The pharmacology of halogenated salicylanilides and their anthelmintic use in animals

G E Swan[a]

ABSTRACT

The halogenated salicylanilides are a large group of compounds developed mainly for their antiparasitic activity in animals. Several halogenated salicylanilides with potent antiparasitic activity have been synthesised of which only closantel, niclosamide, oxyclozanide, rafoxanide and resorantel are commercially available. Closantel and rafoxanide, which represent the most important drugs in the group, are used extensively for the control of *Haemonchus* spp. and *Fasciola* spp. infestations in sheep and cattle and *Oestrus ovis* in sheep in many parts of the world. Niclosamide is used extensively for its anticestodal activity in a wide range of animals. Antiparasitic activity of the halogenated salicylanilides has also been demonstrated against a large number of other internal parasites, in particular haematophagous helminths, and external parasites including ticks and mites, in a variety of animal species. Several cases of toxicity and mortality have been reported for closantel and rafoxanide in sheep and goats. Their unique pharmacokinetic behaviour appears to play an important role in the efficacy and safety of these compounds. The chemical and physical characteristics, mode of action, pharmacokinetics, antiparasitic activity and toxicity of the halogenated salicylanilides in animals are reviewed.

Key words: animals, antiparasitic activity, halogenated salicylanilides, pharmacology, review, safety.

Swan G E **The pharmacology of halogenated salicylanilides and their anthelmintic use in animals.** *Journal of the South African Veterinary Association* (1999) 70(2): 61–70 (En.). Department of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

INTRODUCTION

Salicylanilides are a very large group of compounds, originally developed as fungicides for topical use and as antimicrobial agents in soaps[64]. Later these compounds were shown to possess potent anthelmintic activity of which the niclosamides[54] tribromsalans[8] and clioxanide[9] were some of the 1st agents to be used. Since then, a wide range of halogenated salicylanilide congeners active against helminth parasites have been synthesised[11,12,32,37,66,76,102,110,126]. The halogenated salicylanilides available in South Africa are summarised in Table 1[113].

Halogenated salicylanilides, in particular closantel and rafoxanide, are important anthelmintics that are used extensively in the control of *Haemonchus* spp. and *Fasciola* spp. infestation in sheep and cattle, and *Oestrus ovis* in sheep in many parts of the world. Niclosamide is widely used for the treatment and control of cestode infections in several animal species. Despite being available over many years and the extensive use of these products, no definitive review of these products has been published.

CHEMICAL AND PHYSICAL CHARACTERISTICS

Chemical structure

Salicylanilides, also referred to as benzamides or salicylamides[54], are weak, acidic phenolic compounds. The basic chemical structure consists of a salicylic acid ring and an anilide ring (Fig. 1).

The structural criteria for fasciolicidal action has been described as the need for electron-withdrawing substitutes on both the salicylic and anilide rings, together with a lipophilic group, such as *tert*-butyl in the 3-position[66]. Improved fasciolicidal activity of salicylanilides has been achieved in compounds such as closantel and rafoxanide by the incorporation of an aryl chain in the aniline moiety of the anilide[12]. In both drugs the active pharmacophore is 3,5-diiodosalicyloyol[110].

Depending on the type of structural substitution, salicylanilides have been subdivided into a number of different groups, including halogenated and non-halogenated derivatives, acetylsalicylanilides, dichlorosalicylanilides and benzoylsalicylanilides. Acetylsalicylanilide derivatives, *e.g.* clioxanide, are activated *in vivo*. This is presumably achieved by hydrolysis of the acetyl group to free the hydroxyl derivative[38].

All salicylanilides developed for use as anthelmintics in animals are halogenated and include: clioxanide (*N*-(4′-chlorophenol)-3,5-diiodo-acetylsalicylamide); closantel (*N*-(5-chloro-4[(4-chlorophenyl) cyano-methyl]-2-methylphenyl)-2-hydroxy-3,5-diiodo-benzamide), dibromsalan (4′,5′-dibromosalicylanilide); oxyclozanide (3,3′,5,5′,6-pentachloro-2-hydroxysalicylanilide), niclosamide (5-chloro-N-(2-chloro-4-nitrophenyl-2-hydroxysalicylanilide); rafoxanide (3′-chloro-4′-(*p*-chlorophenoxy)-3,5-diiodosalicyl-

Table 1: **Halogenated salicylanilides registered for use in animals in South Africa.**

Drug	Trade names	Manufacturer/Supplier
Closantel	Flukiver, Seponver	Janssen AH
	Pro-inject Yellow, Prodose Yellow,	
	Ranide Super, Vetdose 4, Vetdose 4 injectable	Logos Agvet
Oxyclozanide	Milborrow Liverfluke Remedy	Bayer AH
Niclosamide	Ex-A-Lint	Hoechst Roussel Vet
	Lintex	Bayer AH
	Prodose Lint	Logos Agvet
Rafoxanide	Nasalcur	Hoechst Roussel Vet
	Ovinox	Bayer AH
	Ranide	Logos Agvet
	Ranox	Pfizer AH
Resorantel	Terenol-S	Hoechst Roussel Vet

[a]Department of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

Received: December 1998. Accepted: May 1999.

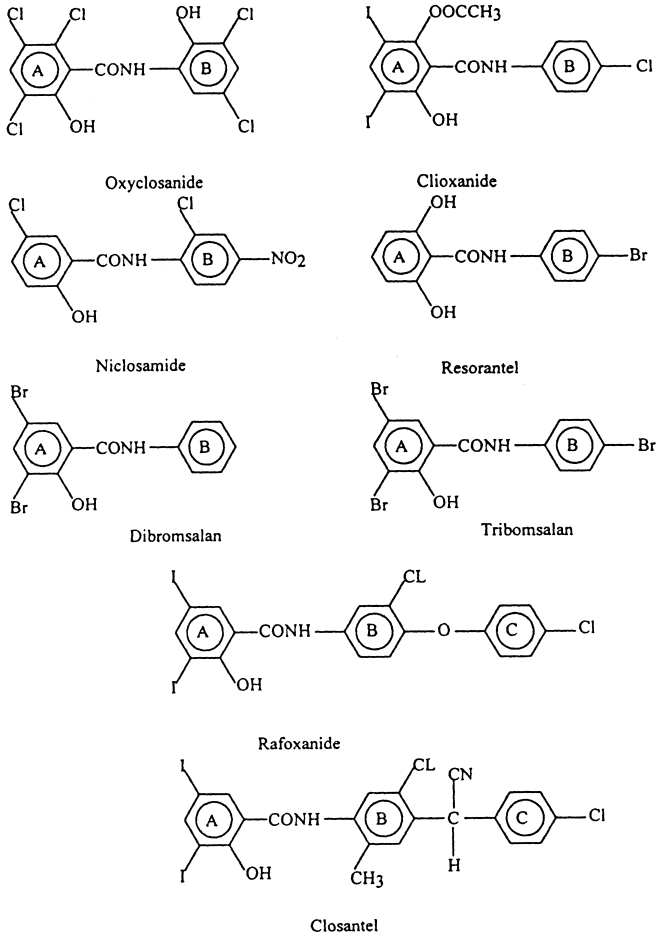

Fig. 1: **Chemical structures of the halogenated salicylanilides developed for use as anthelmintics in animals**[1,14].

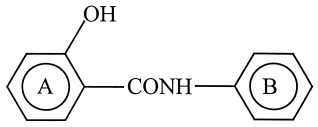

Fig. 2: **Basic structure of salicylanilides: salicylic ring** (A) **and anilide ring** (B)**.**

anilide); resorantel (*N*-(4′-bromophenyl)-6-hydroxysalicylamide) and tribromsalan (3,4′,5-tri-bromosalicylanilide). The chemical structures of these compounds are given in Fig.2.[1,14].

Crystal structure and polymorphic forms

The 2 aromatic rings of the salicylanilide moeity are approximately co-planar with a dihedral angle between them[101]. There are significant conformational differences between the various halogenated salicylanilides and analogues regarding their crystalline forms. Interatomic dimensions in the salicylanilide moiety are consistent within the halogenated salicylanilides. The crystalline forms of closantel have not been reported.

Polymorphic forms of salicylanilides have been characterised by X-ray powder diffraction for oxyclozanide[82]. The polymorphic forms exhibit variable behaviour, both towards aqueous solubility and stability in suspension. Absorbed or occluded impurities are thought to play an important role in the polymorphic behaviour of the drug.

Anthelmintic activity of rafoxanide is inversely related to the size of crystalline particles present in a 2.5 % oral suspension[4].

Physicochemical characteristics

The salicylanilides are generally weakly acidic, highly lipid-soluble compounds. Very little information on the physicochemical characteristics of the individual salicylanilides has been reported. Of the salicylanilide group, rafoxanide and closantel are the most widely used and are therefore more extensively described in literature. Rafoxanide has a molecular weight of 626.01 and a melting point of 173–7 °C[2]. It is a greyish-white crystalline powder, moderately soluble in acetone, chloroform, ethyl acetate, acetonitrile and methanol but insoluble in water[76].

Ultraviolet light absorption of a 0.004 % w/v solution in 0.1 M methanolic hydrochloric acid is in the range 230–350 nm and exhibits maximal excitation at 280 nm and 335 nm. Absorbance at 280 nm is about 0.97 and at 335 nm, about 0.59 [2].

Closantel has a molecular weight of 663.07[14]. It is weakly acidic (pKa = 4.28) and is highly lipid-soluble (log P = 7.15, *n*-octanol/buffer pH 6.98)[72].

MODE OF PHARMACOLOGICAL ACTION

The primary action of salicylanilides has generally been associated with the uncoupling of oxidative phosphorylation. Early *in vitro* studies, using houseflies as well as rat liver mitochondria, showed several salicylanilides as potent inhibitors of this electron transport associated phosphorylation[127].

Salicylanilides were approximately 1000–10000 times more potent than dinitrophenol, an earlier compound known to inhibit oxidative phosphorylation. *In vitro* activity of inhibition of electron transport associated phosphorylation in *Fasciola hepatica* and *Ascaris lumbricoides* were later reported for oxyclozanide, rafoxanide and closantel[24,25,26,62,121,123]. Several workers have subsequently confirmed the proposed mechanism *in vivo*[25,87,123].

In vitro inhibition of succinate dehydrogenase activity with a wide range of salicylanilides[38] and inhibition of the fumarate reductase system by oxyclozanide and rafoxanide in *F. hepatica* (unpublished information as cited in Coles[23]) have also been reported. These effects may be due to the interrelationship of the succinate dehydrogenase activity to the fumarate reductase system and oxidative phosphorylation. It has been suggested that rafoxanide diminishes ATP-synthesis, resulting in increased internal intermediate pools in the pathway. Later, as an independent effect, the further metabolism of succinate is inhibited.

According to the chemiosmotic theory of Mitchell[73], hydrogen-ionophores act by uncoupling of phosphorylation by the translocation of protons through the inner mitochondrial membrane. The maintenance of the proton gradient across the inner mitochondrial membrane is essential for the continued production of ATP. The inner mitochondrial membrane is normally impermeable to protons, but can be rendered permeable by the addition of uncouplers of oxidative phosphorylation that act by destroying the proton gradient. This activity selectively prevents the utilisation of the chemical energy derived from electron transport for the net phosphorylation of ADP to ATP, thus depriving the cell of its normal source of energy[103].

Closantel was shown to have *in vitro* and *in vivo* effects on both the motility and ultrastructure of *F. hepatica*[103]. It induces rapid spastic paralysis of the fluke, severe

Table 2: **Summary of pharmacokinetic parameters reported for closantel, rafoxanide and oxyclozanide in sheep and cattle.**

Drug and species		Dosage		Pharmacokinetic parameter				Reference
	n	Dose (mg/kg)	Route[a]	AUC (µg.d/mℓ)	Cmax (µg/mℓ)	Tmax (h)	T½e*l* (d)	
Closantel — sheep	4	7.5[b]	i.r.	786 ± 335	32 ± 9	64.8 ± 21.6	14.3 ± 1.9	55
	4	3.75[b]	i.m.	575 ± 278	31 ± 4	12 ± 12	12.2 ± 4.6	
	5	10	p.o.	1303 ± 447	47 ± 11	24	23	72
	5	5	i.m.	1027 ± 101	48 ± 4	8–24	16	
	3	5	p.o.	852 ± 215	22 ± 5	–	–	
	3	5	i.m.	1497 ± 428	55 ± 10	–	–	
	3	10[c]	p.o.	1541 ± 272	48 ± 6	–	–	
	3	10[d]	p.o.	1578 ± 223	51 ± 3	–	–	
	5	7.5	p.o.	1035 ± 212	48 ± 5	–	14.5 ± 2.3	75
— cattle	3	10	p.o.	1108 ± 112	51 ± 3	48	–	72
	3	5	i.m.	1205 ± 323	54 ± 5	48	–	
	6	2.5	i.m.	756 ± 333	28 ± 7	–	–	
	3	5	i.m.	1205 ± 323	54 ± 5	–	–	
	3	10[c]	p.o.	1118 ± 327	48 ± 6	–	–	
	3	10[d]	p.o.	1108 ± 112	51 ± 3	–	–	
Rafoxanide — sheep	5	7.5	p.o.	605 ± 79	23 ± 2	–	16.6 ± 1.2	75
	8	15	p.o.	203 ± 74	26±7	39 ± 14	6.5 ± 1.5	114
	10	7.5	p.o.	414 ± 106	35±5	26 ± 2.4	7.5 ± 2.0	115
Oxyclozanide — sheep	5	15	p.o.	51±8	19.0 ± 2.3	–	6.4 ± 0.8	75

[a]p.o. = orally; i.r. = intraruminal; i.m. = intramuscular.
[b]^{14}C-closantel.
[c]Solution; [d]suspension.

sloughing of the integument, swelling of the basal infoldings, mitochondrial deformation and a reduced output of secretory products, especially in the tegumental and gastrodermal cells. The spastic paralysis seen with closantel is similar to that observed with rafoxanide and oxyclozanide[41]. Although these changes have been ascribed to uncoupling of oxidative phosphorylation, the increased muscle tone may be due to an increase in calcium ions within the muscle cells of the fluke[103].

Closantel has also been shown to rapidly decrease intrategumental pH in *Schistosoma mansoni* and *F. hepatica* at concentrations that were lower than those that affect ATP concentration[80]. The sensitivity of flukes to uncoupling agents may be due to the fact that Kreb's cycle activity is restricted to the 'outer layer' of the fluke[119].

PHARMACOKINETICS

Very few pharmacokinetics studies, other than metabolic studies, have been reported for salicylanilides in ruminants[30,31,55,72,75,114,115]. These pharmacokinetic studies involve only closantel, oxyclozanide and rafoxanide. No intravascular pharmacokinetic studies have been reported.

Halogenated salicylanilides generally share common pharmacokinetic features[70]. The anthelmintic activity of these compounds has been directly associated with their pharmacokinetic profile[6,47,66,72,75,86]. A summary of the main pharmacokinetic parameters reported for closantel, rafoxanide and oxyclozanide in sheep and cattle are given in Table 2.

These parameters were derived from both model-independent procedures and non-linear compartmental analysis. The elimination rate constants and half-lives of closantel were determined by a single exponential compartmental model in 2 of the studies[55,72], whereas in 1 study a tri-exponential equation determined the best-fitted curves for closantel, oxyclozanide and rafoxanide[75].

A lag time for oral absorption was included in the model in one of the rafoxanide studies evaluated by a single exponential compartment[114].

Absorption

Following oral administration of closantel and rafoxanide in ruminants, maximum plasma concentrations (Cmax) are reached 24–48 h later[7,114,115]. Hennessy *et al.*[55] reported a longer time of 65 h to maximum plasma concentrations (Tmax), indicating a slower rate of absorption of closantel administered intraruminally in sheep. The rate of gastrointestinal absorption of closantel is slower in goats than in sheep, most likely due to a reduced digesta flow rate[55].

Reduced digesta flow rate resulted in significantly lower peak plasma concentrations and extent of absorption, as measured by area under the drug concentration *versus* time curve (AUC) of rafoxanide administered orally in grazing lambs compared to housed lambs fed hay and concentrates[118]. Peak rafoxanide plasma concentrations were significantly higher in sheep infected with 6-week-old *F. hepatica* as compared to uninfected sheep[75]. A 2–3-fold increase in the bioavailability of rafoxanide was reported in suckling lambs, aged 5–8 weeks of age compared to weaned lambs aged 4–5 months[115].

In ruminants the oral bioavailability of closantel relative to intramuscular administration is approximately 50 %[72]. A similar relative oral bioavailability, based on the difference in efficacy against 6-week-old *F. hepatica*, after oral (36.1 %) and intramuscular (60.4 %) administration at 3 mg/kg, has been proposed for rafoxanide in cattle[75].

The extent and rate of absorption of closantel is dose independent[72]. A linear increase in closantel plasma concentrations was reported in cattle and sheep at doses between 2.5 mg/kg and 10 mg/kg. Niclosamide is restricted to the gastrointestinal tract due to very poor absorption following oral administration[20].

The activity of clioxanide in sheep is reduced if it is passed directly into the abomasum[9,117,123]. It is suggested that deacetylation of clioxanide takes place in the rumen to form a hydroxyl derivative that is more readily absorbed[86,87]. Efficacy of clioxanide is reduced if administered into the abomasum, most likely due to slow deacetylation affecting the extent and rate of absorption, with reduced

persistence of the active constituents in blood. Acetylsalicylanilide derivatives such as clioxanide show poor anthelmintic activity *in vitro,* but *in vivo* are potent anthelmintics[38].

Distribution

Halogenated salicylanilides are extensively plasma-bound and poorly distributed to tissues. Michiels *et al.*[72] reported the ratio of closantel in the plasma relative to that in the lung and kidney tissue is 6–7; 9–12 for heart and liver; 30 for muscle; and about 100 for fat. A plasma to liver concentration ratio of 17.5 for rafoxanide; and a plasma to bile ratio for closantel and rafoxanide of >50 % and 35 %, respectively, have been reported[75].

Extensive binding to plasma albumin (>97 %) has been reported for both closantel and rafoxanide[72,75]. The binding to plasma albumin is characterised by very high affinity and high capacity. Closantel-binding to erythrocytes (*c.* 4 %) and in plasma water (1 %) is limited[72].

The extensive plasma binding of salicylanilides is responsible for the long elimination half-life (T½*el*) associated with these products[75,86,88]. A T½*el* of 2–3 weeks has been reported for closantel and rafoxanide in sheep and cattle, and 6 days for oxyclozanide in sheep[30,31,55,72,75]. The T½*el* for closantel in different animal species varies from 6 days in goats, 1 week in the rat and 10 days in the dog[55,72]. Faster elimination resulted in an almost 3-fold lowering of the AUC in goats[55].

Comparable data in rats and dogs have been reported for rafoxanide[6]. Elimination half-life of closantel and rafoxanide was unaffected by route and dose administered[31,55,72].

It has been suggested[75] that the T½*el* of salicylanilides may be correlated with the turnover of plasma albumin to which they are bound, which is about 16.6 days[56].

Metabolism and excretion

Salicylanilides are poorly metabolised and are excreted mainly unchanged. Only 1.0–2.5 % of rafoxanide is metabolised in the liver in cattle[31] and about 90 % of closantel is excreted unchanged in the faeces and urine in sheep and cattle[72].

A reductive monodeiodination reaction appears as the main metabolic pathway for closantel in sheep, resulting in the formation of 3 and 5-monoiodoclosantel isomers[72]. Similar results have not been confirmed in cattle and goats[99]. Incubation of clioxanide with sheep liver microsomal enzymes revealed that monodeiodination is a non-enzymatic reaction[32]. Monodeiodination was not considered in the metabolic degradation of rafoxanide[29].

Rafoxanide is metabolised to 3,5-diiodosalicylic acid by amide hydrolysis and is found in blood, milk and muscle of cattle[29]. Amide hydrolysis is unlikely in closantel, niclosamide and resorantel owing to steric hindrances by substituents *ortho* to the amide bond[72].

Metabolic dehalogenation of both iodine atoms has not been observed for closantel or other deiodosalicylanilide derivatives[29,34,72]. Rapid and complete photolytic dehalogenation of rafoxanide by exposure to daylight has been reported[44].

Glucuronide formation occurs with salicylanilides and accounts for the presence of an enterohepatic circulation proposed for these compounds[74]. In rats 10–15 % of a salicylanilide dose is reabsorbed from the gastrointestinal tract. A glucuronide metabolite of oxyclozanide has also been identified[11]. Halogenated salicylanilides are not metabolised by glutathione conjugation[33].

Closantel is excreted mainly *via* the faeces. More than 80 % of ^{14}C-closantel was recovered from total 8-week faecal output and less than 0.5 % from the urine in sheep[72]. Within 48 h of dosing *c.* 43 % of the oral and 10 % of the i.m. dose was excreted with the faeces. Thereafter, faecal excretion proceeded more slowly with an average of 1–2 % of the dose per day. A faecal elimination half-life of 15.9–23 days was reported. Two to three percent of closantel is excreted at a maximum concentration of 1 μg/mℓ in milk in cattle. Comparable excretion of rafoxanide occurs in the urine of sheep and milk in cattle[29,30,31,54]. No data on biliary and faecal excretion of rafoxanide have been reported in ruminants. Oxyclozanide is concentrated and excreted in the bile in the environment of adult flukes[11].

In the rat the urine is the most important (>70 %) route for excretion of the salicylanilide derivative of benzanilide, an intermediate compound used in the dye and perfume industry[74]. Only 20 % is excreted in the faeces. Thirty-five percent of the [^{14}C]salicylanilide was excreted in the bile in 24 h. Incubation with rat caecal contents produced no hydrolysis[104]. In the case of rafoxanide there is extensive metabolism in the bile of rats[46].

ANTIPARASITIC ACTIVITY

Salicylanilides have variable activity against a wide range of helminths and a number of ectoparasites. The antiparasitic spectrum of halogenated salicylanilides used in sheep and cattle is summarised in Tables 3 and 4. Except for niclosamide, these products are mainly restricted for use in ruminants, although antiparasitic activity has been shown in a number of other animal species. Niclosamide is recommended for use in ruminants, dogs and cats. Closantel and rafoxanide have the broadest antiparasitic spectrum and are the most widely used of the salicylanilides. Oxyclozanide, niclosamide and resorantel have a very narrow anthelmintic spectrum.

Nematodes

The anthelmintic activity of salicylanilides, except niclosamide and oxyclozanide, is specifically directed towards haematophagous nematodes[86]. Niclosamide and oxyclozanide are not effective against nematodes. Closantel and rafoxanide are highly effective against both immature and mature stages of *Haemonchus contortus, Geigeria pachyscelis* and *Chabertia ovina* in sheep[47,60] and *H. placei, Bunostomum phlebotomum* and *Oesophagostomum radiatum* in cattle[47,98,106]. Activity against the immature parasitic stages are partly due to persistent activity related to their long biological half-lives[70]. Reduced effectivity occurs against non-blood sucking immature *H. contortus* (before 8 days of age)[100] and hypobiotic larval stages[108]. Oxyclozanide is poorly active against *Haemonchus* spp. and is ineffective against *Bunostomum* sp., *Oesophagostomum* sp. and *Chabertia* sp. in either cattle or sheep[126].

Persistent antiparasitic activity of closantel has been reported against a number of different parasite species and in different animals[47,48,50,52]. Activity is maintained against infective 3rd larval stages (L_3) of *H. contortus* and *G. pachyscelis* when administered orally to sheep at 10 mg/kg for up to 7 weeks and 8 weeks before infection, respectively[47]. Prolonged activity has also been reported against L_3 *H. placei, B. phlebotomum* and *O. radiatum* in cattle when administered subcutaneously (s.c.) at 5 mg/kg. Anthelmintic persistence of closantel protects sheep against reinfection for up to 28 days[52].

A single s.c. injection of closantel at 5 mg/kg completely cleared adult *Capillaria bovis* infections in cattle[47].

Marked effectivity against natural and experimentally-induced infestations of *Strongylus vulgaris* was reported in foals given repeated doses of an oral closantel formulation[48,49]. Fourth larval and immature adult stages of *S. vulgaris* present in the mesenteric arteries had been completely cleared in foals treated 5 times with closantel at 20 mg/kg every 2 months starting at 1 month of age and was 86 % effective when foals were given 3 treatments of 8 mg/kg at the same interval. At the higher dose, closantel was also highly effective against adult *S. vulgaris, S.*

Table 3: **Summary of the antiparasitic spectrum of all halogenated salicylanilides commonly used in sheep.**

Type of parasite	Antiparasitic spectrum				
	Closantel	Rafoxanide	Oxyclozanide	Niclosamide	Resorantel
NEMATODES					
H. contortus					
Adult	+++	+++	+		
Immatures	+++	++			
G. pachyscelis					
Adult	+++	+++			
Immature	+++	+++			
C. ovina					
Adult	+++	+++			
Immatures	+++	+++			
TREMATODES					
F. hepatica					
Adult	+++	+++	+++		
Immatures	+++	+++	++		
F. gigantica					
Adult	+++	+++	+++		
Immatures	+++	+++	+++		
Paramphistomum sp.					
Adult				+++	++
Immatures		++		++	++
CESTODES					
Moniezia expansa				+++	++
ECTOPARASITES					
Oestrus ovis	+++	+++			
Linognathus ovillus	+++				
Psoroptes sp.	++				

+Effectivity only demonstrated; ++moderately effective; +++highly effective.

edentatus and *Triodontophorus* spp.

Closantel at 7.5 and 10 mg/kg has marked anthelmintic effect on adult stages of *Ancylostoma caninum* in dogs[50]. The authors propose that the maturation of the larval stage may be affected at 20 mg/kg, although closantel does not affect the abundance of arrested hookworm larvae nor prevents their subsequent development.

Early studies showed that rafoxanide was highly effective against a thiabendazole tolerant *H. contortus* K-strain[40]. Closantel was also shown to have activity against benzimidazole resistant strains of *H. contortus* in sheep[52,53] and against fenbendazole- and levamisole-resistant *H. contortus* strains[120]. Anthelmintic resistance to rafoxanide and closantel has been identified in *H. contortus* in sheep in South Africa[124].

Closantel, in combination with broad spectrum anthelmintics, has been used successfully in a preventative anthelmintic programme to control haemonchosis and trichostrongylosis in sheep and was reported to retard the selection for anthelmintic resistance in *Trichostrongylus* spp.[27].

Eradication of *H. contortus* using closantel in sheep has been proposed[5].

Trematodes

Salicylanilides are effective against a wide range of hepatic and intestinal trematodes in a variety of animals[20,122]. Closantel, rafoxanide and oxyclozanide are of the most important drugs used for the treatment and control of fascioliasis, whereas niclosamide and resorantel form the mainstay of *Paramphistomum* control in ruminants.

Closantel administered at 5 and 10 mg/kg orally in sheep and at 2.5 mg/kg s.c. in cattle is highly effective against adult *F. hepatica*[47,69]. In sheep similar high efficacy occurs at the same dosage against adult and immature *F. gigantica* (8-week-old) and immature *F. hepatica* (4- and 6-week-old)[47]. Only moderate efficacy against immature *F. hepatica* (6-week-old) at 7.5 mg/kg s.c. was reported[47]. Given intramuscularly at 2.5 mg/kg in cattle, closantel is highly effective against adult *F. gigantica,* but only slightly effective (55.8 %) against the 6-week-old immature stages[47].

Closantel is ineffective against immature stages of 2 commonly occurring paramphistome species in Australia[92].

The efficacy of rafoxanide oral suspension at doses of 2.5–20 mg/kg against immature and adult *F. hepatica* and *F. gigantica* has been confirmed in both cattle[63,85,96,98,106,107] and sheep[3,10,19,26,39,60]. An improved efficacy against *F. gigantica* has been ascribed to the more pathogenic nature, voracious feeding habits and higher metabolic rate of this parasite, in relation to *F. hepatica*[10,26,96]. Rafoxanide also appears to be more efficacious in sheep than in cattle against fluke of comparable age[60,106,107]. Administered s.c., rafoxanide injectable solution is approximately 2.5 times more effective than the oral suspension formulation when administered i.r. in cattle against *Fasciola* infestation[96,122]. Rafoxanide at 15 mg/kg orally is moderately effective against immature *Paramphistomum microbothrium*[58] in sheep, but poorly effective up to 9 mg/kg s.c. in cattle[96,98].

Putative efficacy of closantel and rafoxanide against immature *F. hepatica* has been attributed to the persistent plasma concentrations affecting the flukes as they mature[75]. The presence of stunted or arrested fluke forms following closantel and rafoxanide treatment in sheep, is in part also ascribed to the persistent effect of the drug[69,75]. Nevertheless there is other evidence that indicate that these drugs act prior to *F. hepatica* reaching maturity. Campbell *et al.*[19] found that rafoxanide was virtually 100 % effective against 6-week-old fluke when sheep were necropsied 6–10 days after treatment, whereas Maes *et al.*[69] showed that closantel was equally effective against 6-week-old and 8-week-old fluke when necropsied either 1 week or 12 weeks after treatment.

According to Maes *et al.*[69], the flukicidal effect of closantel is related more to peak plasma concentrations and less to residual persistent effect.

Closantel given orally at 15 and 20 mg/kg is highly effective in reducing 8-week-old *Fascioloides magna* infestation in sheep[111,112]. A 7.5 mg/kg dose administered i.m. was equivalent to a 15 mg/kg oral dose[112]. Rafoxanide at 10 and 15 mg/kg administered orally was shown to be 100 % effective against both immature and mature *F. magna*[42]. Oxyclozanide only had a slight effect.

At doses of 10 and 15 mg/kg oxyclozanide is highly effective against mature stages of *F. hepatica* and against the adult and immature (6 weeks) stages of *F. gigantica* in sheep and cattle[11,93,126].

Higher doses are required against 6-week-old *F. hepatica.* It was suggested that the poor activity against immature *F. hepatica* is due to the high plasma binding of oxyclozanide in blood that bathes the immature fluke in the liver parenchyma[11]. According to Froyd *et al.*[43], there is no

Table 4: **Summary of the antiparasitic spectrum of salicylanilides commonly used in cattle.**

Type of parasite	Antiparasitic spectrum				
	Closantel	Rafoxanide	Oxyclozanide	Niclosamide	Resorantel
NEMATODES					
H. placei					
Adult	+++	+	+		
Immatures	+++				
B. phlebotomum					
Adult	+++	++			
Immatures	++				
O. radiatum					
Adult	+++	++			
Immatures	+++				
Capillaria sp.					
TREMATODES					
F. hepatica					
Adult	+++	+++	+++		
Immatures		+++	++		
F. gigantica					
Adult		+++	+++		
Immatures		+++	+++		
Paramphistomum sp.					
Adult	+		+++	+++	+++
Immature		+		+++	+++
CESTODES					
ECTOPARASITES					
Dermatobia sp.	+++				
Hypoderma sp.	+++				
Gedoelstia sp.		++			
Chrysomya bezziana	++				
Boophilus microplus	++				
Amblyomma sp.	++				

[+]Effectivity only demonstrated; [++]moderate efficacy; [+++]highly effective.

correlation between oxyclozanide concentrations in plasma and effectivity against liver fluke as judged by the number of parasites found at slaughter. A fixed dose of 3.4 g of oxyclozanide was shown to have equivalent efficacy in cattle with mass greater than 350 kg in comparison with a dose of 10 mg/kg.

Oxyclozanide has been extensively used for the treatment of paramphistomiasis[122]. Oxyclozanide alone or in combination with levamisole is highly effective against immature and mature paramphistomes at oral doses of 15 and 18.7 mg/kg[91,122]. Rolfe and Boray[91] found that 2 doses of oxyclozanide given 3 days apart was more effective than a single dose against *Calicophoron calicophorum* in cattle. Single doses gave varying activity. According to Van den Bossche *et al.*[122] oxyclozanide does not remove all parasites present in the host. Where complete success is claimed, it is usually on the basis of negative faecal egg counts. In horses, oxyclozanide is successful against *Gastrodiscus aegypticus*[90].

The efficacy of clioxanide against immature and mature *F. hepatica* has been reported by a number of workers[8,9,18,19,81,94]. Large differences in fasciolicidal efficacy of clioxanide, particularly against immature stages occurs between oral, i.r. and i-a routes of administration[8,9,17]. A marked reduction in efficacy occurs following i-a administration. Reduction in efficacy reported after oral treatment is most likely due to a proportion of the drug that bypasses the rumen and which is deposited into the abomasum[9,19]. As described previously, the activity of clioxanide depends on activation by rumen microbes. The efficacy of rafoxanide against *F. hepatica* is not affected when administered either orally, intraruminally or intra-abomasally[17].

Niclosamide at 50–100 mg/kg and resorantel at 65 mg/kg given orally to cattle and sheep are highly effective against immature *P. microbothrium*[45,57]. The effect of niclosamide in calves against the immature stages is erratic and it is not effective against adult stages in both sheep and cattle[20]. Erratic efficacy is also reported for resorantel against both immature and mature paramphistomes in sheep, goats and cattle. However, according to Van den Bossche *et al.*[122] resorantel is the most consistently successful drug used in the treatment of paramphistomiasis in cattle and sheep. Resorantel has also been used for the treatment of *G. aegypticus*[90].

Cestodes

Anticestodal activity of salicylanilides is predominantly restricted to niclosamide and resorantel, although some activity has also been reported for closantel and oxyclozanide. The effect of oxyclozanide against *Moniezia* sp. in sheep has been restricted to reduction in faecal egg counts and voiding of gravid segments[126]. At necropsy it was noted that the scolices had remained *in situ* and were capable of continued growth. Closantel at 40 mg/kg orally and 20 mg/kg administered i.m. has been shown to have high anthelmintic activity against the larval stages of *Taenia pisiformis* in experimentally-infected rabbits[22,47]. No effect against the cysticercus stages in the peritoneal cavity has been found. Activity against *Anoplocephala perfoliata* was demonstrated in horses that had received 5 monthly doses of closantel at 20 and 40 mg/kg[48].

Ectoparasites

Although salicylanilides are primarily effective against helminths, their effect against a number of insects and arthropods has been demonstrated. As far as could be determined, except for *Oestrus ovis,* these activities have not been recognised as official claims by regulatory authorities for these products.

Closantel and rafoxanide are 2 of the major products recommended for the control of *O. ovis* in sheep[47,58,95,108]. According to Snijders *et al.*[108], rafoxanide at 7.5 mg/kg given orally was highly effective against overwintering 1st instar larvae and against 2nd and 3rd larval instars within 96 h of treatment. A residual effect against the re-establishment of *O. ovis* in recently-treated sheep was proposed[58,59]. Closantel is highly effective against *O. ovis* at 5 mg/kg administered orally to sheep[47].

Other than their effect against *O. ovis* in sheep, closantel and rafoxanide have been shown to be effective against a range of other Oestridae in different animals. Studies conducted in foals treated with closantel 3 or 5 times every 2 months at doses between 2–40 mg/kg demonstrated a high efficacy against *Gastrophilus intestinalis* larvae[48,49]. In yearlings, 3 doses of at least 8 mg/kg were required. Closantel has also been shown be effective against *Dermatobia hominis*[21],

Hypoderma bovis[47] and *H. lineatum*[35] in cattle. Effectivity of rafoxanide against *Gedoelstia* in blesbuck[105] and *D. hominis* in cattle[21] has also been reported.Efficacy of closantel against *Chrysomya bezziana* has also been shown[109]. Closantel had no effect on the larvae of *Stomoxys calcitrans* and *Haematobia irritans*[36].

Anti-arthropod activity of closantel has been reported against *Boophilus microplus* in cattle that were naturally infected[67,125] and against *Amblyomma americanum* in experimentally-infected cattle[36,47,67], and against *Linognathus ovillus*[15], *Cochlyomia hominivorax*[47] and *Psoroptes communis* var. *ovis*[83] in sheep. Closantel is active against *Demodex canis* in dogs[68] and *Ornithonyssus sylvarium* when given as a feed additive to chickens[28].

SAFETY AND TOXICITY

Salicylanilides are moderately safe compounds and have safety factors of approximately 3–6 times the recommended dose levels[1,116]. No untoward effects are generally seen with rafoxanide when using single doses of 58 mg/kg in cattle or 45 mg/kg in sheep. However, toxicity has been reported in lambs that had allegedly been treated at the recommended dose[84]. A LD_{50} of rafoxanide for either sheep or cattle has not been determined, but for the rat an oral LD_{50} of approximately 2300 mg/kg has been calculated. According to Adams[1], no adverse effects are seen with closantel in sheep after repeated doses of 10 or 40 mg/kg orally or 5 or 20 mg/kg i.m. every 4 weeks over a 40-week period. Closantel is safe for use in breeding rams, ewes and bulls.

Salicylanilides are potent uncouplers of oxidative phosphorylation[127]. The pharmacokinetic behaviour of salicylanilides most likely contribute mostly to the selective toxicity for parasites.

Clinical signs of toxicity

The classical signs of salicylanilide toxicity in animals include blindness, paresis and ultimately death (Fig. 3). Blindness is an inconsistent toxic effect in cattle. General signs related to uncoupling of phosphorylation, *i.e.* hyperventilation, hyperthermia, convulsions and tachycardia may also be present.

The clinical signs of rafoxanide and closantel toxicity in sheep include inappetence, blindness, mydriasis and ophthalmoscopic papilloedema[13,51,76,78,79,84,89]. Prozesky and Pienaar[89] reported slight ataxia of the hindquarters as accompanying signs, while others[51] reported intense dyspnoea, diarrhoea and recumbency in sheep receiving doses of 450 mg/kg or more of rafoxanide. Susceptibility to rafoxanide toxicity appears to be more severe in sheep infected with liver fluke than in uninfected animals[76].

Fig. 3: **Clinical signs of rafoxanide and closantel toxicity in sheep at more than double the recommended dose (left: weakness and recumbency; right: mydriasis).**

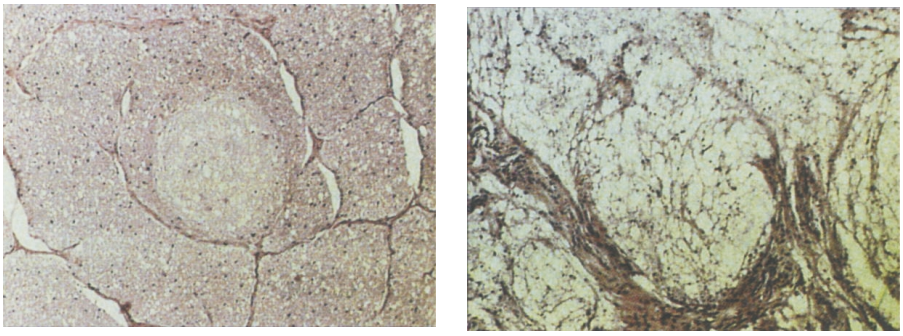

Fig. 4: **Brain and optic nerve histopathological lesions in sheep following rafoxanide and closantel poisoning (left: optic nerve vacuolation; right: vacuolation of white matter of brain).**

Signs of toxicity occur within 24–72 h of treatment[16,51,89]. However, sheep inadvertently overdosed with rafoxanide, at an estimated dose of 450 mg/kg, exhibited signs of toxicity on the same day[79]. Recovery of some affected animals over a period of 3–4 weeks was reported in goats overdosed with closantel[16]. Cattle overdosed with rafoxanide s.c. at 45–60 mg/kg presented signs of tachypnoea, muscle tremors, clonic spasms, opisthotonus, paddling movements of the forelimbs, prolapse of the nictitating membrane and blindness with mydriasis[97]. At an oral dose of 80 mg/kg in cattle, inappetance and diarrhoea are observed[1].

Oxyclozanide signs of toxicity in sheep and cattle include depression, anorexia and diarrhoea at oral doses of 25 mg/kg per day[126]. The toxic manifestations of clioxanide included neuropathy and cerebral oedema in rats[65] and blindness and vacuolation of the white matter of the central nervous system in sheep[77]. Experimental papilloedema induced by rafoxanide in the dog has been reported[13].

Other toxic manifestations included increased glutamic oxaloacetic transaminase and serum alkaline phosphatase, neutrophilia, lymphopaenia, focal hepatic necrosis and lymphoid necrosis in lymphnodes and intestine lymphoid tissue.

Photodermatitis and skin irritation in man have been reported for halogenated salicylanilides which have been incorporated in soaps as antimicrobial agents or when used topically as fungicides[61,64].

Pathological lesions

No gross *post mortem* lesions have been reported in either closantel or rafoxanide toxicity. Histopathologically, symmetrical status spongiosis (Fig. 4), oedema, haemorrhage, demyelination and lytic necrosis of the optic fasciculi, accompanied by neurophagocytosis and chromatolysis, have been reported[79,89,116].

Complete absence of nerve cells in the ganglionic cell layer of the retina has also been reported[89].

Odiawo *et al.*[79] suggests that optic nerve lesions, but not retinal lesions, appear to contribute to blindness in acute poisoning. Dogs given 3–11 doses of rafoxanide orally at 100 mg/kg developed bilateral equatorial cataracts, papilloedema, vacuolation of the optic nerve, optic chiasma, white matter of the brain and spinal cord and focal vacuolation of the sciatic nerve[13]. The pathogenesis of the ocular and neural lesions was ascribed to increased cerebrospinal fluid pressure, probably due to an increased amount of fluid in the cranial cavity, brain swelling and meningeal inflammation around the optic nerve and optic chiasma. Similar findings, *i.e.* vacuolation of the white

matter of the brain and lens opacities, have also been observed in experiments using multiple doses of 250 mg/kg rafoxanide per day in rats[65].

CONCLUSIONS

The halogenated salicylanilides, in particular closantel and rafoxanide, are important anthelmintics that are used extensively in the control of *Haemonchus* spp. and *Fasciola* spp. infestation in sheep and cattle, and *O. ovis* in sheep. Niclosamide and resorantel are used for the control of paramphistomes and cestodes. Halogenated salicylanilides share a number of common pharmacokinetic features, including extensive plasma binding, prolonged elimination half-life and limited metabolism. The persistent anthelmintic effect and predilection against haematophagous parasites substantiate the importance of the pharmacokinetics of these compounds on their efficacy. In addition, the margin of safety of halogenated salicylanilides, as toxicity is dose-dependent, may be affected by changes in bioavailability and in extent of plasma binding. Despite the importance of pharmacokinetics on the efficacy and safety of halogenated salicylanilides, very few studies have been reported that examine factors that potentially may influence their absorption and disposition in ruminants.

REFERENCES

1. Adams H R 1995 *Veterinary pharmacology and therapeutics.* Iowa State University Press, Ames
2. Anon. 1993 *British Pharmacopoeia (Veterinary).* Her Majesty's Stationary Office, London
3. Armour J, Corba J 1970 The anthelmintic activity of rafoxanide against immature *Fasciola hepatica* in sheep. *Veterinary Record* 92: 213–214
4. Banu E, Draghici C, Cilianu S, Leoveanu O, Tamas V, Rizopol D, Neubauer G, Funiera G 1981 Biological availability of the anthelmintic rafoxanide,with reference to structure and crystal size. *Sesuinea scientifica jubiliara '30 de ani de activate a Institului de Cercetari Chimico-Farmaceutice:* 93–96
5. Barger I A, Hall E, Dash K M 1991 Local eradication of *Haemonchus contortus* using closantel. *Australian Veterinary Journal* 68: 347–348
6. Barth D 1974 Rafoxanid Ein neues Leberegelmittel bei Schaf und Rind. *Tierärztliche Umschau* 29: 104–110
7. Booth N H, McDonald L E 1988 *Veterinary pharmacology and therapeutics.* Iowa State University Press, Ames
8. Boray J C, Happich F A, Andrews J C 1965 Tests on the anthelmintic efficiency of 'Hilomid' against immature and mature *Fasciola hepatica* in sheep. *Veterinary Record* 77: 175–176
9. Boray J C, Roseby F B 1969 The effect of route of administration on the efficiency of clioxanide against immature *Fasciola hepatica* in sheep. *Australian Veterinary Journal* 45: 363–365
10. Boray J C, Wolff K, Trepp H C 1973 Testing of new fasciolicides: I. Efficacy and toxicity of rafoxanide in sheep experimentally infected with *Fasciola hepatica* and *F. gigantica. Schweizer Archiv für Tierheilkunde* 115: 367–371
11. Broome A W, Jones W G M 1966 A new drug for the treatment of fascioliasis in sheep and cattle. *Nature* (London) 210: 744–745
12. Brown G R, Chesterson G J, Coles G C 1985 Potentiation of fasciolicidal agents by benzoyl side chains. Synthesis of Benzoylsalicylanilides. *Journal of Medical Chemistry* 28: 143–146
13. Brown W R, Ruben L, Hite M, Zwickey R E 1972 Experimental papilledema in the dog induced by a salicylanilide. *Toxicology and Applied Pharmacology* 21: 533–541
14. Budavari S, O'Neil M J, Smith A, Heckelman P E, Kinneary J F 1996 *The Merck Index.* Merck Research Laboratories, Whitehouse Station, New Jersey
15. Butler A R 1986 Effects of closantel on face lice (*Linognathus ovillus) of sheep. Australian Veterinary Journal* 63: 89–90
16. Button C, Jerrett I, Alexander P, Mizon W 1987 Blindness in kids associated with overdosage of closantel. *Australian Veterinary Journal* 64: 226
17. Campbell N J, Brotowidjoyo M D 1975 The efficiency of clioxanide and rafoxanide against *Fasciola hepatica* in sheep by different routes of administration. *Australian Veterinary Journal* 1: 500–503
18. Campbell N J, Hotson I K 1971 The anthelmintic efficiency of clioxanide and rafoxanide against *Fasciola hepatica* and *Haemonchus contortus* in sheep. *Australian Veterinary Journal* 47: 5–8
19. Campbell W C, Ostlind D A, Yakstis J J 1970 The efficacy of 3,5-diiodo-3'-chloro-4'-(p-chlorophenoxy)-salicylanilide against immature *Fasciola hepatica* in sheep. *Research in Veterinary Science* 11: 99–100
20. Campbell W C, Rew R S 1986 *Chemotherapy of parasitic diseases.* Plenum Press, New York
21. Chaia G, Chiari L, da Silva D C, Guerrero J 1981 Pilot trials on the treatment of *Dermatobia hominis* infections in cattle with closantel. *American Journal of Veterinary Research* 42: 1240–1241
22. Chevis R A F, Kelly J D, Griffin D L 1980 The lethal effect of closantel on the metacestodes of *Taenia pisiformis* in rabbits. *Veterinary Parasitology* 7: 333–337
23. Coles G C 1974 Fluke biochemistry *Fasciola* and *Schistosoma. Helminthological Abstracts* (Series A) 44: 147–162.
24. Corbert J R, Goose J 1971 A possible biochemical mode of action of the fasciolicides nitroxynil, hexachlorophene and oxyclozanide. *Pesticide Science* 2: 119–121
25. Cornish R A, Behm C A, Butler R W, Bryant C 1977 The *in vivo* effects of rafoxanide on the energy metabolism of *Fasciola hepatica. International Journal of Parasitology* 7: 217–220
26. Cornish R A, Bryant C 1976 Changes in energy metabolism due to anthelmintics in *Fasciola hepatica* maintained *in vitro. International Journal of Parasitology* 6: 393–398
27. Dash K M 1986 Control of helminthosis in lambs by strategic treatment with closantel and broad-spectrum anthelmintics. *Australian Veterinary Journal* 63: 4–8
28. De Vaney J A, Ivie G W 1984 Evaluation of closantel as a feed additive for control of northern fowl mites 1983. *Insecticidal Acaricidal Tests* 9: 435–435
29. Dedek W, Grahl R, Schwarz H, Ludwig P 1978 Metabolismus Rückstände und Ausscheidung des Anthelminthikums 131J-Rafoxanid in Blut Milch, Fleisch und Urin am laktierenden Rind. *Archiv für Experimentelle Veterinarmedizin* 32: 951–955
30. Dedek W, Schwarz H, Liebaug E 1976 Untersuchungen zum Abbau und zur Ausscheidung des Anthelminthikums 131J-Rafoxanid an Rind und Schaf. *Archiv für Experimentelle Veterinarmedizin* 30: 423–426
31. Dedek W, Schwarz H, Liebaug E 1977 Abbau un Rückstandsdynamik des Anthelminthikums 131J-Rafoxanid in Blut Milch Fleish und Fett bei unterschiedlicher Dosierung am Rind. *Archiv für Experimentelle Veterinarmedizin* 31: 365–368
32. Douch P G C 1979 The metabolism of the anthelmintics clioxanide and resorantel and related compounds *in vitro* by *Moniezia expansa, Ascaris suum* and mouse- and sheep-liver enzymes. *Xenobiotica* 9: 263–268
33. Douch P G C, Buchanan L L 1978 Glutathione conjugation of some xenobiotics by *Ascaris suum* and *Moniezia expansa. Xenobiotica* 8: 171–176
34. Douch P G C, Gahagan H M 1977 Metabolism of niclosamide and related compounds by *Moniezia expansa. Xenobiotica* 7: 301–307
35. Drummond R O 1984 Cattle – *Hypoderma lineatum* animal systemic insecticide test 1983. *Insecticidal Acaricidal Tests* 9: 419–420
36. Drummond R O, Miller J A 1985 Systemic activity of closantel for control of lone star ticks, *Amblyomma americanum* (L) on cattle. *Experimental and Applied Acarology* 1: 193–202
37. Düwel D 1970 Ein neues Zestizid: Terenol seine Wirkung gegen Bandwürmer von Laboratoriums- und Haustieren. *Deutsche Tierärztliche Wochenschrift* 77: 97–101
38. Düwel D, Metzger H 1973 26-Dihydroxybenzoic acid anilides as fasciolicides. *Journal of Medical Chemistry* 16: 433
39. Edwards C M, Parry T O 1972 Treatment of experimentally-produced acute fascioliasis in sheep. *Veterinary Record* 90: 523–526
40. Egerton J R, Yakstis J J, Campbell W C 1970 The efficacy of rafoxanide (3,-diiodo-3'-chloro-4'-(p-chlorophenoxy)salicylanilide against *Haemonchus contortus* in sheep. *Research in Veterinary Science* 11: 382–383
41. Fairweather I, Holmes S D, Treadgold L T 1984 *Fasciola hepatica:* motility response to fasciolicides *in vitro. Experimental Parasitology* 57: 209–224
42. Foreyt W J, Todd A C 1974 Efficacy of rafoxanide and oxyclozanide against *Fascioloides magna* in naturally infected cattle. *American Journal of Veterinary Research* 35: 375–377
43. Froyd G 1969 The efficacy of oxyclozanide in heavy cattle. *Veterinary Record* 85: 705–707
44. Gabrio T, Scheybal A, Konrad H, Dedek W, Schwarz H 1978 Residues in milk after application of the fasciolicides rafoxanide and hexachloro-p-xylene. *Nahrung* 22: 409–414
45. Gaenssler J G, Reinecke R K 1970 The anthelmintic efficacy of resorantel. *Journal of the South African Veterinary Medical Association* 41: 211–214
46. Galtier P, Larrieu G, Franc M 1985 Incidence of experimental fascioliasis on hepatic disposition of $^{[3H]}$Tetracycline and $^{[14C]}$Rafoxanide in rats. *Journal of Pharmaceutical Science* 74: 1004–1006

47. Guerrero J 1984 Closantel: A review of its antiparasitic activity. *Preventive Veterinary Medicine* 2: 317–327
48. Guerrero J, Michael B F, Rohovsky M W, Campbell B P 1983 The activity of closantel as an equine antiparasitic agent. *Veterinary Parasitology* 2: 71–77
49. Guerrero J, Newcomb K, Seibert B P, Michael B F 1985 Activity of closantel in the prevention of *Gastrophilus* and *Strongylus vulgaris* larval infections in equine foals and yearlings. *American Journal of Veterinary Research* 46: 16–18
50. Guerrero J, Page M R, Schad G A 1982 Anthelmintic activity of closantel against *Ancylostoma caninum* in dogs. *Journal of Parasitology* 68: 616–619
51. Guilhon J, Jolivet G, Barnabe R 1971 Etude de la toxicité aiguë pour le mouton d'un nouvea fasciolicide: le 3,-diiodo-3'-chloro-4'-(p-chlorophenoxy)-salicylanide. *Bulletin Academie Veterinaire* 44: 33–37
52. Hall C A, Kelly J D, Whitlock H V, Ritchie L 1981 Prolonged anthelmintic effect of closantel and disophenol against a thiabendazole selected resistant strain of *Haemonchus contortus* in sheep. *Research in Veterinary Science* 31: 104–106
53. Hall C A, McDonell P A, Graham J M 1980 Anthelmintic activity of closantel against benzimidazole resistant strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep. *Australian Veterinary Journal* 56: 461–462
54. Hecht G, Gloxhuber C 1960 Experimentelle Untersuchungen mit N-(2'-Chlor-4'-Nitrophenyl)-5-Chlorsalicylamid, enem neuen bandwurmmittel: toxicologische Untersuchungen. *Arzneimittel-Forsch* 10: 884–885
55. Hennessy D R, Sangster N C, Steel J W, Collins G H 1993 Comparative pharmacokinetic disposition of closantel in sheep and goats. *Journal of Veterinary Pharmacology and Therapeutics* 16: 254–260
56. Holmes P H, Dargie J D, Maclean J M, Mulligan W 1968 Albumin and globulin turnover in chronic ovine fascioliasis. *Veterinary Record* 83: 227–228
57. Horak I G 1964 Studies on paramphistomiasis. IV. The anthelmintic efficacy of Lintex and Freon against *Paramphistomum* spp. in sheep and cattle. *Journal of the South African Veterinary Medical Association* 33: 161–166
58. Horak I G, Louw J P, Raymond S M 1971 Trials with rafoxanide. 3. Efficacy of rafoxanide against the larvae of the sheep nasal bot fly *Oestrus ovis* Linné 1761. *Journal of the South African Veterinary Association* 42: 327–339
59. Horak I G, Snijders A J 1974 The effects of *Oestrus ovis* infestation on merino lambs. *Veterinary Record* 94: 12–16
60. Horak I G, Snijders A J, Louw J P 1972 Trials with rafoxanide. 5. Efficacy studies against *Fasciola hepatica, Fasciola gigantica, Paramphistomum microbothrium* and various nematodes in sheep. *Journal of the South African Veterinary Association* 43: 397–403
61. Ison A E, Tucker J B 1967 Delayed reactions in positive photopatch tests to halogenated salicylanilides. *Archives of Dermatology* 96: 59–61
62. Kane H J, Behm C A, Bryant C 1980 Metabolic studies on the new fasciolicidal drug closantel. *Metabolic and Biochemical Parasitology* 1: 347–355
63. Knapp S E, Presidente P J A 1971 Efficacy of rafoxanide against natural *Fasciola hepatica infections in cattle. American Journal of Veterinary Research* 2: 1281–1291
64. Kraushaar A 1954 Chemotherapeutic activity of halogenated salicylanilides depending on the constitution. *Arzneimittel-Forsch* 4: 548–551
65. Kurtz S M, Schardein J L, Fitzgerald J E, Kaump D H 1969 Toxicologic studies with a halogenated salicylanilide. Abstracts: 8th Annual Meeting of the Society of Toxicology. *Toxicology and Applied Pharmacology* 14: 18
66. Lee R M 1973 The relationship between biological action and structure of some fasciolicidal salicylanilides. *Parasitology* 67: 13–14
67. Lombardero O J, Luciana C A 1982 Efectos del closantel inyectable 5 % sobre la oviposicion y la eclosion de huevos de *Boophilus microplus* en vacunos naturalmente infestados. *Gaceta Veterinaria* 44: 310–313
68. Losson B, Benakhla A 1980 Efficacite du closantel dans le traitment de le gale demodectique du chien. *Annales de Medica Veterinaria* 24: 521–526
69. Maes L, Lauwers H, Deckers W, Vanparijs O 1988 Flukicidal action of closantel against immature and mature *Fasciola hepatica* in experimentally infected rats and sheep. *Research in Veterinary Science* 44: 229–232
70. McKellar Q A, Kinabo L D B 1991 The pharmacology of flukicidal drugs. *British Veterinary Journal* 47: 306–321
71. Metzger H, Düwel D 1973 Investigations of metabolism in the liver fluke (*Fasciola hepatica*) as an aid to the development of new anthelmintics. *International Journal of Biochemistry* 4: 133–143
72. Michiels M, Meuldermans W, Heykans J 1987 The metabolism and fate of closantel (Flukiver) in sheep and cattle. *Drug Metabolism Reviews* 18: 235–251
73. Mitchell P 1961 Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. *Nature* 191: 144–148
74. Mitchell S C, Norbury H M, Waring R H, Gadsden P M, Wood P B 1982 A comparison of the metabolism and elimination of benzanilide and salicylanilide in the rat. *Xenobiotica* 12: 93–99
75. Mohammed-Ali N A K, Bogan J A 1987 The pharmacodynamics of the flukicidal salicylanilides rafoxanide closantel and oxyclozanide. *Journal of Veterinary Pharmacology and Therapeutics* 10: 127–133
76. Mrozik H, Jones H, Friedman J, Schwartzkopf G, Schardt R A, Patchett A A, Hoff D R, Yakstis J J, Riek R F, Ostlind D A, Plishker G A, Butler R W, Cuckler A C, Campbell W C 1969 A new agent for the treatment of liver fluke infection (fasciolíasis). *Experientia* 25: 883–1001
77. O'Brien J J 1970 Toxicological aspects of some modern anthelmintics. *Australian Veterinary Journal* 46: 297–300
78. Obwolo M J, Odiawo G O, Ogaa J S 1989 Toxicity of a closantel-albendazole mixture in a flock of sheep and goats. *Australian Veterinary Journal* 66: 229–230
79. Odiawo G O, Ogaa J S, Ndikuwera J, Moulton J E 1991 Acute amaurosis in sheep due to rafoxanide neurotoxicity. *Zimbabwe Veterinary Journal* 22: 64–99
80. Pax R A, Bennett J L 1989 Effect of closantel on intrategumental pH in *Schistosoma mansoni* and *Fasciola hepatica. Journal of Parasitology* 5: 169–171
81. Pearson I G, Whitlock H V, De Goosh C P, Farrington K J, Jones R C, Haigh J A 1970 Clioxanide a new anthelmintic active against *Fasciola hepatica* and *Haemonchus contortus* in sheep. *Australian Veterinary Journal* 46: 480–486
82. Pearson J T, Varney G 1973 The anomalous behaviour of some oxyclozanide polymorphs. *Journal of Pharmacology and Pharmaceutics* 25: 62–70
83. Perez A A, Marti V J, Romero J, Aloisi G 1982 Actividad de closantel (R-31520) en sarna ovina naturalmente adquirida. *Gaceta Veterinaria* 44: 683–685
84. Pienaar J G 1977 Nuwere veterinêre neuropatologiese toestande in Suid-Afrika. *Journal of the South African Veterinary Association* 48: 13–18
85. Presidente P J A, Knapp S E 1972 Anthelmintic effect of rafoxanide against immature *Fasciola hepatica* in calves. *American Journal of Veterinary Research* 3: 1603– 1610
86. Prichard, R. K. 1978 Anthelmintics. Refresher course for veterinarians – refresher course in the therapeutic jungle. University of Sydney: 421–463
87. Prichard R K 1978 The metabolic profile of adult *Fasciola hepatica* obtained from rafoxanide-treated sheep. *Parasitology* 76: 277–288
88. Prichard R K, Hennessy D R, Steel J W, Lacey W 1985 Metabolite concentrations in plasma following treatment of cattle with five anthelmintics. *Research in Veterinary Science* 39: 173–178
89. Prozesky L, Pienaar J G 1977 Amaurosis in sheep resulting from treatment with rafoxanide. *Onderstepoort Journal of Veterinary Science* 44: 257–260
90. Roberts H M, Adams W J E, Danks D C 1976 *Gastrodiscus aegypticus:* a therapeutic trial. *Rhodesian Veterinary Journal* 6: 73–76
91. Rolfe P F, Boray J C 1986 Chemotherapy of paramphistomum in cattle. *Australian Veterinary Journal* l4: 328–332
92. Rolfe P F, Boray J C 1993 Comparative efficacy of moxidectin, an ivermectin/clorsulon combination and closantel against immature paramphistomes in cattle. *Australian Veterinary Journal* 70: 265–266
93. Roy R M, Sukhla S S 1971 Oxyclozanide activity against *Fasciola gigantica* in naturally infected buffalo, cattle sheep and goats. *Tropical Animal Health and Production* 3: 26–31
94. Samson K S, Wilson G I, Allen R W 1969 Sheep liver fluke anthelmintics: preliminary experiments with eight compounds against mature and immature *Fasciola hepatica. American Journal of Veterinary Research* 30: 807–810
95. Sanyai P K, Maru A, Gupta A K 1986 The use of injectable rafoxanide against natural ovine nasal myiasis. *Veterinary Parasitology* 19: 127–131
96. Schröder, J 1981 Injectable rafoxanide solution as an anthelmintic for cattle. M.Sc. thesis, University of Pretoria
97. Schröder J 1982 The safety of injectable rafoxanide in cattle. *Journal of the South African Veterinary Association* 53: 29–31
98. Schröder J, Honer M R, Louw J P 1977 Trials with rafoxanide. 8. Efficacy of an injectable solution against trematodes and nematodes in cattle. *Journal of the South African Veterinary Association* 48: 95–97
99. Short C R 1994 Consideration of sheep as a minor species: comparison of drug metabolism and disposition with other domestic

ruminants. *Veterinary and Human Toxicology* 36: 24–40
100. Sinclair K B, Prichard R K 1975 The use of disophenol in studies of the pathogenicity of the arrested 4th-stage larvae of *Haemonchus contortus* in sheep. *Research in Veterinary Science* 19: 232–234
101. Sindt A C, Mackay M F 1980 Flukicides. IV. Crystal and molecular structure of 3′-Chloro-4′-(p-chlorophenoxy)-3,5-diiodosalicylanilide (Rafoxanide). *Australian Journal of Chemistry* 3: 203–207
102. Singh H, Singh A K, Sharma S, Iyer R N, Srivastava O P 1977 Synthesis of 5-chloro-3′-nitro-4′-substituted salicylanilides a new series of anthelmintic and antimicrobial agents. *Journal of Medical Chemistry* 20: 826–829
103. Skuce P J, Fairweather I 1990 The effect of the hydrogen ionophore closantel upon the pharmacology and ultrastructure of the adult liver fluke *Fasciola hepatica*. *Parasitological Research* 76: 241–250
104. Smith G, Griffiths L A 1974 Metabolism of N-acylated and O-alkylated drugs by the intestinal microflora during anaerobic incubation *in vitro*. *Xenobiotica* 4: 477–487
105. Snijders A J, Horak I G 1972 Trials with rafoxanide. 4. Efficacy against the oestrid fly *Gedoelstia hässleri* in the blesbuck (*Damaliscus dorcas philipsi* Harper, 939). *Journal of the South African Veterinary Medical Association* 43: 295–297
106. Snijders A J, Horak I G 1975 Trials with rafoxanide. 7. Efficacy against *Fasciola hepatica, Haemonchus placei* and *Bunostomum phlebotomum* in cattle. *Journal of the South African Veterinary Association* 46: 265–267
107. Snijders A J, Horak I G, Louw J P 1971 Trials with rafoxanide. 2. Efficacy against *Fasciola gigantica* in cattle. *Journal of the South African Veterinary Medical Association* 42: 253–257
108. Snijders A J, Horak I G, Louw J P 1973 Trials with rafoxanide. 6. The effect of repeated and single treatments with rafoxanide against *Haemonchus contortus* and *Oestrus ovis* in sheep. *Journal of the South African Veterinary Association* 44: 251–263
109. Spradbery J P, Owen I L 1990 Efficacy of closantel against infestations of screw-worm fly *Chrysomya bezziana*. *Australian Veterinary Journal* 17: 340–340
110. Srivastava R P, Sharma S 1990 Synthesis of 2,5-disubstituted benzimidazoles 1,3,4-thiadiazoles and 3,5-diiodosalicylanilides as structural congeners of rafoxanide and closantel. *Pharmazie* 45: 34–37
111. Stromberg B E, Schlotthauer J C, Conboy G A 1984 The efficacy of closantel against *Fascioloides magna* in sheep. *Journal of Parasitology* 70: 446–447
112. Stromberg B E, Schlotthauer J C, Seibert B P, Conboy G A, Newcomb K M 1985 Activity of closantel against experimentally induced *Fascioloides magna* infection in sheep. *American Journal of Veterinary Research* 46: 2527–2529
113. Swan G E 1998 *Index of Veterinary Specialities*. MIMS Division of Times Media, Pretoria: 41–42
114. Swan G E, Botha C J, Taylor J H, Mülders M S G, Minnaar P P, Kloeck A 1995 Differences in the oral bioavailability of three rafoxanide formulations in sheep. *Journal of the South African Veterinary Association* 66: 197–201
115. Swan G E, Mülders M S G 1993 Pharmacokinetics of rafoxanide in suckling and weaned lambs following oral administration. *Journal of the South African Veterinary Association* 64: 67–70
116. Swan G E, Schröder J 1981 A safety trial with rafoxanide in sheep. *Journal of the South African Veterinary Association* 2: 123–125
117. Symonds L E A, Roseby F B 1969 The use of clioxanide as an anthelmintic in sheep infected with *Haemonchus contortus*. *Australian Veterinary Journal* 45: 385
118. Taylor S M, Mallon T R, Blanchflower W J, Kennedy D G, Hewitt S A 1993 Effects of dietary variations on plasma concentrations of oral flukicides in sheep. *Journal of Veterinary Pharmacology and Therapeutics* 16: 48–54
119. Tielens A G M, van den Heuvel J M, Van den Bergh S G 1984 The energy metabolism of *Fasciola hepatica* during its development in the final host. *Molecular Biochemistry in Parasitology* 13: 301–307
120. Uppal R P, Yadav C L, Bhushan C 1993 Efficacy of closantel against fenbendazole and levamisole resistant *Haemonchus contortus* in small ruminants. *Tropical Animal Health and Production* 25: 30–32
121. Van den Bossche H 1972 *Comparative biochemistry of parasites.* Academic Press, New York
122. Van den Bossche H 1983 Worm diseases: Economic aspects of anthelmintic treatment. In Ruckebush Y, Toutain P-L, Gouritz G D (eds) *Veterinary pharmacology and toxicology.* MTP Press, Lancaster, UK: 221–224
123. Van den Bossche H, Verhoeven H, Vanparijs O, Lauwers H, Thienpont D 1979 Closantel a new antiparasitic hydrogen ionophore. *Archives Internationales de Physiologie et de Biochemie* 87: 851–852
124. Van Wyk J A, Malan F S 1988 Resistance of field strains of *Haemonchus contortus* to ivermectin, closantel, rafoxanide and the benzimidazoles in South Africa. *Veterinary Record* 123: 226–228
125. Vasconcelos O T, Rocha U F, Ferreira F A, Costa A J, Moraes F R, Christoforo M T 1987 Acção anti-ixodídica do closantel sombre *Boophilus microplus* (Canestrini). *Ars Veterinaria* 3: 243–256
126. Walley J K 1966 Oxyclozanide (3,3′,5,5′,6-pentachloro-2,2′-dihydroxybenzanilide—Zanil) in the treatment of the liver fluke *Fasciola hepatica* in sheep and cattle. *Veterinary Record* 78: 267–276
127. Williamson R L, Metcalf R L 1967 Salicylanilides: A new group of active uncouplers of oxidative phosphorylation. *Science* 58: 1694–1695