 2	236	210	212	198	186	171	154	126^{ab}	107^{ab}	78^{ab}	78^{ab}	75^{ab}	71^{ab}	71^{ab}	62^{ab}	49^{ab}
Statistical Significance[1]	NS	NS	NS	NS	NS	NS	NS	0.05	0.04	0.006	0.02	0.003	0.004	0.01	0.01	0.04

[1] NS Not significant at $P<0.05$
Means with different letters are significantly different from each other

TABLE 9 **MEAN (+/- STANDARD DEVIATION) ABOMASAL AND PROXIMAL DUODENAL TISSUE GASTRIN CONCENTRATIONS (PICOMOLES/GRAMME OF MUCOSA) IN CONTROL AND MOXIDECTIN TREATED CALVES**

Treatment	Abomasum - Antrum (picomoles/gramme)	Proximal Duodenum (picomoles/gramme)
Control	1148 (+/- 404)	32.3 (+/- 10.8)
Formulation 1	1250 (+/- 441)	37.3 (+/- 21.5)
Formulation 2	1323 (+/- 467)	50.9 (+/- 17.9)
Statistical Significance	NS[1]	NS

[1] NS Not significant at $P<0.05$

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APPENDIX 1.1 DATA USED FOR ALLOCATING CATTLE TO GROUPS

Calf No.	FEC[1] Day -2	FEC Day -1	Mean FEC	FEC Rank	Weight (Kg)	Weight Rank	Mean Rank	Group	Dose (ml)	Batch No.
1	100	100	100	3	168.0	10	6.5	2	17	A56
2	250	350	300	12	173.0	11	11.5	2	17	A56
3	800	850	825	23	149.5	4	13.5	1	15	(19)
4	450	200	325	14	174.0	13	13.5	2	17	A56
5#	150	400	275	11	193.5	22	16.5	3	-	
6	600	450	525	19	192.0	21	19.5	3	-	
7	550	250	400	15	173.5	12	13.5	3	-	
8	300	0	150	5	185.5	19	11.5	3	-	
9†	2350	4750	3550	-	-	-	-	2	14	A63
10	550	350	450	16	159.0	5	10.5	2	16	A56
11†	250	200	225	-	204.5	-	-	1	20	(19)
12	1050	500	775	22	148.5	2	12.0	2	15	A63
13	300	100	200	6	193.5	22	13.5	1	19	(13)
14	150	250	200	6	159.5	7	6.5	3	-	
15†	100	50	75	-	198.5	-	-	3	-	
16	500	600	550	21	149.0	3	12.0	3	-	
17	100	50	75	2	196.5	24	12.0	1	20	(13)
18	200	400	300	12	183.5	18	14.5	3	-	
19†#	0	150	75	-	202.0	-	-	3	-	
20	450	500	475	17	159.0	5	11.0	1	16	(19)
21	100	450	225	10	187.5	20	19.5	1	19	(19)
22	50	50	50	1	180.0	17	8.0	3	-	
23	500	550	525	19	175.0	14	16.0	1	18	(13)
24	800	1000	900	24	176.0	15	19.0	2	18	A56
26	200	50	125	4	160.0	8	6.0	1	16	(19)
27	550	400	475	17	177.0	16	6.0	2	18	A63
28	200	200	200	6	164.5	9	7.5	1	16	(13)
29	200	200	200	6	118.5	1	3.5	2	12	A56

[1] FEC - Faecal Egg Count.

† Spare animals for substitution in case of death.

** 1 = Formulation 1 AC 7215/26/(13), (19).
2 = Formulation 2 AC 7215/34/A56,A63.
3 = Control

No. 19 was substituted for No.5 at the end of the trial because No.5 had had a zero FEC since Day +5.

APPENDIX 1.2.1 INDIVIDUAL FAECAL EGG COUNTS FOR THE CONTROL GROUP

	Day of sampling																	
Calf No.	**-36**	**-21**	**-14**	**-2**	**-1**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+10**	**+12**	**+14**	**+15**	**+16**
6	0	200	100	600	450	300	150	250	150	200	150	100	0	100	50	200		
7	50	1900	300	550	250	800	700	500	950	450	1350	1150	1100	800	850	ND[2]	2300	
8	0	100	0	300	0	300	150	150	0	0	0	50	100	100	0	ND	ND	200
14	0	200	100	150	250	100	350	50	50	100	150	200	0	250	250	350		
16	0	700	350	500	600	500	1700	1250	1150	2550	4280	4750	3500	1400	2300	3950		
18	0	250	250	200	400	300	100	200	550	450	500	300	200	500	250	ND	1200	
19	0	50	100	0	150	500	350	300	300	250	100	150	50	50	0	350		
22	0	150	150	50	50	250	400	350	50	0	0	50	0	100	50	ND	ND	0
Arith Mean[1]	**6**	**444**	**169**	**294**	**269**	**381**	**488**	**381**	**400**	**500**	**816**	**844**	**619**	**406**	**469**	**1213**	**1750**	**100**

[1] Arithmetic Mean
[2] ND Not Done

APPENDIX 1.2.2 INDIVIDUAL FAECAL EGG COUNTS FOR THE FORMULATION 1 GROUP

	Day of sampling																	
Calf No.	**-36**	**-21**	**-14**	**-2**	**-1**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+10**	**+12**	**+14**	**+15**	**+16**
3	0	450	0	800	850	400	50	50	150	50	0	0	0	0	0	ND	0	
13	0	300	250	300	100	250	0	50	0	0	0	50	0	50	0	ND	ND	50
17	0	0	0	100	50	0	0	50	0	0	0	0	0	0	0	ND	0	
20	0	300	ND[2]	450	500	250	500	300	50	50	100	0	0	0	0	0		
21	0	450	100	100	450	250	100	0	0	0	0	0	0	0	0	ND	ND	0
23	0	50	350	500	550	100	300	200	0	100	0	0	0	0	0	ND	0	
26	0	150	50	200	50	100	0	0	0	0	0	0	0	0	0	0		
28	50	50	200	200	200	300	50	50	0	0	0	0	0	0	0	ND	ND	0
Arith Mean[1]	**6**	**219**	**136**	**331**	**344**	**206**	**125**	**86**	**25**	**25**	**13**	**6**	**0**	**19**	**0**	**0**	**0**	**25**

[1] Arithmetic Mean
[2] ND Not Done

APPENDIX 1.2.3. INDIVIDUAL FAECAL EGG COUNTS FOR THE FORMULATION 2 GROUP

	Day of sampling																	
Calf No.	**-36**	**-21**	**-14**	**-2**	**-1**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+10**	**+12**	**+14**	**+15**	**+16**
1	0	200	250	100	100	600	0	0	0	0	0	0	0	0	0	ND	ND	0
2	0	800	50	250	350	300	300	200	300	50	50	50	ND	50	300	ND	650	
4	0	100	0	450	200	ND[2]	0	0	0	0	0	0	0	0	0	ND	0	
9	0	3250	100	2350	4750	ND	6350	2150	1100	650	600	400	150	100	0	ND	ND	
10	0	250	0	550	350	3750	600	250	300	250	50	150	50	50	0	ND	ND	300
12	50	250	700	1050	500	350	250	600	1150	1250	1750	250	350	400	350	900		
24	50	4450	700	800	1000	650	350	450	700	150	150	0	0	0	50	ND	50	
27	0	100	150	550	400	150	0	0	0	50	0	0	50	0	0	ND	ND	0
29	0	50	ND	200	200	100	200	200	1650	900	250	50	50	100	0	0		
Arith Mean[1]	**13**	**771**	**264**	**494**	**388**	**842**	**213**	**213**	**513**	**331**	**281**	**63**	**71**	**75**	**125**	**450**	**233**	**100**

[1] Arithmetic Mean
[2] ND Not Done

APPENDIX 1.3.1 TOTAL ABOMASAL WORM COUNTS IN THE CONTROL GROUP

	Ostertagia spp.			*Trichostrongylus axei*
Calf Number	**Adults**	**Early L4**	**Late L4**	**Adults**
6	7340	100	40	480
7	41800	3200	760	6440
8	17460	20540	2360	9480
14	1520	14440	340	100
16	800	960	80	3620
18	8260	8720	2300	5440
19	6540	36820	9580	4080
22	1060	5500	1700	2560
Geometric Mean	**4973.1**	**4481.8**	**723.8**	**2229.5**

APPENDIX 1.3.2. TOTAL ABOMASAL WORM COUNTS AFTER TREATMENT WITH FORMULATION 1

	Ostertagia spp.			*Trichostrongylus axei*
Calf Number	**Adults**	**Early L4**	**Late L4**	**Adults**
3	0	100	0	60
13	0	0	0	0
17	0	0	0	40
20	0	0	0	0
21	0	0	0	0
23	0	0	0	0
26	0	0	0	0
28	0	0	0	0
Geometric Mean	**0**	**0.8**	**0**	**1.7**

APPENDIX 1.3.3 TOTAL ABOMASAL WORM COUNTS IN THE AFTER TREATMENT WITH FORMULATION 2

	Ostertagia spp.			*Trichostrongylus axei*
Calf Number	**Adults**	**Early L4**	**LL4**	**Adults**
1	0	0	20	0
2	0	120	0	240
4	0	0	0	0
10	0	20	0	140
12	0	0	0	0
24	0	0	0	20
27	0	20	0	0
29	0	0	0	0
Geometric Mean	**0**	**2.9**	**0.5**	**4.4**

APPENDIX 1.3.4 TOTAL SMALL INTESTINAL WORM COUNTS IN THE CONTROL GROUP

	Cooperia spp.			*Trichostrongylus* spp.
Calf Number	**Adults**	**Early L4**	**Late L4**	**Adults**
6	100	60	0	0
7	28220	9100	700	0
8	20	0	0	20
14	3260	1220	40	60
16	13220	1500	20	0
18	4700	80	0	0
19	8480	27060	1060	0
22	6940	960	140	180
Geometric Mean	**2272.3**	**463.1**	**22.4**	**3.7**

APPENDIX 1.3.5 TOTAL SMALL INTESTINAL WORM COUNTS IN CALVES AFTER TREATMENT WITH FORMULATION 1

	Cooperia spp.			*Trichostrongylus* spp.
Calf Number	**Adults**	**Early L4**	**Late L4**	**Adults**
3	0	520	20	0
13	3980	0	0	0
17	0	0	0	0
20	3120	20	0	0
21	420	20	0	0
23	20	0	0	0
26	40	0	0	0
28	680	4920	280	0
Geometric Mean	**85.2**	**12.5**	**2.0**	**0.0**

APPENDIX 1.3.6 **TOTAL SMALL INTESTINAL WORM COUNTS IN CALVES AFTER TREATMENT WITH FORMULATION 2**

	Cooperia spp.			*Trichostrongylus* spp.
Calf Number	**Adults**	**Early L4**	**Late L4**	**Adults**
1	280	0	0	0
2	9600	10840	120	0
4	0	0	0	0
10	5140	3620	820	0
12	10800	77000	440	0
24	3160	20	0	0
27	280	0	0	0
29	240	4800	0	0
Geometric Mean	**649.6**	**152.2**	**8.0**	**0.0**

APPENDIX 1.3.7 TOTAL LARGE INTESTINE WORM COUNTS IN THE CONTROL GROUP

	Oesophagostomum radiatum	*Trichuris* sp.
Calf Number	**Adults**	**Adults**
6	0	5
7	35	0
8	0	0
14	0	0
16	0	0
18	0	0
19	0	0
22	0	15

No statistical analyses able to be performed due to insufficient worm numbers.

APPENDIX 1.3.8 TOTAL LARGE INTESTINE WORM COUNTS IN CALVES AFTER TREATMENT WITH FORMULATION 1

	Oesophagostomum radiatum	*Trichuris* sp.
Calf Number	**Adults**	**Adults**
3	0	0
13	0	0
17	0	0
20	0	0
21	0	0
23	0	0
26	0	0
28	0	0

No statistical analyses able to be performed due to insufficient worm numbers.

APPENDIX 1.3.9 TOTAL LARGE INTESTINE WORM COUNTS IN CALVES AFTER TREATMENT WITH FORMULATION 2

	Oesophagostomum radiatum	*Trichuris* sp.
Calf Number	**Adults**	**Adults**
1	0	0
2	0	0
4	0	0
10	0	0
12	0	5
24	0	0
27	0	0
29	0	0

No statistical analyses able to be performed due to insufficient worm numbers.

APPENDIX 1.3.10 SPECIATION OF ABOMASAL WORMS FROM CALVES IN THE CONTROL GROUP

Calf Number	*Ostertagia ostertagi*	*Ostertagia lyrata*	*Trichostrongylus axei*
6	122/125	3/125	11/11
7	106/107	1/107	114/114
8	93/101	8/101	104/104
14	34/36	2/36	-
16	13/13	-	70/70
18	93/100	7/100	102/102
19	93/102	9/102	68/68
22	19/22	3/22	48/48

APPENDIX 1.3.11 SPECIATION OF ABOMASAL WORMS FROM CALVES TREATED WITH FORMULATION 1

Calf Number	*Ostertagia ostertagi*	*Ostertagia lyrata*	*Trichostrongylus axei*
3	-	-	1/1
13	-	-	-
17	-	-	1/1
20	-	-	-
21	-	-	-
23	-	-	-
26	-	-	-
28	-	-	-

APPENDIX 1.3.12 SPECIATION OF ABOMASAL WORMS FROM CALVES TREATED WITH FORMULATION 2

Calf Number	*Ostertagia ostertagi*	*Ostertagia lyrata*	*Trichostrongylus axei*
1	-	-	-
2	-	-	5/5
4	-	-	-
10	-	-	4/4
12	-	-	-
24	-	-	1/1
27	-	-	-
29	-	-	-

APPENDIX 1.3.13 SPECIATION OF SMALL INTESTINAL WORMS FROM CALVES IN THE CONTROL GROUP

Calf Number	*Cooperia oncophora*	*Cooperia mcmasteri*	*Cooperia punctata*
6	-	-	-
7	113/132	19/132	-
8	-	-	-
14	49/54	5/54	-
16	87/105	18/105	-
18	46/53	3/53	4/53
19	78/90	9/90	3/90
22	119/136	16/136	1/136

APPENDIX 1.3.14 SPECIATION OF SMALL INTESTINAL WORMS FROM CALVES TREATED WITH FORMULATION 1

Calf Number	*Cooperia oncophora*	*Cooperia mcmasteri*	*Cooperia punctata*
3	-	-	-
13	23/24	1/24	-
17	-	-	-
20	34/38	4/38	-
21	3/3	-	-
23	1/1	-	-
26	1/1	-	-
28	7/7	-	-

APPENDIX 1.3.15 SPECIATION OF SMALL INTESTINAL WORMS FROM CALVES TREATED WITH FORMULATION 2

Calf Number	*Cooperia oncophora*	*Cooperia mcmasteri*	*Cooperia punctata*
1	-	-	-
2	99/111	12/111	-
4	-	-	-
10	90/105	15/105	-
12	55/65	10/65	-
24	12/16	4/16	-
27	2/2	-	-
29	2/2	-	-

APPENDIX 2.1 PEPSINOGEN ASSAY

Pepsinogen concentrations were determined by converting the proenzyme to pepsin with 0.1 M hydrochloric acid, and measuring the amount of tyrosine released from serum protein.

Into duplicate new 5ml plastic tubes was added 1.5 ml of 0.1 M HCl and 1 ml of distilled water. To the control tube was added 2 ml of 10% trichloroacetic acid (TCA) and the tube was vortexed. 0.5ml of sample was added to both the control and the test tubes and each was vortexed immediately. Tubes were covered with cling film and the test tube was incubated for three hours at 37^0 C before the addition of TCA. Both duplicates were allowed to stand for 30 minutes and revortexed just prior to centrifugation at 3500 rpm for 10 minutes. From each duplicate, 2 ml of supernatant was removed and added to 4 ml of 0.5 M NaOH. A further 1 ml of Folin-Ciocalteau reagent was added to each of the duplicates. After 6 minutes of colour development, absorbence was measured at 700 nm using a SP6-550 UV/VIS Pye Unicam spectrophotometer. The amount of tyrosine present was equated with absorbence by use of a standard curve. The difference between the control and test tubes was attributed to the pepsin activity from serum pepsinogen. Sample pepsinogen activity was expressed as IU tyrosine/litre of serum. This is defined as the quantity of enzyme in one litre of serum to release 1 micromole of tyrosine from albumin at 37^0 C and at pH 1.5-2.1 (optimal pH for pepsin) in a reaction period of one minute.

The standard curve was linear and was derived by reading the absorbence of solutions of known concentrations of tyrosine. The minimum standard (0 micromoles) was made by adding 2 ml of distilled water to 4 ml of 0.5 N NaOH. The maximum standard (100 micromoles) was made by adding 0.8 ml of 10% TCA, 0.2 ml of distilled water and 1 ml of tyrosine stock solution to 4 ml of 0.5 M NaOH.

Standard curve for pepsinogen assay

Stock Tyrosine Dilution	Absorbence
0	0
0.1	0.035
0.2	0.073
0.3	0.108
0.4	0.142
0.5	0.179
0.6	0.212
0.7	0.243
0.8	0.281
0.9	0.318
1.0	0.334

The pepsinogen activity of the sample and serum controls was calculated as follows:

$$\frac{\text{Optical Density Test - Optical Density Control}}{\text{Optical Density Standard - Optical Density Blank}} \times \frac{0.2^{1}}{} \times \frac{1000}{0.2^{2}} \times \frac{1}{180}$$

= **IU tyrosine / litre at 37^{0} C**

180 = time (minutes of incubation)

^{1} = moles tyrosine in standard

^{2} = volume of sample coloured by reagent

APPENDIX 2.2.1 MEAN SERUM PEPSINOGEN CONCENTRATIONS (IU TYROSINE/LITRE) FOR THE CONTROL GROUP OF CALVES

	Day of sampling															
Calf Number	**-4**	**-2**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**	**+14**
6	2.54	3.43	2.71	3.03	1.81	2.66	2.44	2.79	1.87	1.66	2.22	1.59	1.34	1.62	1.72	0.87
7	0.70	0.85	0.54	0.67	0.67	0.65	1.24	1.10	1.07	1.13	0.78	1.44	1.18	1.23	0.88	0.93
8	1.72	2.01	2.34	1.94	1.57	1.49	3.28	0.79	2.02	2.09	1.69	2.14	1.97	2.04	ND	ND
14	2.22	2.66	1.69	1.49	1.14	1.00	1.66	1.66	1.40	1.51	0.45	1.71	ND[1]	1.25	1.44	0.79
16	2.74	2.79	1.89	1.56	1.34	0.82	0.77	1.15	0.92	0.72	0.65	0.89	0.50	1.00	0.84	0.54
18	1.79	2.51	0.99	1.32	1.14	1.37	1.20	1.91	1.56	1.61	1.69	1.37	1.06	0.79	1.02	0.94
19	2.67	3.09	2.50	1.75	1.74	1.47	1.37	2.03	2.60	1.85	1.98	1.93	1.10	0.97	0.70	0.64
22	1.39	1.49	1.54	1.29	1.17	1.00	1.32	1.05	1.44	1.30	0.88	1.56	2.04	1.54	1.00	0.37
Mean	**1.97**	**2.35**	**1.77**	**1.63**	**1.32**	**1.31**	**1.66**	**1.56**	**1.61**	**1.48**	**1.28**	**1.58**	**1.31**	**1.31**	**1.09**	**0.72**
Standard Deviation	0.71	0.86	0.75	0.68	0.38	0.63	0.81	0.67	0.54	0.43	0.68	0.38	0.54	0.41	0.37	0.22

[1] ND Not analysed

APPENDIX 2.2.2 MEAN SERUM PEPSINOGEN CONCENTRATIONS (IU TYROSINE/LITRE) FOR THE CALVES TREATED WITH FORMULATION 1

	Day of sampling															
Calf Number	**-4**	**-2**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**	**+14**
3	2.66	2.98	1.58	1.52	1.82	1.45	1.23	1.44	0.96	0.97	1.23	0.94	0.72	0.45	0.72	0.51
13	2.01	2.01	1.34	1.50	1.69	1.80	0.89	2.06	1.48	2.38	0.75	1.88	1.56	1.63	1.71	1.10
17	1.23	1.44	1.58	0.89	0.99	0.83	1.10	1.51	0.96	0.77	0.75	0.99	0.65	1.05	0.59	0.73
20	2.02	1.62	1.42	1.61	1.37	1.35	1.24	ND[1]	0.85	0.97	ND	1.00	0.82	0.90	0.59	0.67
21	0.16	1.31	1.00	0.88	0.94	1.13	0.69	0.81	1.31	1.16	0.50	0.10	1.29	0.75	0.86	0.72
23	0.97	1.40	0.81	1.04	0.86	0.80	0.85	1.02	0.24	0.65	1.50	0.43	0.32	0.75	0.69	0.35
26	1.77	1.79	1.15	0.80	0.99	0.89	1.67	0.75	1.64	1.35	1.74	1.71	0.87	1.32	0.75	0.99
28	1.54	1.96	0.82	0.84	0.90	0.60	0.46	0.70	0.62	0.55	0.85	0.57	0.44	0.18	0.21	0.28
Mean	**1.67**	**1.81**	**1.21**	**1.13**	**1.20**	**1.11**	**1.02**	**1.19**	**1.01**	**1.10**	**1.04**	**1.05**	**0.83**	**0.88**	**0.77**	**0.67**
Standard Deviation	0.56	0.54	0.31	0.35	0.38	0.40	0.38	0.50	0.46	0.58	0.45	0.50	0.42	0.46	0.42	0.29

[1] ND Not analysed

APPENDIX 2.2.3 MEAN SERUM PEPSINOGEN CONCENTRATIONS (IU TYROSINE/LITRE) FOR THE CALVES TREATED WITH FORMULATION 2

	Day of sampling															
Calf Number	**-4**	**-2**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**	**+14**
1	0.81	1.02	0.81	1.15	1.79	1.51	0.80	0.88	2.10	1.24	1.00	1.32	1.08	1.13	1.07	ND
2	4.11	4.61	2.02	2.27	1.92	2.57	1.44	1.66	1.44	2.09	0.94	1.62	1.12	1.00	1.02	0.79
4	2.84	2.76	1.62	1.49	1.59	1.32	0.80	1.30	0.94	1.15	1.02	1.09	0.77	0.82	1.12	0.69
10	1.47	1.20	1.20	0.82	0.92	0.85	1.21	0.80	0.85	0.89	0.86	1.09	1.20	0.22	0.37	ND
12	1.42	1.82	0.57	0.74	0.67	0.79	ND[1]	0.93	1.10	0.74	0.26	0.54	ND	0.80	0.54	0.40
24	2.74	3.33	1.89	1.32	1.67	2.02	1.28	1.71	1.12	1.82	2.38	1.64	1.40	1.04	1.19	ND
27	3.03	2.84	2.19	1.66	1.47	1.05	0.96	1.13	1.05	1.42	1.55	1.20	1.12	1.20	1.31	ND
29	1.31	1.51	0.77	0.79	0.67	0.37	0.54	1.26	0.70	0.78	0.83	0.16	0.70	0.29	0.32	0.35
Mean	**2.22**	**2.39**	**1.38**	**1.28**	**1.34**	**1.31**	**1.00**	**1.21**	**1.12**	**1.27**	**1.10**	**1.08**	**1.06**	**0.81**	**0.87**	**0.56**
Standard Deviation	1.13	1.23	0.63	0.53	0.51	0.72	0.32	0.34	0.41	0.49	0.62	0.51	0.25	0.37	0.39	0.21

[1] ND Not analysed

APPENDIX 3.1 GASTRIN RADIOIMMUNOASSAY

This is a modification of the method of (Hansky and Cain, 1969) by (Simpson *et al.*1993).

Assay Buffer: 0.02 M Veronal buffer pH 8.6 containing per litre:

- 4.12 g Na barbiturate
- 0.744 g barbitone
- 5 g bovine serum albumin
- 100 mg thimerosal
- 10 mg neomycin

Tracer: Synthetic non-sulphated human G-17 (Research Plus, Bayanne, NJ, USA) was labelled with I^{125} using the chloramine T method. The label was purified on a Sephadex G10 column followed by separation on a Diethylaminoethyl cellulose column with a NaCl gradient from 0 to 1 M. The tracer for the assay contained 1200-1600 cpm per 500 µl.

Antiserum: Antibody (Ab) 74 (the kind gift of Dr Hansky) which binds equally with human, porcine and ovine sulphated and non-sulphated G14, G17 and G34 was used in a final dilution of 1:100,000 with 1:400 normal rabbit serum (NRS). The solution for the assay contained 1:250 NRS and 1:40000 Ab.

Standards: Synthetic non-sulphated human G-17 was made up in assay buffer in concentrations of 0, 2, 5 10, 20, 50, 100 and 200 pM.

Second antibody: Serum from sheep immunized with rabbit gamma globulin was used as the precipitating antibody. This was standardized against Donkey anti-rabbit globulin (IDS, England).

Assay procedure: Assays were conducted in triplicate.

Assay tubes contained:

(i) Totals - 500 µl tracer
(ii) Non Specific Binding - 100 µl buffer, 400 µl NRS without Ab, 500 µl tracer
(iii) Standards - 100 µl standard solution, 400 µl Ab, 500 µl tracer
(iv) Samples - 100 µl sample, 400 µl Ab, 500 µl tracer

The tubes were incubated for 2 days at 4°C. 200 µl of the second Ab was added to all tubes except

(i) and incubated for a further 3 days at 4°C. Tubes were centrifuged at 2000 rpm for 30 minutes, the supernatant discarded and the pellet counted for 5 minutes in a gamma counter. Gastrin concentrations were determined from a standard curve and expressed as pM.

APPENDIX 3.2.1 **MEAN SERUM GASTRIN CONCENTRATIONS (PICOMOLES/LITRE) FOR THE CONTROL GROUP OF CALVES**

	Day of sampling															
Calf Number	**-4**	**-2**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**	**+14**
6	153	145	183	164	191	117	135	118	111	104	99	119	95	86	93	77
7	149	141	159	165	205	237	261	178	199	286	320	230	200	176	121	145
8	147	189	201	174	172	181	160	139	111	126	99	119	95	86	93	77
14	204	207	217	189	148	172	147	121	121	96	88	92	81	ND	85	ND
16	234	338	305	248	198	228	252	224	150	100	136	107	146	140	77	69
18	185	191	251	293	263	251	210	171	161	233	129	135	122	87	66	62
19	169	307	375	372	444	216	180	96	95	95	88	63	63	39	34	29
22	151	208	181	225	ND[1]	144	ND	134	99	82	87	63	63	ND	53	42
Mean	**174**	**216**	**234**	**229**	**232**	**193**	**192**	**147**	**130**	**140**	**130**	**116**	**108**	**88**	**78**	**71**
Standard Deviation	32	71	73	74	100	48	50	41	36	76	79	53	47	48	27	37

[1] ND Not analysed

APPENDIX 3.2.2 **MEAN SERUM GASTRIN CONCENTRATIONS (PICOMOLES/LITRE) FOR THE CALVES TREATED WITH FORMULATION 1**

	Day of sampling															
Calf Number	**-4**	**-2**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**	**+14**
3	334	ND[1]	347	201	129	131	129	148	140	81	80	63	65	50	44	55
13	90	75	83	74	52	52	56	55	47	50	43	47	44	43	45	41
17	72	77	50	47	46	53	ND	44	50	42	45	47	39	37	29	38
20	239	226	207	174	158	119	122	78	83	46	42	61	45	58	35	24
21	75	88	56	67	57	49	52	35	30	30	35	37	36	48	38	40
23	219	226	ND	363	226	262	189	153	98	74	78	68	70	53	47	42
26	163	159	252	239	253	172	166	99	83	62	56	51	54	43	48	49
28	117	159	118	143	149	146	108	76	68	45	40	33	47	ND	45	33
Mean	**164**	**144**	**159**	**163**	**134**	**123**	**117**	**86**	**74**	**54**	**52**	**50**	**50**	**47**	**41**	**40**
Standard Deviation	94	66	113	106	79	73	51	45	35	17	18	13	12	7	7	10

[1] ND Not analysed

APPENDIX 3.2.3 MEAN SERUM GASTRIN CONCENTRATIONS (PICOMOLES/LITRE) FOR THE CALVES TREATED WITH FORMULATION 2

	Day of sampling															
Calf Number	**-4**	**-2**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**	**+14**
1	149	136	112	185	223	99	213	233	151	158	135	93	62	64	68	45
2	ND[1]	264	251	ND	280	254	202	150	96	84	98	85	85	69	68	65
4	382	196	213	213	190	171	117	79	74	72	66	65	57	65	29	67
10	260	352	228	227	198	211	238	144	212	80	90	97	89	82	84	38
12	167	163	191	216	213	130	152	143	81	77	83	72	66	64	53	48
24	203	102	188	208	136	242	159	128	92	66	53	54	45	44	42	36
27	223	285	320	179	119	87	77	70	76	49	51	48	50	ND	42	35
29	271	182	193	158	128	ND	71	68	80	39	53	88	114	109	114	59
Mean	**236**	**210**	**212**	**198**	**186**	**171**	**154**	**127**	**107**	**78**	**78**	**75**	**71**	**71**	**62**	**49**
Standard Deviation	78	84	60	25	55	68	62	55	49	36	29	18	23	20	27	13

[1] ND Not analysed

APPENDIX 3.3 ABOMASAL AND PROXIMAL DUODENAL TISSUE GASTRIN CONCENTRATIONS (PICOMOLES/GRAMME) IN CONTROL AND MOXIDECTIN TREATED CALVES

Calf Number	Abomasum - Antrum (picomoles/gramme)	Proximal Duodenum (picomoles/gramme)
Control		
6	609	36.1
7	1471	38.5
8	931	11.9
14	1025	30.8
16	926	28.0
18	1067	38.6
19	1939	48.1
22	1221	26.2
Mean (+/- Standard deviation)	**1149 (+/-404)**	**32.3 (+/- 10.8)**
Formulation 1		
3	1218	78.2
13	1427	17.4
17	944	42.7
20	1032	19.9
21	990	49.0
23	2275	42.7
26	1072	11.3
28	1050	37.5
Mean (+/- Standard deviation)	**1251 (+/- 441)**	**37.3 (+/- 21.5)**
Formulation 2		
1	925	63.4
2	1998	61.1
4	625	62.8
10	1640	49.5
12	1370	71.6
24	1286	19.3
27	973	49.7
29	1769	30.2
Mean (+/- Standard deviation)	**1323 (+/- 467)**	**50.9 (+/- 17.9)**
Statistical Significance	**NS[1]**	**NS**

[1] NS Not significant at P<0.01