Concentrations of pesticides in fleeces after pour-on and saturation dipping of Merino sheep

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ABSTRACT

A year-long farm trial was performed in 2003/04 to determine the relative efficacies of commercial formulations of diflubenzuron and triflumuron lousicides and measure pesticide concentrations in different parts of the fleece throughout the year. Significant differences were found between the concentrations of pesticides present on samples of fleeces taken from the back and side initially ($P<0.05$), 5 months ($P<0.001$), and 12 months ($P<0.05$) after pour-on treatments. The bulk of the pesticides from pour-on treatments remained in the zone where they had been applied and little moved to the sides of the sheep. Five months after application pesticide concentrations were significantly higher ($P<0.001$) in staple tip compared with butt samples on both back and side fleece samples for pour-on or jetting treatments, confirming recent predictions that diffusion of pesticides in greasy wool will be limited. Concentrations in butt samples from 5 months onwards were near or less than the mean LD95 doses reported for diflubenzuron for susceptible – 0.31 mg/kg, 95% confidence limits (CL) 0.16-1.25 mg/kg – and resistant lice populations – 4.4 mg/kg, 95% CL 1.4-71 mg/kg. The results offer a possible explanation for the anecdotal reports of poor efficacy of some pour-on louse treatments and treatment failures on farm.

Keywords: Louse control; pour-on treatments; saturation treatments; pesticide concentrations; pesticide residues.

INTRODUCTION

Farmers use pour-on or saturation formulations of pesticides to control lice on sheep. Pour-on formulations result in very high concentrations of pesticide in the wool where they are applied, and rely on movement of this pesticide from there to other parts of the body and fleece for their efficacy. There have been reports of farmers finding louse control on Merino sheep difficult, particularly when using pour-on treatments on sheep shorn with winter combs. In the past, lice have developed resistance to synthetic pyrethroids (SPs) and this has resulted in low efficacy (Johnson *et al.*, 1990). About 10 years ago the insect growth regulator (IGR) compounds diflubenzuron and triflumuron appeared on the market and are now commonly used in the place of the SPs. Anecdotal information from Merino farmers in New Zealand suggests the pour-on IGR products appear to be becoming less effective in controlling lice for a full year when applied off-shears – the current recommendation. Low effectiveness of IGR pour-on products has also recently been reported in Australia (James & Levot, 2005). Lack of control can mean re-treatments are needed before shearing and consequently elevated residue levels appear in wool clips. Also, there is a continued expense on farm to treat animals each year.

One hypothesis developed to explain the resistance by sheep lice to the SPs (Johnson *et al.*, 1990) attributed it to the pour-on backline application technique used to apply the pesticides. Studies have shown that the bulk of the SPs from a pour-on treatment remained where they were applied and very little moved from the backline zone after application (Johnson *et al.*, 1990; Johnson *et al.*, 1995a). Concentrations of pesticide were relatively uneven compared with saturation treatments, being very high along the backline but much lower elsewhere. Resistance to SP compounds could develop in lice exposed to sub-lethal concentrations as a result of these treatments and product failure could result. Similar situations with pour-on backline treatment with the IGRs could explain reports of low efficacy and the possible emergence of resistance (James & Levot, 2005).

A year-long farm study was undertaken to examine the relative effectiveness of pour-on and jetting methods of application of triflumuron and diflubenzuron for louse control in Merino sheep. As part of this study, pesticide concentrations were measured in different parts of the fleece on occasions throughout the year to determine what role they might play in the performance of different formulations. This paper reports on the results of analysis of data on pesticide concentrations in fleeces measured in the study. A limited amount of the data has been reported previously (Rankin *et al.*, 2005). The full data set includes data for alternative treatments included to test their effectiveness, and data from samples collected to examine the variability in pesticide concentrations between replicate samples.

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MATERIALS AND METHODS

Trial methodology

The trial methodology has been described previously (Rankin *et al*., 2005). The trial was carried out on a lice-infested Merino flock from August 2003 to August 2004 on a property near Cromwell in the South Island of New Zealand. Five hundred lousy animals were selected before shearing from a ewe flock of about 2500 animals and divided into 10 treatment groups at random. In early September, after the initial louse count, all the sheep were shorn - two groups using flat combs and the remainder using winter combs – and the pesticide treatments applied.

Treatments

Treatments (Table 1) were applied 24 hours after shearing by pour-on or saturation treatment methods using commercially available products. Saturation treatments were applied by hand-jetting or using an automatic jetting race. All treatments were applied as recommended on the product labels and in the 2003 IVS Annual (Nottingham, 2003) where applicable, unless otherwise stated.

Pour-on treatments 1, 2, 5, and 6 applied 20 mL of a 25g/L solution/suspension of triflumuron or diflubenzuron whereas treatment 3 delivered 24 mL of diflubenzuron. Treatments using diflubenzuron were applied in one pass down the backbone of the animal from head to tail using an NJ Phillips automatic drenchmaster gun fitted with a Cooper's T-bar nozzle. Triflumuron was applied in two passes adjacent to each other on either side of the backbone (10 mL each) from head to tail, using the applicator gun supplied.

Hand-jetting treatments were applied with a Dutjet handpiece, using approximately 2 litres of dip wash per animal. Hand-jetting treatments were applied with dilute solutions containing either 0.625 g/L of diflubenzuron or 0.48 g/L of triflumuron. They were made with one pass just either side of the backline of the animal from the back of the head to the rump; one pass below these from the shoulder to the rump on each side; and one pass up one side from the bottom of the brisket up underneath the neck and then down the other side of the brisket.

Treatments 3 and 9 were 'off-label'. Treatment 3 was recommended (Stuart Edwards, WoolPro/Meat and Wool Innovation, personal communication) and applied as two bands (12 mL of diflubenzuron solution/suspension in each) from head to tail on either side of the backbone. Treatment 9 was included as we previously found it to be effective at controlling lice on crossbred sheep and was carried out as recommended for crossbred sheep.

A modified CraigCo automatic jetting race (AJR) with 3 mm diameter nozzles was used to apply a nominal 2 litres of mixed dip wash formulation of chlorfenvinphos (1.0 g/L) and diflubenzuron (0.375 g/L) for treatment group 10 and to the remainder of the farm flock. The AJR unit contained two parallel sets of four nozzles side-by-side, in the direction of travel on top of the unit; one set of three nozzles mounted vertically on each side; and two sets of three bottom nozzles each in a row at right angles to the direction of travel, with the front set slightly angled forwards and the back set angled approximately 45° forwards.

Animals were allowed to stand in separate groups for 24 hours after treatment.

Table 1: Treatments applied and estimates ± standard errors for parameters from regression analysis of natural logarithm transforms of pesticide concentrations for different treatment groups according to the relation $\log_e(\text{concentration}+1)_t$ = Constant + Effect for side + Slope × Time(t(days)). The effect of side was not significant where no such values are given.

Treatment group	Application method	Shearing comb	Pesticide	Constant	Effect for side	Slope	R^2	Significance
1	Pour-on	Flat	Diflubenzuron	8.70 ± 0.36	-3.21 ± 0.42	-0.0119 ± 0.0015	94.7	P<0.001
2	Pour-on	Winter	Diflubenzuron	8.56 ± 0.42	-3.31 ± 0.49	-0.0120 ± 0.0017	93.0	P<0.001
3	Pour-on	Winter	Diflubenzuron	9.05 ± 0.14	-2.74 ± 0.16	-0.0128 ± 0.0006	99.1	P<0.001
4	Hand-jetting	Winter	Diflubenzuron	6.28 ± 0.20	-	-0.0117 ± 0.0009	97.2	P<0.001
5	Pour-on	Flat	Triflumuron	8.97 ± 0.97	-4.20 ± 1.14	-0.0118 ± 0.0040	74.5	P<0.05
6	Pour-on	Winter	Triflumuron	9.30 ± 0.73	-4.83 ± 0.85	-0.0119 ± 0.0030	86.8	P<0.01
7	Hand-jetting	Winter	Triflumuron	6.47 ± 0.40	-	-0.0123 ± 0.0018	90.7	P<0.01
8	Hand-jetting	Winter	Diflubenzuron	6.90 ± 0.24	-	-0.0102 ± 0.0011	94.9	P<0.001
9	Hand-jetting	Winter	Ivermectin	3.36 ± 0.59	-	-0.0080 ± 0.0026	63.1	P<0.05
10	Automatic jetting race	Winter	Chlorfenvinphos	7.11 ± 0.47	-	-0.0163 ± 0.0021	92.5	P<0.001
			Diflubenzuron	5.91 ± 0.28	-	-0.0105 ± 0.0012	93.4	P<0.001

Collection and analysis of wool samples

Wool samples for pesticide analysis were collected from 10 animals selected at random from each group 24 hours, 27 days, 5 months and 12 months after treatment. Only pour-on treatment groups were sampled at 27 days. Backline samples were taken immediately adjacent to the backline at the middle of the sheep and mid-side samples from the standard mid-side site. Areas measuring 40 mm x 40 mm were shorn down to skin level using Oster Golden A5 clippers.

Samples from 10 sheep were combined to make composite samples for analysis. When sampling animals 24 hours and 27 days after treatment, samples were taken from both sides of animals and combined appropriately to give enough wool for side samples for analysis. For the samples taken 5 months and 12 months after treatment, individual staples were cut in half transversely to provide tip and butt portions for analysis.

To provide estimates of sampling variation, at 5 months 20 extra sheep from treatment groups 2, 6, 7, and 8 were sampled in a similar manner. The samples from these 20 extra sheep from each group were combined at random into 4 groups of five, to provide a further 4 sets of composite samples per treatment for pesticide analysis.

Representative, uniformly blended, composite samples for each treatment group, with butt and tip separate where relevant, were made by mini-coring (Van Pelt *et al.*, 1982) and air blending (IWTO-19-03, 2003). Pesticides were analysed at Canesis Network Ltd laboratories using solvent extraction, followed by GCMS or HPLC analysis. Detection limits were 0.1 mg/kg for diflubenzuron, triflumuron and chlorfenvinphos and 0.4 mg/kg for ivermectin.

Statistical analysis

Regression analysis

Only single analytical determinations were made for the 10-sheep composite samples.

Rates of decrease in concentration of pesticides in the fleeces were determined using regression analysis (GenStat, 2006) assuming an exponential rate of decrease. Concentration data were transformed to natural logarithms [$\log_e$(concentration+1)] and regressed against time since application to give a straight line relationship. Where butt and tip pesticide concentrations were measured for specific samples these data were first averaged to obtain an overall pesticide concentration before transformation. The significance of the effect of different sampling sites (back or side) was examined by adding sampling site as a fixed effect. Where this was not significant sample site was ignored. The slopes of the two lines for concentrations on back and side with time were examined to determine if the rates of decrease in concentration differed between the back and side of the animals. No such significant differences were detected. The half-life was calculated as $T_{½}=\log_e(0.5)/\text{slope}$, where slope was that from the relevant regression equations.

Analysis of variance

Pesticide data from samples collected 5 months after treatment were analysed by analysis of variance (GenStat 2006) after log transformation [$\log_e$(concentration+1)] to normalise the residuals. Initial analysis showed there were no significant differences between the concentrations in the 10-sheep composite samples and the four 5-sheep composites within each of the four treatment groups, and so the data were combined for analysis.

RESULTS

Predicted concentrations of diflubenzuron and triflumuron on the backs of the sheep immediately after pour-on treatments (time zero) were not significantly different, as were those at the sides (Tables 1 and 2). Concentrations at the back were much higher than those at the side sites in pour-on treatments 1, 2, 3, and 6 ($P<0.05$). In contrast, after jetting treatments 4, 7 and 8 with either pesticide there were no differences in initial pesticide concentrations at the two sites. Predicted concentrations one year after treatment at back sites were higher than those found at the side sites in treatments 1, 2, and 3 ($P<0.05$; Table 2).

Rates of decrease in triflumuron or diflubenzuron concentrations – represented by the slopes of the regression equations in Table 1 - on the backs and sides for both pour-on and jetting treatments were not significantly different. The mean slopes (±sd) of the regression equations for diflubenzuron and triflumuron treatments were -0.01152 (±0.000875) and -0.01198 (±0.000238), respectively. Calculated mean half-lives were 60 days (95% confidence limits (CL) 52-71 days) for diflubenzuron and 58 days (95% CL 56-60 days) for triflumuron. The calculated half-life for chlorfenvinphos was 43 days (95% CL 34-57 days).

Data in Table 3 show mean log transformed pesticide concentrations and the least significant difference ($P\leq0.05$) in the four treatment groups 2, 6, 7 and 8, five months after application of pesticides. Back transformed predicted concentrations are shown in parentheses. Concentrations of pesticides in the staple tip from the back of pour-on treatments were significantly

Table 2: Predicted mean concentrations (mg/kg) and 95% confidence limits of the initial (time = 0 days) and final concentrations after one year (time = 365 days) at the back and side sites obtained from the regression model. Where no data are given at the side site, data are the same as those for the corresponding back site.

Treatment group	Initial pesticide concentrations				Pesticide concentrations after 1 year			
	Back		Side		Back		Side	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
1	6,030	12,260 – 2,962	242	724 – 80	77	278 – 21	2.1	13 – 0
2	5,240	12,090 – 2,270	190	691 – 51	65	295 – 14	1.4	14 – 0
3	8,520	11,270 – 6,440	549	841 – 359	80	133 – 48	4.2	8.5 – 0
4	533	795 – 357	-	-	6.4	14.7 – 2.4	-	-
5	7,860	54,720 – 1,130	117	2,350 – 5	107	3,490 – 2.3	0.6	103 – 0
6	10,940	47,100 – 2,540	86	820 – 8	143	1,950 – 10	0.1	25 – 0
7	644	1,440 – 289	-	-	6.2	32 – 0.6	-	-
8	991	1,600 – 613	-	-	23	57 – 8.6	-	-
9	28	93 – 7.8	-	-	0.6	13.4 – 0	-	-
10	1223	3,130 – 477	-	-	2.3	18 – 0	-	-
	368	644 – 210	-	-	7.0	22 – 1.7	-	-

Table 3: Mean natural logarithm transformations of pesticide concentrations in samples from four treatment groups 5 months after treatment. Back-transformed pesticide concentrations (mg/kg) are given in parentheses.

		Body site					
		Back		Side			
Pesticide	Application method	Staple tip	Staple butt	Staple tip	Staple butt	LSD	Significance (overall)
Diflubenzuron	Hand-jet	5.78 (322)	1.08 (1.9)	5.45 (231)	1.16 (2.2)		
	Pour-on	6.74 (840)	2.84 (16.2)	3.62 (36.2)	0.36 (0.4)		
Triflumuron	Hand-jet	5.31 (200)	1.34 (2.8)	3.80 (43.7)	0.91 (0.70)	0.77	$P<0.01$
	Pour-on	7.92 (2,760)	3.52 (32.6)	2.35 (9.5)	0.77 (0.80)		

higher than concentrations in the tip samples from the side for both pesticides ($P<0.001$). There was no significant difference for the hand-jetting application of diflubenzuron ($P>0.05$) but for triflumuron this difference was significant ($P<0.001$). Concentrations of pesticides in the back tip and side tip samples in the four treatment groups were significantly higher than those in their respective butt samples ($P< 0.001$ in all cases). There was no difference in concentrations in the butt portion of the back and side samples of the hand-jetted groups ($P>0.05$). Differences in residue concentrations between the tip and butt on the back were greater than on the side. This difference was larger with pour-on treatments than with the hand-jetting treatments.

DISCUSSION

Similar initial concentrations of diflubenzuron and triflumuron, found at the back or side sites for both pour-on and hand-jetting treatment groups, were partly expected, as the same or similar amounts of each pesticide were applied in the different treatment groups for each application method. The initial concentration of ivermectin (28 mg/kg) from treatment 9 was much lower than the mean of 723 mg/kg from the hand-jetting treatments 4, 7, and 8. However, as about one-twentieth the amount of ivermectin was used in treatment 9 compared with that in other groups, this is expected.

Higher concentrations of diflubenzuron and triflumuron at the back compared with the side sites of pour-on treatment groups 1, 2, 3, and 6 immediately after treatment showed that these two pesticides did not spread evenly around the sheep after treatment. One year after application this pattern of concentrations was still present in treatment groups 1, 2 and 3, although concentrations were much lower. Jetting treatments showed a more even distribution of the pesticides on the backs and sides of the sheep both initially and one year later. This was expected as the pesticides were applied over a larger area of the sheep than for the pour-on treatments.

Lower pesticide concentrations in the butt of the staple compared with the tip at the back and side analysed 5 months after treatment, and still lower concentrations after 12 months, suggest that diffusion of both diflubenzuron and triflumuron along the greasy fibre is limited (Rankin, 2005). This behaviour is similar to that observed for deltamethrin after pour-on (Johnson *et al.*, 1995a) and plunge-dipping treatments (Johnson *et al.*, 1995b), and triflumuron after pour-on treatment (Russell & Nunn, 2001).

The observations also suggest that any movement of diflubenzuron and triflumuron around the sheep after pour-on treatment occurs during or shortly after application. A similar effect was observed by Johnson *et al.* (1995a) with deltamethrin. Pour-on formulations moved from the area of application within 24 hours of treatment and peak concentrations appeared on the upper and lower flanks about 4-11 days later. The mode of spread of pesticides applied by pour-on treatment is not known (Sinclair, 1985) but it seems likely that transcutaneous absorption through the skin and distribution of pesticides to the sebaceous glands in the skin, which excrete woolgrease onto the wool, is crucial for spread of the pesticides to wool on the sides of the animals.

The half-lives calculated for the decay of diflubenzuron and triflumuron were averages over a growing season, uncorrected for wool growth. They were higher than those observed for a range of organophosphate pesticides by Rammell & Bentley (1989) who found half-lives in crossbred wool fleeces varied (30-52 days (back), 39-58 days (side) in winter; 11-21 days (back), 13-24 days (side) in summer). The seasonal differences were attributed to photo degradation and temperature effects (Rammell & Bentley, 1990). Diflubenzuron is reasonably stable in sunlight (Tomlin, 1994), so such a half-life might be expected. The half-life for chlorfenvinphos was similar to the mean winter value (42 days) found by Rammell & Bentley (1989) in crossbred sheep and higher than the mean of the winter and summer values (24 days) that might approximate a half-life over a year. These differences in decay rates presumably arise from differences in fleece architecture between the breeds and possibly environmental factors.

The concentrations of diflubenzuron and triflumuron in back or side samples one year after treatment in the jetting groups were of the same order of magnitude as those observed in New Zealand Merino wool clips (Rankin *et al.*, unpublished results) and those observed by Russell & Nunn (2001) and Morcombe *et al.* (1999) in Australian Merino wool clips. Mass weighted mean pesticide concentrations for the pour-on treated groups were also of the same order of magnitude, and assumed the width of each band of pesticide was 80 mm and the remaining pesticides were spread over about 600 mm of the girth of the sheep.

Concentrations of diflubenzuron in butt samples from 5 months onwards, especially on the sides of animals, were close to or lower than the mean LD95 doses reported for susceptible - 0.31 mg/kg (95% CL 0.16-1.25 mg/kg) - and resistant lice populations - 4.4 mg/kg (95% CL 1.4-71 mg/kg) (James & Levot, 2005). Such low concentrations in the butt samples would have limited ability to kill lice. As this is the zone where louse nymphs feed, there would be reduced efficacy of treatments. Such effects are likely to decrease long-term parasite control and hasten the development of resistance in parasites to the chemicals involved.

The limited movement and diffusion of pesticides could explain (a) the less effective louse control with pour-on treatments compared to either of the jetting system treatments in the recent farm trial (Rankin *et al.*, 2005) and (b) why none of the treatments eradicated lice a year after treatment. The concentrations of pesticide were initially more uniform in the jetting than in the pour-on treatments. Thus control of parasites on the sides of jetted animals was better than for pour-on treated animals, resulting in better overall control. After treatment lice were often found on the sides of pour-on treated animals - where concentrations of pesticides were lowest. For all treatments, however, concentrations of pesticide in the wool near the skin decreased as the wool grew, to concentrations below those required to kill lice.

The limited spread of diflubenzuron and triflumuron after pour-on applications and similar decay rates during the trial may reflect the similar chemical structures and physical properties of these two pesticides. Both compounds belong to the benzoyl urea class of pesticides and are relatively water insoluble and lipophilic, with K_{ow} (octanol/water partition) logP values of 3.89 and 4.91, respectively - similar to that of deltamethrin (K_{ow} logP=4.6; Tomlin, 1994).

Other researchers (Johnson *et al.*, 1995a; Russell & Nunn, 2001) have raised concerns about the limited movement and diffusion of pesticides and the impact of this on parasite control and development of resistance. This study has shown that the IGRs diflubenzuron and triflumuron show similar behaviour to the SPs when applied in pour-on treatments. Thus, like the SPs, resistance to these IGRs may be developing through the use of the pour-on technique. The results from this study are important for researchers studying treatment efficacy and farmers trying to control pests

(Rankin, 2005; Rankin *et al.*, 2005). If researchers do not recognise that pesticide concentrations may be variable along a staple length they may incorrectly assume enough pesticide is present to control pests. Farmers alone may not necessarily be responsible for failures of louse control treatments when using pour-on IGR products, because the treatment method itself may lead to problems after continued use (Johnson *et al.*, 1995a). The sheep parasiticide industry may need to take a lead and find better ways to apply chemicals to where they are needed, and to maintain suitable concentrations in appropriate fleece zones. This will help improve louse control and help prevent premature development of resistance in external parasites.

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