Cypermethrin loss from sheep fording a stream

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1 INTRODUCTION

Sheep can suffer from a number of skin parasites that cause ill health and suffering, thus it is essential to prevent and control such infections. Dipping is the most effective method to treat a wide range of parasites[1], but there have been many pollution incidents over the past few years involving cypermethrin that have been thought to result from the use of sheep dips. Consequently, the market authorisation for cypermethrin-based sheep dips was suspended in February 2006.

Environment Agency investigations indicate a number of potential routes of exposure, including: 1) cypermethrin contained in runoff from hard surfaces within the farmyard, and 2) removal from the fleece when sheep enter streams. The current guidelines advise that sheep should be kept away from water courses for at least 2 weeks after dipping "where possible", thus acknowledging that this is not always practical. However, even after 2 weeks sheep dip product may remain on the fleece, particularly cypermethrin persists in the fleece for at least 3 months following dipping[2]. It is therefore feasible that fording streams could contribute to pollution incidents, but the magnitude of loss, and therefore the significance of this source is unknown.

This study was therefore undertaken to quantify losses of cypermethrin from fleece when sheep ford a stream and to assess the influence of drying time since dipping on such losses. In addition, cypermethrin loading to the farmyard from drips from the fleece was investigated. The significance of the farmyard as a source of cypermethrin is being investigated in a separate study (VMD02051).

2 METHODOLOGY

2.1 Removal from fleece and the influence of drying time

The test material was a microemulsion concentrate containing cypermethrin (high cis 80/20) 10% w/w for the control of blowfly strike, lice, keds and ticks (Auriplak supplied by Virbac UK). The dip solution was prepared by the farmer. Two litres of dip concentrate was diluted into 1000 L of water in a 350 gallon (1591 L) plastic, circular bath. Two samples (1 L) of the dip solution were taken before dipping began, and two samples were taken at the end of the dipping day for analysis. No further dip was added to the bath during the day, as the dip volume did not decrease significantly.

[1] www.defra.gov.uk/animalh/welfare/farmed/advice/posters.pdf

[2] www.noahcompendium.co.uk/Bimeda/documents/4029.html

The sheep were a mixture of pure Swaledales and Swaledale-Blueface Leicester mules, and they had approximately 3 weeks fleece growth since shearing. The sheep were dipped on 7 August 2006 by the farmer and his assistant at specified intervals following normal practice. After leaving the bath, the sheep remained in the initial drip pen (drain pen - where any dip draining from the sheep flows back into the bath) for 10 minutes in line with current guidelines; the end of the 10 minutes was the benchmark 'zero' hours drying time. The sheep were then directed into pens a few metres from the initial holding area where they stood for either 0.25, 0.5, 1, or 2 hours (Plate 1). Additionally, sheep were held for 4 hours in a fold or barn, or for 8, 24, and 48 hours in a barn. Three sheep were assigned to each pen and there were three replicates per dripping time, giving a total of 72 sheep.

After the required drying time, the sheep from a single pen/fold/barn were herded approximately 15 m through the yard (Plate 2), through the farmer's (empty) footbath and through a water-filled metal trough (simulating a stream) (Plate 3) after which the sheep returned to the field. The farmer's footbath was used solely as a funnel to get the sheep through the trough of water easily; the sheep were familiar with the footbath, they could not turn around once in it, and there was then only the "stream" between them and the open field. Creating this environment for the sheep negated the need for any handling of the animals.

The simulated stream consisted of a metal trough, 1.8 m long x 0.6 m wide x 0.3 m deep containing 100 L of water giving a water depth of 9.3.cm. Although 9.3 cm was an arbitrary depth it was a reasonable value and it approximated to the water depth (3") that the farmer considered, through experience, that the sheep were comfortable with; a greater depth could be dangerous for a sheep to cross under real conditions, and where a stream is deeper it tends to be narrower and sheep would jump across rather than go through a stream out of preference. The water depth also depended on the dimensions of the trough and the volume of water used. The dimensions of the trough were determined by the availability of standard materials (2.43 x 1.52 m metal sheets) and the fact that it had to be long enough that the sheep would not attempt to jump across to avoid the water. The volume of water was determined by the availability of water carriers that could be easily transported within the farmyard, and the need for a rapid turn-around between replicates when performing the experiment. Twenty-five litre jerry cans were identified as ideal for the task, thus four jerry cans were used to fill a single trough. Consideration was also given to the amount of waste-water generated, thus 100 L was deemed to be the most suitable volume on all counts. The jerry cans were filled with tap water in preparation for the experiment the day before dipping.

Plate 1 Sheep in pens for specified drying time

Plate 2 Sheep trotting through the yard towards the footbath and "stream"

Plate 3 The simulated “stream” after sheep have been through

After the sheep had exited the trough, the water was stirred and a sample was transferred to a glass bottle (1L) using a glass jug. The remaining water was pumped directly into waste containers for later disposal. The samples were stored in a cool box prior to transfer to the laboratory for analysis. The glass jug and the trough were cleaned with methanol and dried between sampling events; two troughs were used during the study. The trough was put back in place after cleaning and filled with another 100 L of water. Water added to the trough without sheep entering it was used to create control samples at the end of the day. This would provide an indication of whether any cross-contamination was occurring on re-using the troughs between tests. In addition, samples containing theoretically-known amounts of cypermethrin were created. Two volumes (5 ml and 9 ml) of a 50 µg ml^{-1} stock solution were added to the trough using a pipette. The water was then agitated and sampled as described above. This was conducted in duplicate.

2.2 Loading to the farmyard

Initially it was intended that any dip product released from the fleece after dipping but before fording the simulated stream would be collected on large metal trays for the different drying times. However, it became apparent that this caused too much distress to the sheep and this section of the study was aborted.

In order to gain some information on the loading of cypermethrin to farmyards after dipping, an alternative protocol was devised whereby cypermethrin was sampled from the concrete surface. Three main areas for comparison of loadings were identified: 1) PENS - the 5 pens used for holding the sheep for up to 2 hours, 2) NON-PEN – the area where the sheep walked to get to the pen and out to get to the trough, but where they did not stand there for any length of time, and 3) DRIP PEN - the area adjacent to the bath where the sheep stood for 10 minutes immediately after dipping.

Acetone (20 ml) was applied to an area of approximately 15 x 15 cm. A cotton swab was used to wipe and remove the acetone. This action was repeated to the same area using a further 20 ml of acetone, and within each pen/ non-pen area/drip pen, six areas were swabbed giving a total of 12 swabs used for a single composite sample.

There was a total of five pens. For one of the pens, the six sub-samples were split into three with each bottle containing swabs from two 15 x 15 cm areas. This was done to provide an indication of inter-pen variation in cypermethrin quantities. The findings could then provide some benchmark when comparing cypermethrin quantities from the non-pen, pen and drip areas. The farmyard swab samples were taken at the end of the day after dipping had ceased. The samples collected from the farmyard were: 2 x non-pen; 2 x drip area; 4 x pen ; 3 x 1 pen (inter-pen variation).

It is acknowledged that there would probably be some loss of cypermethrin to the concrete through sorption, but as all the surfaces sampled were concrete, comparisons between the sampled areas should be valid. Likewise, although the data would not provide figures of total loading (due to losses to the concrete), the data should be representative of the quantity of cypermethrin that may be available for removal from the farmyard. Similarly, although the farmer had dipped with cypermethrin the day before this experiment, and in the past (*ca*. 6 years ago), it is unlikely that losses to the concrete differed between sampling areas, thus comparisons between the sampled areas could be made. Swab samples from the farmyard surface were not taken prior to dipping as there had been no intention to sample the concrete at the start of the study, and it could not be foreseen that these samples would be required. To provide an estimate of the quantity of cypermethrin lost to the concrete a separate test was undertaken where a known amount of cypermethrin was applied drop-wise using a pipette to a 15 cm^2 and the area sampled after 1 hour as described above. Two volumes (1ml and 3ml) of a 50 µg ml^{-1} stock solution were used and the tests were duplicated.

2.3 Chemical analysis

Water samples (500 ml) were partitioned twice (2 x 50 ml) using dichloromethane to extract cypermethrin. Samples were partitioned for 1 minute and left to stand for 10 minutes. The extract was concentrated to dryness and re-dissolved in acetone prior to analysis by GC-MS. Control samples for water consisted of water collected from the trough without sheep entering it (see section 2.1). Acetone (500 ml) was used to extract cypermethrin from the swabs. The samples were shaken vigorously for 2 minutes and then placed in an ultrasonic bath for 30 minutes. An aliquot of the extract was transferred to a 2 ml amber vial for analysis by GC-MS. Control samples consisted of methanol-washed swabs.

Each batch contained a determination of at least one control sample extract and one fortified control extract that were prepared concurrently with the samples. The fortified control extract was prepared by adding a known amount of a cypermethrin solution to a control sample. Calibration standards were produced in the range of 0.01 µg ml^{-1} to 15.0 µg ml^{-1} in acetone using a stock solution of 1000 µg ml^{-1} of cypermethrin in acetone. All standards and stock solutions were stored within the range of –20 ± 5 °C. The instrumental conditions used for analysis are listed below:

Autosampler:	Hewlett Packard 6890 Series
GC:	Hewlett Packard 5890 with electronic pressure control
Detector:	HP 5971 MSD
Injector:	Split/splitless operated in splitless mode.
Column:	J&W DB5-MS 30m (nominal) x 0.25mm i.d., 0.25 µm film thickness
Injector Temperature:	250°C
Detector Temperature:	300°C

Oven temperature Program:	Initial temperature 100°C held for 1 min, then 20°C/min until 300°C, held for 5 mins.
Carrier Gas:	Helium in constant flow mode with inlet pressure of 6.4 psi at 100°C
Injection Volume:	2 µL
Ions detected:	m/z 163, 165, 181*, 209 * ion used for quantification

Linear regression was used to determine the best-fit straight line for the plot of peak area versus concentration of the external standard solutions. This best-fit line was then used to determine the concentration of cypermethrin for each sample and recovery sample.

All calibration graphs were linear over the standard ranges with the correlation coefficients ranging from 0.970 to 1.000. Recoveries were within the range of 75.2 to 162.8 %. The mean recovery values and relative standard deviations (% RSD) were also calculated from the individual recovery values and are shown in the Appendix. The lowest calibrated levels (LCL) were 10 ng L^{-1} for water samples and 5 µg for the swabs.

2.4 Data analysis

The data were analysed using the Tukey Method in Genstat v9 and linear regression in Microsoft Excel v9. Statistical analysis was performed on the raw data. To illustrate the results and for extrapolation of the results, the raw data was amended by multiplying the recorded concentration by the volume of water (100 L) and dividing this value by three, to give the mass of cypermethrin removed per sheep. Similarly, the raw swab data quantified the mass of cypermethrin in a total area of ca. 6 x 15 cm^2; the results were therefore converted to give a value in µg m^{-2}.

The theoretical starting concentration of the dip solution was calculated from: 1L of dip product that is 10% w/w in 500 L water equals 100 g of product in 500 L = 200 mg L^{-1}, assuming 1 ml = 1g. The theoretical total mass of cypermethrin in the dip bath was therefore 200 g.

3 RESULTS

3.1 Cypermethrin loss from fleece when fording a stream

The results (Figure 1) clearly illustrate that large quantities of cypermethrin are removed from the sheep when they ford water with a maximum loss of 1.5 mg of cypermethrin from a single sheep/replicate. Even after a drying time of two days, an average of nearly 0.2 mg per sheep could be removed. Significantly ($p<0.05$) more cypermethrin was removed after 0.25h compared to drying times of 4 hours and above. There was also significantly ($p<0.05$) less removal after 24h drying compared to 0.5 and 1h. The total mass of cypermethrin recovered in the water samples for the 72 sheep was 41 mg. The raw data for all the samples are given in the Appendix. Cypermethrin was not detected above 0.01 µg L^{-1} in most of the control water samples (taken to measure potential cross-contamination due to re-use of the trough), but some control samples contained 22 µg L^{-1} representing a maximum of 0.5% of the total detected. The variability between replicates was relatively low giving confidence in the findings.

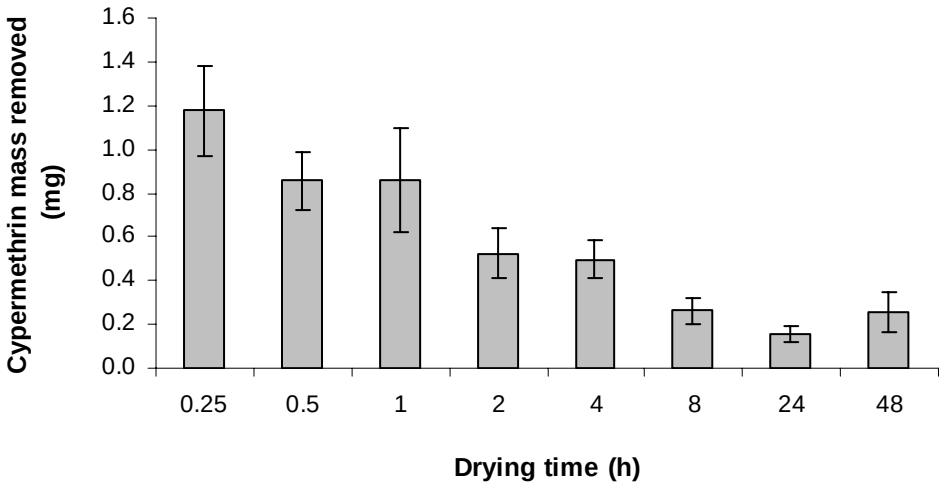

Figure 1 Quantity of cypermethrin removed <u>per sheep</u> during fording and the effect of drying time showing the mean ± 1 s.e.

3.2 Cypermethrin on the farmyard surface

The order in which the sheep were held in the pens was partly random, but it did depend on the position of the sun, and the sheep were kept out of the sun where possible for their comfort. The usage of the pens and the quantity of cypermethrin detected on the concrete surface is summarised in Table 1.

Table 1 Usage of the pens for holding sheep and the mass of cypermethrin detected therein

	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5
Drying time tests (h) in pen	0.5	1; 0.25; 0.5	2; 2; 0.5	0.25; 2; 0.25	1; 1
Total dripping time (h)	0.5	1.75	4.5	2.5	2
Total no. sheep	3	9	9	9	6
Mass of cypermethrin (mg m^{-2})	0.91	2.56	3.05	2.44	4.18

The detection of less cypermethrin in the pen that held only three sheep for half an hour compared to the other pens indicates that cypermethrin can continue to be released from the fleece even after the sheep have been left to drip for 10 minutes prior to being moved to the farmyard. However, a more specific relationship between the number of sheep/drying time and cypermethrin loading of the farmyard could not be established. Excluding the data from pen 1, there was significantly ($p < 0.05$) more cypermethrin removed from the pen area compared to the non-pen area supporting the general supposition that cypermethrin is lost from the fleece in the second holding area, although there are still some losses in the non-pen area even though the sheep may not be resident there for any length of time. Figure 2 illustrates that cypermethrin in the drip pen near the bath is ten times greater than that of the pen and non-pen area. Extrapolating the data by multiplying the quantities detected by the dimensions of the areas sampled, the calculated total masses of cypermethrin on the farmyard surface were *ca*. 150 mg in the drip pen; 30 mg in the pen area and 12 mg in the non-pen area.

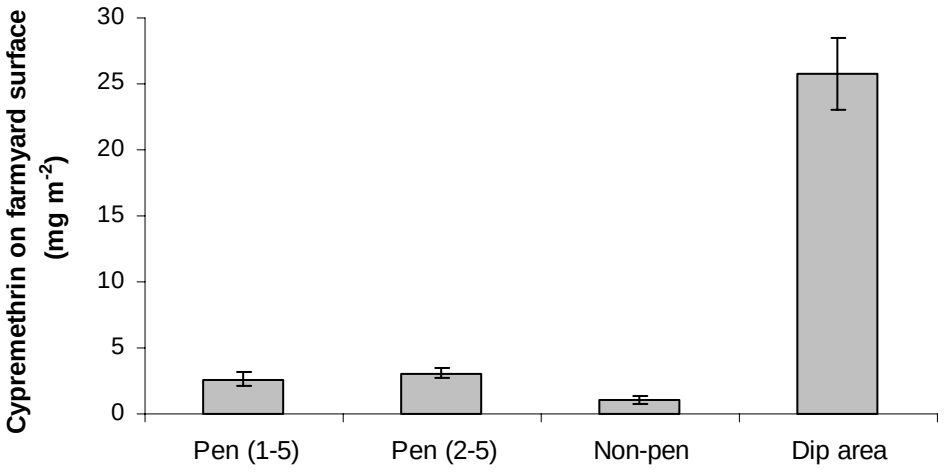

Figure 2 Cypermethrin quantities on the farmyard surface showing the mean ± 1 s.e.

The results from the swabs after applying a known amount of cypermethrin are inconsistent. Three swabs gave a value of ca. 120 µg which is higher than the 50 µg applied for two of them and lower than the 150 µg for the other swab. The other value of 175 µg was higher than the 150 µg applied. These findings could indicate that low levels of cypermethrin remain in the concrete after dipping and are removed in the presence of an organic solvent. Although attempts were made to sample an area where sheep were less likely to have been, this was difficult in practice as there was a limited area of the farmyard where the concrete was the same as the pen and non-pen area previously sampled.

The theoretical concentration of the dip solution was 0.2 g L^{-1}. This compares to the quantified concentrations of 0.04 and 0.03 g L^{-1} prior to dipping and 0.13 and 0.12 g L^{-1} after dipping The difference between the duplicate samples for each time point is small indicating that the results are valid. However, the concentrations are lower than expected, and it would ordinarily be expected that the concentration would be lower at the end of the day as the compound binds to faeces and other organic material entrained in the dip bath. Although the dip was mixed after preparation (using a crook-like pole), it is possible that this was not thorough and the solution was agitated more strongly when the sheep were in the bath.

3.3 Environmental significance

A similar approach to that used in the FOCUS surfacewater model, where the quantity of pesticide is diluted in a 100 m stretch of stream (volume = 30,000 L), was used to assess the significance of the quantities of cypermethrin removed in relation to water quality. The Environmental Quality Standard (EQS) of cypermethrin is 0.002 µg L^{-1} therefore only 0.06 mg of cypermethrin needs to enter the stream (30,000 L) in order to equal the EQS.

4 DISCUSSION

The results clearly demonstrate that cypermethrin is removed from sheep when they enter water. Whilst increasing the length of the drying time from a few hours to a few days did reduce the quantity of cypermethrin removed, the quantity removed was still in the order of hundreds of micrograms. Assessing the environmental significance of the findings illustrated that even after 2 days drying it could take only a few sheep to pollute the water, and a single sheep escaping to a watercourse within a few hours after dipping could potentially raise cypermethrin levels above the EQS. Even though the assessment approach was simple and no consideration was given to sorption to the sediment, dissipation, or dilution, it must be remembered that the results illustrated in Section 3 are for a single sheep with a short fleece. It is possible that, even accounting for dissipation within the stream, an entire flock fording a stream could contribute to cypermethrin pollution incidents. The basic

assessment of the environmental significance of the findings indicates that it may be prudent to assess the risks more accurately.

It could be argued that the water trough did not wholly represent a stream, as the water was static rather than dynamic, and it is possible that the mass of cypermethrin removed in the experiment was slightly less than in a dynamic system due to the absence of any erosive power. However, it is unlikely that any differences warrant the commitment of resources to pursue this matter further.

It is worthy of note that tail length could influence extrapolation of the findings. Swaledales have long tails (and horns) whereas cross-breds have short tails (see Plate 2 – the sheep at the front of the line is a cross-bred; the back sheep is a Swaledale). It is feasible that a significant amount of dip product can be soaked up by the tail and the product could then be released if the tail drags in water. This supposition is partly based on anecdotal evidence of farmers having to replace/top-up footbath water frequently when Swaledales are treated and the tail can be seen dragging through the solution. Similarly, the length of the fleece could be of significance. In the summer, sheep are often dipped a few weeks after clipping; some re-growth is required for the dip product to be retained on the sheep. In the autumn, the sheep will have a fuller fleece, and the longer the fleece, the more dip product that could be retained. In autumn there may be a higher loading to the farmyard compared to the summer (farmer's observation), and potentially, there will be more dip product available for removal from the fleece if it enters the water. Conversely, however, there may also be water available for dilution in the stream. In the absence of scientific evidence these theories are speculative and there is no information that could provide a guide as to the extent to which fleece length, or water depth, influence cypermethrin losses. The benefit of investigating losses from sheep with short fleece is that the data have illustrated the great extent to which contamination can occur, even in the absence of a full fleece. Nevertheless, as these variables can differ between flocks and the age of the animals, consideration should be given to such factors when extrapolating the results of this study to other farms/sheep breeds.

Small quantities of cypermethrin (23 µg L^{-1}) were detected in some of the control water samples indicating that the troughs may not have been thoroughly cleaned. However these concentrations represented 0.05 to 0.5% of the total detected for the relevant samples, indicating that it is unlikely that this contamination significantly affected the results. In addition, the spikes used for quality assurance purposes were effectively redundant. These levels were chosen on the grounds that they were considered to be close to environmentally-relevant concentrations, and there were no data to indicate that the values used (2.5 and 4.5 ng L^{-1}) would not fall within the range of the results. In practice, the values used were gross underestimates of the quantities of dip removed. Unfortunately, due to the nature of such field work, it was not possible to repeat the spikes. It was also considered not

worth the resources to reduce the calibration limit for spike levels that were so low compared to the working data. However, given the very high quantities of cypermethrin recovered and the low variability between replicates, there is no reason to believe that the main finding (significant quantities of cypermethrin are removed from sheep fording streams) is not valid.

The results of the test where known amounts of cypermethrin were applied to the surface and then removed by swabbing, did not demonstrate a relationship between loading and recovery, hence the results do not clarify the extent to which cypermethrin may bind to concrete surfaces. Furthermore, as the farmer had dipped with cypermethrin on the day prior to the current study, and had used cypermethrin-based dips in the past (ca. 6 years ago), it is not possible to categorically state that all the cypermethrin recovered from the concrete surface was from the current study. Nevertheless, it is possible to compare the data for the pen, non-pen and drip pens relatively as it is unlikely that any losses to the concrete would significantly differ between sampling areas, and the swab data provided useful information on the order of magnitude of cypermethrin on the concrete, data that were previously non-existent. Comparing the non-pen and the pen area, mean cypermethrin quantities detected were 1.0 and 2.6 mg m^{-2} respectively. The thirty-six sheep stood in the pen area therefore contributed 1.6 mg m^{-2}, a total of 8 mg. Even accepting the limitations of the swab data, the presence of sheep on the farmyard after draining can contribute milligrams of cypermethrin to the surface, thus the farmyard may also be a significant source of cypermethrin for surface water pollution. Furthermore, although the results indicated that the drip area had much higher quantities of cypermethrin per *unit* area than the farmyard, the dimensions of the farmyard/second holding area can be much larger than the drip pen so the total amount of cypermethrin on these surface may not differ as greatly as first appears. The overall hazard that the farmyard source poses may then depend on the proximity of the yard and associated drains to a surface water body. The issue of the farmyard as a source of cypermethrin has been considered as part of a Defra-funded study and the results are reported elsewhere[3].

[3] Sinclair et al., 2007. Cypermethrin losses from sheep farms. Report to VMD.

5 CONCLUSIONS

- Cypermethrin losses from fleece ranged from 1.5 mg (0.25 h drying time) to 0.09 mg (24 h drying time) **per sheep**.

- There were significantly lower ($p<0.01$) (but still high; > 0.15 mg per sheep) losses after drying times of 1 and 2 days compared to a few hours.

- Given the magnitude of cypermethrin losses after a drying for two days, it may be advisable to investigate sheep dip losses at intervals of 1 or 2 weeks, and even up to several months after dipping. Such **data are required to provide evidence-based guidance on the amount of time that sheep must be kept away from watercourses post-dipping**.

- As little as 0.06 mg of cypermethrin in a 100 m stretch of steam may be sufficient to exceed the EQS.

- Sheep fording a stream could contribute significantly to pollution incidents.

- The fleece contributes to contamination of the farmyard even after the recommended 10 minute dripping time.

- The farmyard may also be a significant potential source of contamination for surface waters.

6 RECOMMENDATIONS

The study has demonstrated that cypermethrin is easily removed from sheep fleece as they ford water. Diazinon will be the only sheep dip available for use once cypermethrin stocks are depleted, but it is not known to what extent this may contribute to pollution incidents from fording water. Diazinon is more soluble than cypermethrin and it has a lower potential for partitioning to organic matter thus it may be more readily removed. Similar tests to that described here are required to provide an indication of the significance of fording as a contributor to diazinon contamination of surface waters.

Considering the findings from the current study it may be advisable to extend the drying times to include 1 week and 2 weeks, particularly as the current guidance proposes that sheep should be kept

out of surface water for at least 2 weeks. It is possible that even after 2 weeks, sheep dip losses from the sheep may cause exceedance of the EQS, particularly given that the manufacturers state that the chemical can stay in the fleece for at least 3 months. It may therefore be prudent to also quantify losses from sheep after several months in order to establish the length of time that the sheep may be a contaminant source in relation to surface water exposure. One possible disadvantage of the longer drying times is that the results may over-estimate the quantity of dip available for removal if the sheep are kept indoors and therefore not exposed to the elements and/or dew-damp grass. However, in order to make direct comparisons between the shorter drying times (undertaken on a dry day) and the longer drying times, the sheep need to be kept indoors to reduce such variability. To investigate the extent to which dip may be removed by other processes such as rainfall or dew, tests could be performed for the 1 and 2-week drying period using sheep that are housed both indoors and outdoors, with the outdoor animals being held in large pens but away from surface water.

The results of the current study will need to be considered alongside the results of the farmyard monitoring study. Both these studies are providing new data and they should be used together to focus any further research requirements. It is likely that higher tier risk assessments will be required to extrapolate the findings more accurately.

7 ACKNOWLEDGEMENTS

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Opinions expressed within the report are those of the authors and do not necessarily reflect opinions of the sponsoring organisation. No comment within this report should be taken as an endorsement or criticism of any compound or product.

8 APPENDIX

Table 2 Recovery and %RSD of Cypermethrin from Fortified Water Samples

Fortification level (ng/L)	Recovery (%)	Mean recovery (%)	%RSD
50	116.1 137.5 146.7	133.4	11.8
5000	128.2 119.2 170.8	139.4	19.8
50000	119.1 117.8 110.5	115.8	4.0

Table 3 Recovery and %RSD of Cypermethrin from Fortified Swab Samples

Fortification level (µg*)	Recovery (%)	Mean recovery (%)	%RSD
10	85.4 85.1 91.4	87.3	4.1
250	86.3 87.4 87.4	87.0	0.7
4000	75.2 80.1 78.5	77.9	3.2

* based on 12 swabs per fortified sample in 500 ml extraction solvent

Table 4 Cypermethrin concentrations of dip solution

Sample number	Cypermethrin residue (g/L)	Fortification level (ng/L)	Recovery (%)	Control residue (ng/L)
1/DIP	0.04250	200	127.7	< 10
2/DIP	0.02910	200	127.7	< 10
26/DIP	0.13251	200	127.7	< 10
27/DIP	0.12149	200	127.7	< 10

Table 5 Raw data for the water samples

Sample number	Source (drying time (h) – Replicate No.)	Time of fording	Cypermethrin residue (ng/L)	Fortification level (ng/L)	Recovery (%)	Control residue (ng/L)
7/WT	0.5 - 1	08:35	18044	200	98.4	< 10
9/WT	1 - 1	09:10	17426	200	98.4	< 10
11/WT	0.25 - 1	09:40	36515	200	98.4	< 10
13/WT	2 - 1	11:00	11334	200	98.4	< 10
15/WT	2 - 2	11:30	13354	200	98.4	< 10
17/WT	0.25 - 2	12:00	23995	200	98.4	< 10
19/WT	1 - 2	12:30	20136	200	98.4	< 10
21/WT	0.5 - 2	13:00	30506	200	99.8	22.75*
25/WT	2 - 3	14:25	22399	200	99.8	22.75*
29/WT	0.25 - 3	14:55	45339	200	99.8	22.75*
31/WT	0.5 - 3	15:25	28417	200	99.8	22.75*
34/WT	1 - 3	16:00	39959	200	99.8	22.75*
35/WT	4 - 1	15:45	13948	200	99.8	22.75*
36/WT	8 - 1	17:05	4541	200	99.8	22.75*
37/WT	8 - 2	17:35	10715	200	147.3	< 10
38/WT	8 - 3	18:00	8284	200	147.3	< 10
39/WT	4 - 2	16:15	19726	200	147.3	< 10
40/WT	4 - 3	15:50	11064	200	147.3	< 10
43/WT	24 - 1	09:05	2631	200	147.3	< 10
44/WT	24 - 2	09:25	5541	200	162.8	< 10
45/WT	24 - 3	10:00	5859	200	162.8	< 10
46/WT	48 - 1	09:15	12692	200	114	< 10
47/WT	48 - 2	09:45	3026	200	114	< 10
48/WT	48 - 3	10:10	7528	200	114	< 10

Table 6 Raw data for water samples with known theoretical concentration

Sample number	Theoretical concentration (ng/L)	Cypermethrin residue (ng/L)	Fortification level (ng/L)	Recovery (%)	Control residue (ng/L)
3/WT	2.5	< 10	200	91.7	< 10
4/WT	2.5	< 10	200	91.7	< 10
22/WT	2.5	18.4	200	99.8	22.75*
24/WT	4.5	63.23	200	99.8	22.75*
41/WT	4.5	32.6	200	147.3	< 10
42/WT	4.5	24.28	200	147.3	< 10

Table 7 Raw data for swab samples

Sample No.	Cypermethrin residue (µg)	Source	No. swabs	Fortification level µg/L)	Recovery (%)	Control residue (µg/mL)
32	815	Pen 1	12	200	92	< 0.01
33	2306	Pen 2	12	200	92	< 0.01
5	2741	Pen 3	12	200	94.2	< 0.01
6	2197	Pen 4	12	200	94.2	< 0.01
8	875	Pen 5a	4	200	94.2	< 0.01
10	1239	Pen 5b	4	200	94.2	< 0.01
12	1644	Pen 5c	4	200	94.2	< 0.01
14	1179	Non-pen rep1	12	200	94.2	< 0.01
16	694	non-pen rep2	12	200	94.2	< 0.01
18	25624	Dip area Rep 1	12	200	94.2	< 0.01
20	20694	Dip area Rep 2	12	200	94.2	< 0.01

Table 8 Raw data for swab samples with known theoretical concentration

Sample No.	Cypermethrin residue (µg)	Theoretical concentration (µg)	No. swabs	Fortification level µg/L)	Recovery (%)	Control residue (µg/mL)
61	120	50	12	200	92	< 0.01
62	175	150	12	200	92	< 0.01
63	123	150	12	200	92	< 0.01
64	124	50	12	200	92	< 0.01