

**LIVESTOCK PRODUCTION PROGRAMME/ANIMAL HEALTH
PROGRAMME**

**ENVIRONMENTAL RISKS OF INSECTICIDE-
TREATED CATTLE IN SEMI-ARID LIVESTOCK
SYSTEMS**

FINAL TECHNICAL REPORT

R7539



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This project was accomplished through collaboration between the Natural Resources Institute, UK, the Tsetse and Trypanosomiasis Control Branch, Zimbabwe, and ECO-MARK (Agro-Evo) Zimbabwe. Funding was provided by the Animal Health and Livestock Production Programmes of DFID.

Executive Summary

- Pyrethroids applied to cattle for the control of tsetse fly can contaminate dung sufficiently to affect fauna utilising dung as a resource. Reduced abundance of insects, dispersal of dung and productivity of pasture are potential impacts of insecticide treated cattle.
- Work conducted at Rekomitjie Research Station (Mana Pools, Zimbabwe) confirmed the presence of pyrethroid residues in dung from a range of cattle treatments and products. Within days of treatment, between 0.01 and 0.1 ppm (wet wt) of pyrethroids were present in dung and there was no detectable loss of residues for 60 days in the field.
- These concentrations of deltamethrin were toxic to dung fauna for up to fifteen days from when the dung was dropped. Dung beetles and muscid fly larvae were susceptible to a range of pyrethroids, including formulations of deltamethrin, alphacypermethrin, cyfluthrin, cypermethrin and flumethrin. Deltamethrin was the most toxic to beetles and fly larvae. Adult muscoids and earthworms were less susceptible.
- A bioassay for dung residues was developed from laboratory and field trials with dung fauna. The true dung beetles (*Scarabaeidae*) and the larvae of *Musca lusoria* provided rapid and reliable assays of pyrethroid residues, saving the cost and wait of chemical analysis.
- Pat dispersal was studied using dung spiked with pyrethroids but no marked effects on dispersal rates were evident unless the insecticide was 10-100 times the LD50. Pat dispersal was also effected by trampling, bird, rodent and termite activity. Dispersal by *Microtermes spp.*, which were responsible for a significant proportion of long-term disposal, was affected when pyrethroid residues reached 1ppm but not at 0.1ppm.
- Deltamethrin residues were bound strongly to dung and were not readily transported to underlying soil.
- Predictive population modelling indicated that when cattle treatments occurred over wide-areas and for many months, the effects of residues on the abundance and distribution of dung fauna could be serious, threatening the slow breeding species (large dung beetles) and cattle frequenting muscoids.
- The route of contamination from treatment to dung was elucidated using mostly pour-on and dip formulations of deltamethrin (Spot-on and Decatix respectively) and employing the bioassay to ascertain the levels of contamination in the dung produced. Recommended applications of Spot-on and Decatix and other permutations showed the role of positioning of products and of grooming.
- By using Decatix instead of Spot-on, or by applying either to restricted areas of the body, the environmental risks of treated cattle to dung fauna were reduced. The optimum strategy may be to apply Decatix (shallow dipped or sprayed) only to the legs or belly, where most of the tsetse feed.

- Restricted applications reduce risks to dung fauna without compromising efficacy to tsetse. Significant cost savings (up to 90%) would accrue from reduced insecticide use and the switch from expensive pour-on formulations to water-based dip and spray products.
- Pyrethroid contamination of blood and milk was negligible (but restricted sample no.)
- Livelihoods of resource-poor farmers benefit through improved cattle production, stock and cultural assets, food security and opportunities for trading products.

[Three scientific papers have been produced as a result of this work – one published and two in draft. A forth is expected in August]

Background

Millions of square miles of E,W,S and central Africa are infested with tsetse flies whose transmission of trypanosomes seriously constrain livestock and crop production. State veterinary departments lack the funds and infrastructure to undertake widespread tsetse control in most of sub-saharan Africa. The promotion of community-based tsetse and trypanosomosis control by state and donor was seen as the solution to these resource constraints. One approach to tsetse control that has gathered momentum over the last ten years is the application of insecticide to cattle, either via pour-ons, dips or spray races. In rural areas, many livestock owners do not have access to cattle dips or spray races, leaving pour-ons as the Hobson's choice for rural-poor farmers in communal areas.

Both the EC and DFID support the development of insecticide-treated cattle techniques and Zimbabwe, Tanzania, Zambia, Uganda, Ethiopia and Burkina-Faso already have tens of thousands of insecticide treated cattle each.

Veterinary pesticides, especially the anti-parasitic avermectins, have been associated with negative faunal and ecosystem impacts in a wide geographical range of pasture systems. The environmental impacts of pyrethroids, which are now widely employed in pour-on and dip formulations for control of ectoparasites and flies, have not been a focus of livestock related research. Reports from farmers, research stations and the recent literature now point to a causal relationship between the use of insecticide treated cattle (for tick and tsetse control) and the mortality of dung beetles (Scarabaeidae). For example, deaths or reduced reproduction have been shown for Scarabidae and Muscidae in Australia (Wardhaugh et al., 1998), Denmark (Sommer et al., 2001) and Zimbabwe (Vale et al., 1999). Population modelling (Wardhaugh et al., 1998) suggests that the observed toxicities could reduce markedly the abundance of the insects, so upsetting their importance in dung dispersal and pasture productivity (Fincher, 1981).

The demand for research into side-effects of treated cattle is primarily driven by donors (especially DFID) and development agencies, who subscribe to the precautionary principle of protecting health and environments where risk cannot be assigned full scientific certainty. Responsible veterinary departments, such as those in member states of the Regional Tsetse and Trypanosomiasis Control Programme for Southern Africa (RTTCP) also recognised the need for the research, which emanated after trials in Zimbabwe showed high insecticide residue levels of 'pour-on' in dung (RTTCP 1999).

Dung beetles are important decomposers of animal dung and benefit plant and soil by recycling nutrients, aerating the soil, improving OM content, water retention and plant root penetration. Most work on dung beetles has focussed on the avermectins but the broad spectrum pyrethroids in faecal residues have the potential to disrupt a much wider fauna associated with dung, such as termite and fly spp. that also assist in the dissipation and incorporation of dung in soil.

Insecticides may gain entry to the animal via grooming and absorption through the skin. The presence of insecticides in the alimentary canal raises questions of consumer safety in the event that unacceptable residue levels in livestock products like milk and meat might accrue.

Project Purpose

To ensure that the promotion of insecticide-treated cattle for the control of tsetse and trypanosomiasis is ethical and the technology sustainable, such that residues in livestock products are not detrimental to consumer and environmental health. The project sought to understand the transport and fate of insecticide residues in cattle dung, the ecological significance of residues for dung fauna and dung dispersal, and to offer mitigation measures that reduce any negative environmental impacts.

Research Activities

The primary aim of the first year's activities was to confirm that contamination of dung was occurring at levels that were detrimental to non-target fauna and function. The second year would establish the route by which dung was contaminated, the chemical fate, and any perturbations on fauna. The third year would investigate ways of avoiding or reducing the contamination.

The categories of research activities listed below broadly cluster together a range of experiments: in practice there is considerable overlap.

- ❖ *Activity 1. Relationship between compound, application technique and residues in animal products.*
- ❖ *Activity 2. Fate of insecticide residues from deposition to dissipation in soil.*
- ❖ *Activity 3. Degradation of dung*
- ❖ *Activity 4. Acute toxicity of dung residues to non-target organisms (NTO)– key species and population level.*
- ❖ *Activity 5. Insecticide perturbation on ecological functions*
- ❖ *Activity 6. Mitigation options.*

General methods

All biological work was performed from January 2000 to March 2003 at Rekomitjie Research Station in the Mana Pools Game Reserve of the Zambezi Valley, Zimbabwe, where wild animals are abundant. The station maintains a herd of about 50 cattle that graze in the surrounding savannah woodland.

Local agro-chemical and public health companies provided the formulated products.

Insecticides

Pyrethroids were obtained as commercial formulations. These and their recommended rates of application to cattle were as follows:

- ❖ Decatix – 50 g l⁻¹ suspension concentrate of deltamethrin, to be diluted with water to a concentration of 0.05 g l⁻¹ as a cattle spray, or 0.0375 g l⁻¹ as a dip.
- ❖ SpotOn – 10 g l⁻¹ solution of deltamethrin in oil, as a pour-on applicable at 0.1 ml of formulation per 1 kg of body weight.
- ❖ Renegade – 15 g l⁻¹ suspension concentrate of alphacypermethrin, as a pour-on applicable at 0.15 ml per kg.

- ❖ Cylence – 10g l⁻¹ solution of cyfluthrin in oil, as a pour on applicable at 0.15 ml per kg.
- ❖ Sentinel – 150 g l⁻¹ emusifiable concentrate of cypermethrin, to be diluted with water to a concentration of 0.15 g l⁻¹ as a cattle spray or dip.
- ❖ Bayopet (concentrated form of Drastic Deadline) – 400 g l⁻¹ emusifiable concentrate of flumethrin. In dips or sprays for cattle the recommended concentration of flumethrin is 0.8 g l⁻¹.

At the recommended rates of application the quantity of active ingredient (ai) deposited per 100 kg of body weight would be 0.04 g for deltamethrin as Decatix, 0.10 g for deltamethrin as SpotOn, and 0.23, 0.15, 0.12 and 0.64 g for the formulations of alphacypermethrin, cyfluthrin, cypermethrin and flumethrin, respectively.

Chemical assays

Insecticide treatments were applied at 09.00h in the morning, to cattle of 395-485 kg restrained in separate cattle crushes. The cattle were then kept in separate but similar conditions and grazed during the day but held over night in pens that were washed down daily (concrete walls and floors). Part of the sample of dung dropped by each animal each day was weighed before and after drying to constant weight in a ventilated oven, to measure the proportion of dry matter. The remainder of each sample was deep frozen and sent to the Tobacco Research Board, Zimbabwe, or the Natural Resources Institute, UK, for residue analysis. Unless stated otherwise, all reported concentrations of insecticide in dung refer to mg kg (fresh wt) dung⁻¹

Bioassays

It was impracticable to maintain the many regularly treated animals needed to supply the large amounts of contaminated dung required for bioassays. Hence, contaminated dung was obtained by “spiking” the dung of untreated animals. For the oil formulations, ie, SpotOn and Cylence, the spike consisted of insecticide diluted with sunflower oil and then mixed into dung at the rate of 1 ml per kg. For the other, water-based formulations the spike was aqueous and mixed into dung at 10 ml per kg. Control dung was tested with and without the diluent alone, but since this showed no diluent effect the data were pooled.

Test insects for laboratory bioassays were freshly collected from the wild at 5-10 kg mounds of uncontaminated cattle dung. Field bioassays were performed within artificial pats, 15 cm in diameter and containing of 800 g of fresh dung, placed 10-15 m apart within 1.5 km of the station. Indices of insect abundance were produced by the numbers of insects found in uncontaminated pats, and in six pitfall traps operated for one day per week in most weeks between January 2000 and March 2001. The pit was lined with a plastic pot, 15 cm wide and 17 cm deep, provided with a non-return funnel and with dung suspended 5cm above in a netting bag.

Dipteran maggots in pats consisted mostly of *Eumusca lusoria* (Wied.) (Diptera: Muscidae). Beetle samples from the pats and traps contained some Histeridae, Carabidae and Staphylinidae, but were mostly Scarabaeinae, with the following genera being most numerous.

Aphodiinae and *Pedaria*-(many spp, not identified).

Anachalcos convexus,

Catharsius spp.

Copris – many spp., including *C. amyntor* Harold and *C. elphenor* Klug
Digitonthophagus – *D. gazella* Fabricius
Garreta – *G. nitens* Oliver
Onthophagus – many spp., including *O. vinctus* Er.
Onitis – many spp., including *O. uncinatus* Klug and *O. viridulus* Boheman
Sisyphus – mostly *S. goryi* Harold and *S. impressipennis* Lansberg

Dry matter

Dung collected in wet and dry seasons was dried to constant weight on tared glass dishes at 110°C in an oven.

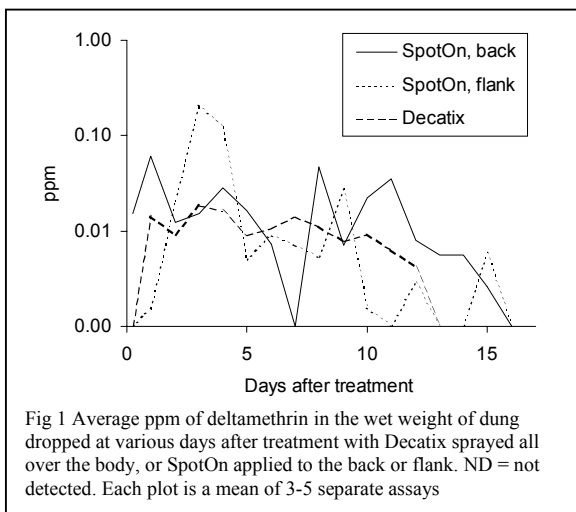
Results DM At the peak of the hot season of September to November, when the cattle were feeding mostly on dry grass, the proportion of dry matter in fresh dung was usually 20-24%. With the lush food available in the wetter weather, from late December to April, the dry matter declined, to be usually 15-19%.

Experiments and Results

Activity 1 Relationship between compound, application technique and residues in animal products.

Oxen were treated with deltamethrin at quarterly intervals between January and October 2000. At each time, one ox was sprayed all over with Decatix, another had SpotOn applied along the back, as normally recommended, and another had the SpotOn dose divided equally between each flank, as sometimes suggested to bring the insecticide closer to those lower parts of the body where most tsetse feed (Vale et al., 1999). A fourth ox was an untreated control. Treatment allocations changed between quarters, so that four different animals were used for each treatment type. Analyses were performed on dung collected a few hours before treatment and at intervals of 1-2 days for 16-17 days after treatment. No insecticide was detected in dung from the control animal, or from the other animals immediately prior to treatment -- the limit of detection being about 0.005 ppm.

Insecticide was always found in dung from treated animals on at least some of the days post-treatment. There was no evidence that the degree of contamination differed consistently between each animal, or from season to season, so the data for all animals and seasons were pooled within treatments. The results (fig. 1) indicate that Decatix produced the lowest contamination; SpotOn applied to the back gave peak contamination almost immediately, whereas the peak was delayed for a day or two when SpotOn was put on the flanks.



Residues in ox blood and milk

A veterinarian took blood samples at intervals from oxen that had been treated with Spot-on some days before. No residues of deltamethrin were found in the blood samples (detection limit 0.0007ug g⁻¹).

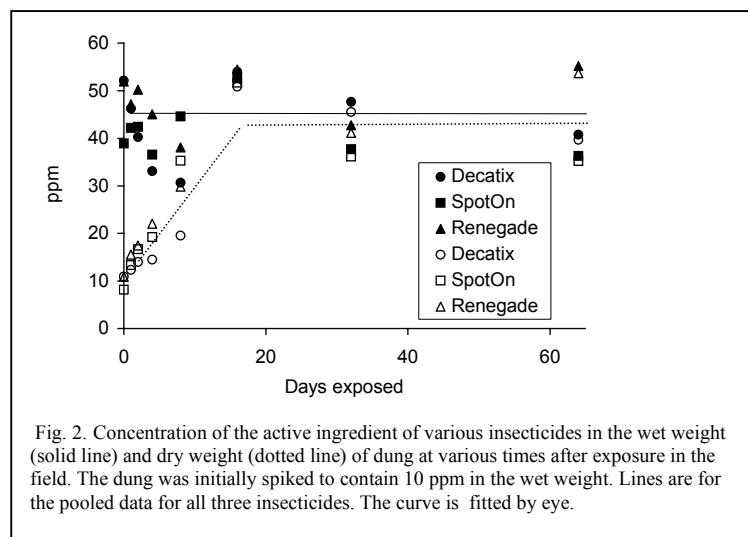
Milk samples were only taken once over a short period of days following treatments with Spot-on and Decatix. Extreme care was taken to reduce the likelihood of mechanical contamination from sampling procedures (for both blood and milk). Milk samples were taken during the F&M outbreak in UK and samples had to be pasteurised by heat before shipping. Although residues were found at or below detection limits, some doubts over a possible loss of deltamethrin by volatilisation when heated to 60C was noted. The difficulties of shipping cattle products to UK persisted and the trial was not resumed. A possible solution would have been sterilisation of milk by formalin – but shipping and DEFRA regulations scuppered the plan.

Activity 2. *Fate of insecticide residues from deposition to dissipation in soil.**Pyrethroids in dung*

Samples of cow dung were spiked at 1ug deltamethrin g⁻¹ wet wt (Spot-on formulation). Fourteen sub-samples of 20g were exposed for 21 days (max) to ambient conditions outside the research lab. A roof was provided to keep off the rain but it was high enough to allow UV radiation. Two samples were analysed for deltamethrin residues at day zero, 4, 8, 12, 16, 20, and 21. Percentage recovery of deltamethrin was between 88% and 98% (no significant difference) indicating that no degradation of deltamethrin was taking place in cow dung for up to 21 days. [Checks of spiking recovery rates accompanied all experiments = 93%-105%]

Dissipation of deltamethrin to soil Dissipation and transport of deltamethrin from dung to soil was evaluated with dung spiked at 1 and 0.1 ug g⁻¹ wet wt. with Spot-on and Decatix formulations. After preparing four chosen savanna sites by loosening soil, three 300g replicates of spiked dung were placed at each site and watered three times a day to emulate wet season punishment i.e. the worst –case scenario. After 3 days the the soil was sampled under the pats (top 5cm soils; diameter 30cm),

sieved and analysed for deltamethrin. Very little deltamethrin had leached into the soil after 4 days of exposure (0.006ug g⁻¹ d.w. for Spot-on and Decatix under 1ppm spiked pats; ten times less (at lower detection limit) in soils under 0.1ppm spiked pats – the highest level that has been found in cattle dung). [lower limits of detection: 7ng g⁻¹ wet wt].

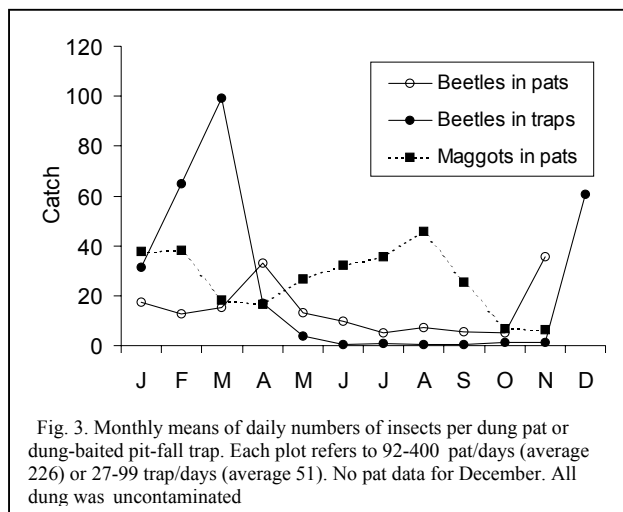


Pyrethroid stability in dung

The fact that pyrethroids are not quickly degraded in dung was also suggested by the daily collection of dead beetles found near contaminated pats for about week after pat production. The numbers of beetles found dead is affected by many factors, such as the drying of the pats and the succession of colonisation, so confusing the indications for chemical stability. To allow direct chemical assays, control dung was spiked with deltamethrin (Decatix and SpotOn) and alphacypermethrin (Renegade) and placed as artificial pats in the field in August 2000. The spiking concentration was high, at 10 ppm, so that proportional changes in concentration could be measured more confidently. Whole pats were removed for analysis after 1, 2, 4, 8, 16, 32 and 64 days. The results (fig. 2) suggest no change in the concentration of pyrethroids in the dry matter, although the concentration in the wet weight increased 4.3 times because the pats dried during the hot rainless period of exposure.

Activity 3 Degradation of dung

Since all test insects were wild-caught their seasonal availability determined their availability for use. Availability was investigated by deploying 1-2 uncontaminated pats at 08.00h and 15.00h daily in most months between Jan and Nov 2002, and counting the numbers of insects found in the pats a day after deployment. At all seasons the number of beetles in pats deployed in the afternoon was greater than in pats deployed in the morning, on average 12% greater. In contrast, the number of muscoid larvae in the afternoon-deployed pats was on average 52 % less than in the morning-deployed.



the month to month patterns in numbers were the same for the morning and afternoon, and so the data for both times of day were combined. Moreover, the indications were the same in each year, so the data for all years were combined. The results (fig. 3) are shown with data from the pitfall traps.

In theory, the number of beetles caught in traps should be greater than the number counted in pats, because traps are intended to catch all arriving insects whereas the counts at pats are reduced by insects leaving. However, theory was invalidated by observations that many beetles, especially the smaller ones, flying away from the traps, having not fallen into the pit. This may explain why the pat counts were greater than trap catches during dry weather, from April to October, when the predominant beetles were small dung-dwelling species such as *Aphodius*. Trap catches were greater than the pat counts in the wet weather, when many large dung-rolling and tunnelling beetles occurred, but this may not be due entirely to the higher efficiency of their trapping. The rollers

(Telecoprids) and tunnelers (Paracoprids) can remove much dung from a pat, so perhaps reducing its effectiveness as an attractant and dwelling for other beetles. (As the pats dry out, so their attractiveness for all except the Endocoprids) is reduced, which is why the afternoon pats are more attractive, as evaporation of the volatiles is less).

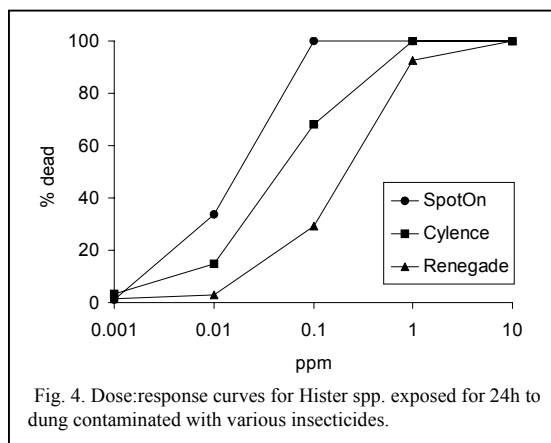
Activity 4. *Acute toxicity of dung residues to non-target organisms (NTO)– key species and population level.*

The main indications from Fig 3 (beetles in pats) are that bioassays should rely on beetles and maggots in the wet season, and more on maggots for most of the dry season.

A series of rapid and reliable bioassays was developed which were used to elucidate the sensitivity and impacts of insects that use dung as a resource. Laboratory test organisms were chosen according to role, availability and abundance and easy of handling observed under Activity 3.

Laboratory Bioassays

Replicated groups of 10-20 adults of each of six taxa of beetles, and of muscoid (as we are not sure of their composition) larvae were placed with 200 g of contaminated dung in enamel dishes 25 cm in diameter and 8 cm deep, covered in netting (in separate pots). The total numbers of insects of each taxa/group used with each concentration of each insecticide averaged 85 (range 40-200). After 24h the insects were removed from the dung and examined to assess the number mortally affected, i.e. dead or moribund, as evidenced by failure to escape from a tilted pot in two hours. Of the insects classed as dead, the proportion moribund was 9.8% for maggots and 59.6% for beetles. The proportion of untreated control deaths was less than 1% for each group, so no correction for controls was made. With the treated insects the deaths showed a typical sigmoid relationship with the log of insecticide concentration (Finney, 1964), as shown in fig. 4 for *Hister* spp. The LD_{50}^{24} was calculated by probit analysis (*loc. cit.*) and was mostly of the order of 0.1 ppm. Hence, the significance of observed effects of various insecticides and test insects on the



LD_{50}^{24} was calculated by accumulating the values of chi-squared for the homogeneity of the distributions of affected and unaffected insects at concentrations of 0.01, 0.1 and 1 ppm.

The results (table 1) show significant effects of insecticide type, with flumethrin being the usually the least toxic and deltamethrin, as SpotOn, being on average 15 times more toxic (although not *Digitonthphagus*).

Deltamethrin in Decatix was one sixth as toxic as in Spoton. With each insecticide there were significant differences between insects. The predatory *Hister* spp. (Histeridae) were more susceptible than some of the true dung-feeding insects.

Sisypus goryi and *S. impressipennis* were chosen for bioassay work, as both were abundant, and produced dung balls. Production rates of balls in bioassay dishes were found to be sensitive to insecticide. For example, in SpotOn tests the average number of balls produced by 10 beetles was 0.48 per beetle for the control, and 0.42, 0.08, 0.02, and 0.00 at concentrations of 0.001, 0.01, 0.1, and 1 ppm, respectively. The wet weight of the balls was not significantly affected by any type of insecticide, and averaged 0.37g (N = 527). However, these data ignore the fact that at high concentrations of insecticide many small incomplete balls seemed to be produced.

Insect	Insecticide					
	SpotOn	Decatix	Renegade	Cylence	Sentinel	Bayopet
<i>Copris amyntor</i>	0.04	0.17	0.08	0.11	0.21	0.62
<i>Digitonthphagus gazella</i>	0.01	0.27	0.12	0.04	0.61	1.52
<i>Hister spp.</i>	0.01	0.12	0.19	0.05	0.06	0.13
<i>Onitis westermanni</i>	0.14	0.45	0.50	0.10	0.16	1.04
<i>Sisypus goryi</i>	0.13	1.82	1.22	0.11	1.13	0.78
Miscellaneous beetles**	0.02	0.04	0.08	0.10	0.06	0.27
Muscid larvae	0.11	0.37	0.32	0.29	0.45	1.56
Geometric mean	0.042	0.252	0.223	0.097	0.234	0.634
Relative toxicity	1.00	0.17	0.19	0.43	0.18	0.07

Table 1. LD50 of muscid larvae and adults of various beetles exposed for 24h to dung spiked with different insecticides* (in each column and each row the LD50 values are significantly heterogenous at $P < 0.001$)

** Scarabidae, including *Euoniticellus intermedius* and *Kheper* spp.

Field Bioassays

Adult beetles

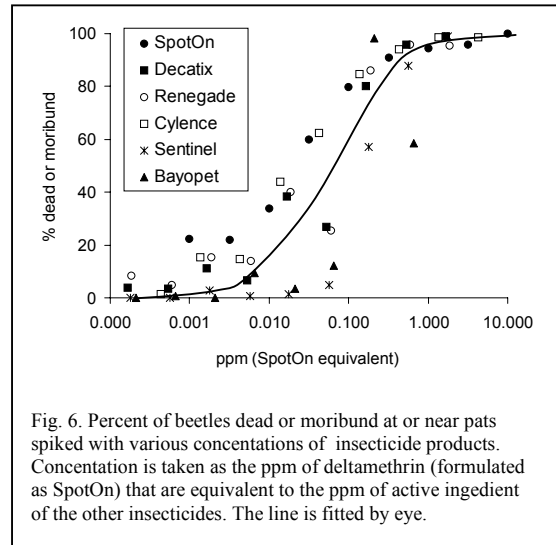
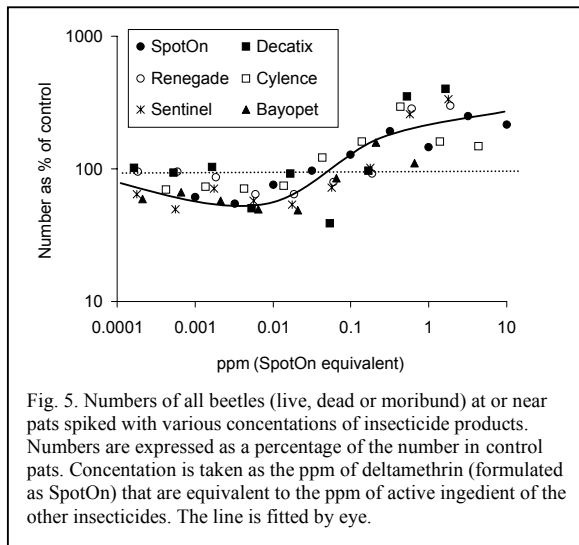
Spiked and untreated control pats were placed individually in the centre of plots of bare soil, 1m in radius, with a range of 4-46 (average 19) pats used to assay each concentration of each insecticide. Pats were visited 2-3 times during the following 24h to remove and record dead and moribund beetles found on the plots. Such visits were necessary to pre-empt much removal by ants and other predators. One of the visits was always at 20.00h, ie, an hour or so after the dusk flight period (peak arrival of beetles at the pats when most beetles were found). At 24h after deployment the dung was broken up and inspected, to record the numbers and condition of beetles in it. At the uncontaminated pats, 91% (N = 141) of the dead and moribund beetles were inside the pats. In contrast, at the contaminated pats most dead and moribund beetles (79%, N = 7710) were outside, mainly within 10 cm. The edge of contaminated pats often became "shredded", apparently because many beetles kept burrowing in and out as if being temporarily repelled after entry.

The various insecticides produced a similar pattern of effects, although the concentration of insecticide associated with a particular level of mortality obviously varied between insecticides, as expected from the laboratory indications of relative toxicity. Hence, to expose the basic pattern, all insecticide concentrations were plotted as their effective equivalent concentration of Spot-On formulated deltamethrin, using the relative toxicity data of Table 1.

With the untreated control, the total number of beetles either alive and moribund and in positions within and around the pats averaged 16.4 per pat. The numbers in the 'spiked' pats (fig. 5) began to decline as a percent of the untreated control when the concentration of active ingredient increased to a deltamethrin equivalent of about

0.001 ppm, and were halved at a concentration of 0.01 ppm. Thereafter, the numbers increased rapidly. The percentage of dead and moribund beetles among the total was always low with the control, averaging 3.9%. With the spiked pats (fig. 6) the percentage started to rise at about 0.01ppm, reaching 50% at about 0.1 ppm, i.e., approximately the LD₅₀ indicated by laboratory work (Table 1, SpotOn).

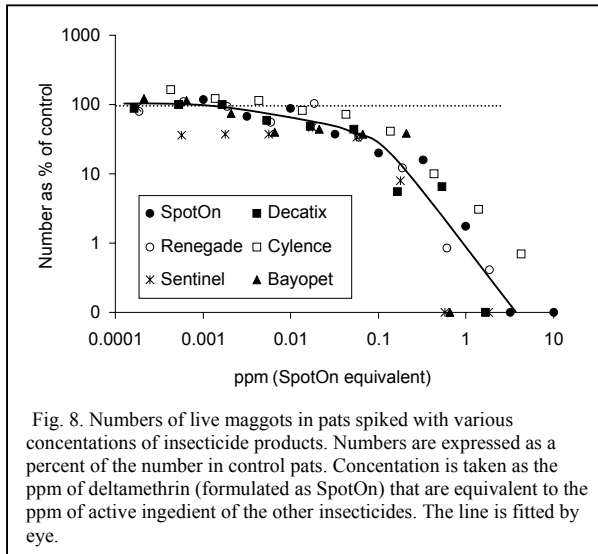
It appears that the pyrethroids are mildly repellent at concentrations of about one tenth of that required to produce detectable mortalities. Once high mortality starts the number of beetles at the pats increases substantially. However, there is no need to suppose that heavy contamination attracts beetles to a pat. This can be explained by the fact that by killing the beetles merely precludes the departure of many beetles from the dung that normally stay only briefly.



Predation of beetles

The extent to which dead and moribund beetles were removed from the pats and surrounding plots affects the precision of the field bioassays and the degree to which knocked down insects can be regarded as effectively killed. Predation rates were examined in February and March 2003. At 10.00h, 240 dead beetles were placed on patches of bare ground, 25 cm in radius and 1 m apart. The beetles were small (5-7 mm from head to tip of abdomen), medium (8-14 mm) or large (15-25mm) and were killed by brief dipping them in boiling water, or by overnight exposure to dung contaminated with 1 ppm of deltamethrin, as SpotOn. This made six treatment groups, of 80 beetles in each. The beetles were examined at 1, 2, 4, 10 and 24 h after deployment, to record the number of beetles removed, i.e., not within 25 cm of the deployment position. Most of the beetles were removed by ants.

The rates of removal (fig. 7) were examined by a chi-squared test on the numbers present and absent after 24h. This indicated significant ($P < 0.001$) heterogeneity, due mainly to the relatively slow loss of large beetles, especially those killed by insecticide. The size effect is attributable to the fact that the small and medium beetles could be taken away intact, whereas the larger ones had to be dismembered first. It seems that the presence of insecticide interfered with the dismemberment but had little effect on intact transportation.



started to decline at concentrations of a.i. equivalent to 0.01 ppm of SpotOn-formulated deltamethrin, and were halved at concentrations of about 0.1 ppm, ie, the LD50 evident in laboratory assays (table 1).

Adult flies

Some of the above effect on larval numbers could be due not to the death of larvae but to adult females declining to breed in contaminated pats. Repellence of Spoton and Decatix to adult Diptera was investigated by placing contaminated and uncontaminated pats 30 m apart, and counting the numbers of muscoids and Scathophagids seen on each at two-minute intervals, for one hour on each of three separate days with each insecticide at each concentration. The results did suggest some repellence, but only at 10 ppm, when the total number recorded on the treated pats were 322 muscids and 1015 scathophagids, as against 444 and 1804, respectively, on the uncontaminated pats. Such poor evidence of repellence would be expected if most flies normally stayed only briefly at pats.

The ability of the deltamethrin-contaminated pats to knock down adult flies was investigated using glass tubes, 2.5 x 7.5 cm, with netting covering one end. The open end of a tube was placed over single muscoids seen on field-deployed pats, so that the floor of the tube was dung. The fly then spent some of its time on the dung and some on sides or top of the tube. After the fly had rested on the dung for a cumulative minute the tube and fly were removed and the fly was transferred to another similar tube that had not touched the dung. After corking the base of this second tube it was held in a polystyrene box until transferred to the laboratory, where knockdown was recorded 2 h later, i.e., about 3 h after exposure. The results for Spoton were similar to those for Decatix, so the data for both insecticides were combined, giving sample sizes of 122-232 for each concentration. The percent knockdown in the untreated controls at 0.001, 0.01, 0.1, 1 and 10 ppm were 0, 2, 4, 14, 22 and 51% respectively, suggesting that adults were far less susceptible than the maggots considered above.

Assays with adult muscoids are unlikely to provide sensitive indications of pat contamination.

Muscid larvae

In the above work the numbers of muscoid larvae in the pats were also recorded at 24h. The proportion of dead and moribund larvae was misleading since such individuals soon rot as to be unrecognisable. There are also highly predatory fly larvae inhabiting the dung, which will have an effect on total larval numbers found. Hence, it is best to focus only on the numbers of live larvae. In the control pats the numbers averaged 24.7. In the spiked pats (fig. 8) the numbers

Colonisation by fly larvae (maggots)

Since the maggots seem highly susceptible to contamination it is unfortunate that their susceptibility can be scored only indirectly, by measuring the reduction in pat colonisation. Given the seemingly random elements in the number of maggots per pat, many pats need to be examined to get a reliable indication of, say, a 20% reduction. In contrast, the beetle work suggested that relatively few pats need to be examined to indicate more directly that mortality has risen by 20%, since the percentages seemed little affected by variations in the numbers of insects per pat.

The maggot assay might be improved by identifying and removing the causes of much of the apparent randomness in maggots per pat. Work to elucidate to elucidate some of the parameters showed that much of the variation in colonisation was due to changes in the dung itself. For example, some animals could consistently produce dung that became well colonised, and then, often in just one or two days, the dung would become colonised consistently poorly, despite high colonisation of dung from other animals. Usually, the reduction occurred when the proportion of dry matter increased to above about 25%, and the colonisation returned to former levels when, eventually, the dung became more watery. The mere addition of water to dry dung did not ensure good colonisation.

Activity 5: Insecticide perturbation on ecological functions

A significant part of this planned activity had to be abandoned because of the political difficulties in Zimbabwe. [Measurement of soil fertility (N,P,K; and soil permeability) in two adjacent farms - one treating cattle with pour-ons the other cattle ranch not - was precluded as farmers abandoned their normal practices in the anticipation of farm take-overs] The programme manager was notified and the resources were devoted to pat dispersal and ecological modelling (paper appended).

Pat dispersal

The effect of contamination on the overall rate of pat dispersal was gauged as follows: Pats spiked with Decatix and SpotOn were deployed in the bush and 10 or 100 days later they were recovered - lifting what remained of them within 50 cm of the deployment site. The remains were often found fragmented, mixed with soil and honeycombed with burrows, so making it difficult to assess objectively how much of the original pat remained. This was overcome by first drying and grinding the soil/pat mix and putting it in a plastic tub of 1120 ml capacity. Dried, ground and sieved soil from the vicinity of the deployed pats was then added to the tub, tapping frequently to ensure that the added soil filled all available space. The tub and its contents were then weighed. Given that the specific gravity of dry soil-free dung was 0.42 compared to 1.61 for soil, it was possible to calculate the volume of dung present. The amount of dung lost during deployment was then calculated by reference to the average volume of pats that had been kept in the laboratory, and hence subject to no dispersal.

Samples of 58 uncontaminated pats were studied in the wet and dry seasons. Sample sizes were 29 for each concentration of each insecticide. The degree of pat dispersal of each sample was indicated by its average percent lost. As it was difficult to compare these averages *per se*, since within samples the distribution of the individual percents was often bimodal, peaking near 10 and 90%, statistical comparisons were made of the proportion of individual values below 50%. This indicated no significant ($P > 0.05$) differences between SpotOn and Decatix and so data for both insecticides

were combined, giving samples of 58 pats per concentration, i.e., the same as for the uncontaminated controls.

Dispersal of the untreated control pats showed no significant seasonal effect at 100 days, although at 10 days the dispersal in the dry season was significantly less than in the wet season (fig. 9). This effect may be associated with larger beetles since these insects seemed to produce most of the short term dispersal and they were relatively scarce in the dry season. At both seasons and both durations of deployment, dispersal was not reduced until insecticide concentration reached 0.1 ppm. None of the observed reductions were significant at 10 days in the dry season. At 10 days and 100 days in the wet season the reductions were significant at concentrations at and above 0.1 ppm. At 100 days in the dry season the reductions were significant only at 1 ppm and above.

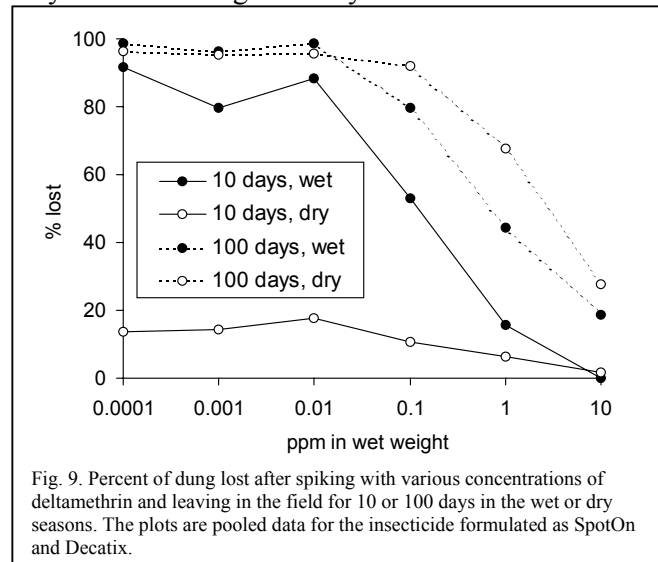


Fig. 9. Percent of dung lost after spiking with various concentrations of deltamethrin and leaving in the field for 10 or 100 days in the wet or dry seasons. The plots are pooled data for the insecticide formulated as SpotOn and Decatix.

The insecticide concentration needed to halve the normal degree of pat dispersal was around 0.5 ppm, i.e., about 10 times more than might have been expected from the previous indications of the concentrations needed to halve the number of active beetles and muscoid larvae in pats. This is partly explicable by the fact that not all pat dispersal was due to insects. For example, some pats were trampled by elephants, others were broken apart by birds and rodents, and fragments were borne away by wind and rain. Moreover, much of the dispersal caused by insects may, especially in dry season, be due to creatures other than beetles and flies, i.e., termites.

Termites

To focus on dispersal by termites alone, plastic plant-pots of 540 ml capacity were level-filled with dung and topped by aluminium foil to protect against wind, rain, beetles, flies and small vertebrates. The pots were set into the soil, so that the top was at ground level. This ensured that the dung could not be trampled by large animals. Termites could access the dung via six “drainage” holes, 5 x 10 mm, in diameter at the base of the pot, below ground. To compensate for the small area of contact between the dung and soil, and for any resulting delay in dung location by termites, 25 ml of dung were placed immediately below the pot and connected to four channels of dung, 1 cm in diameter and 25 cm long, radiating away from the pot about 5 cm below ground.

Potted dung was deployed for 100 days in the dry season, during which time it was occupied mainly by *Microtermes* spp. (Isoptera: Termitidae). As in the previous study there was no effect of SpotOn as against Decatix, so the data for both insecticides were combined, giving samples of 24 pots for each concentration of deltamethrin and for the untreated control. The loss of untreated control dung averaged 64%, indicating that termites can be responsible for most of the long term dispersal. For the contaminated pats the losses at 0.001, 0.01, 0.1, 1 and 10 ppm were 68, 69, 64, 40,

and 8 respectively, with losses becoming significantly less than the untreated control at 1 ppm ($P < 0.5$). A little more than 1 ppm seemed necessary to halve the dispersal.

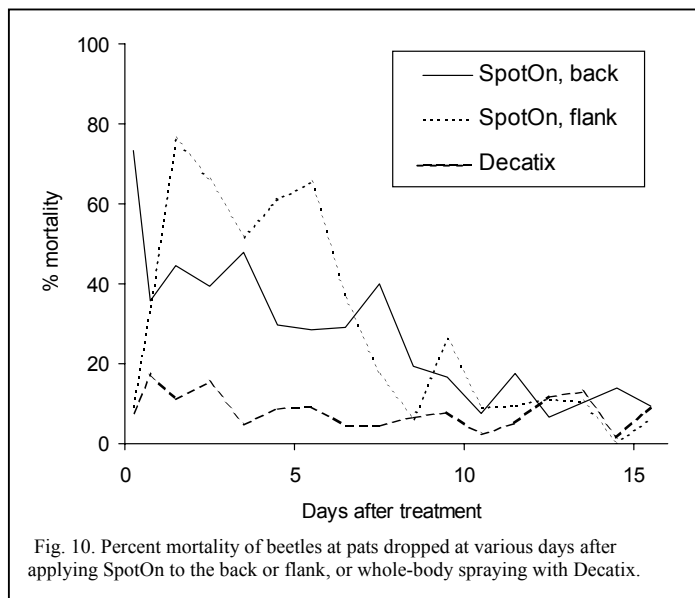
Activity 6. Mitigation options.

Options to reduce the toxicity and level of contamination in dung included the investigation of alternative insecticides, formulations and applications. Beetle and maggot bioassays of dung were used to gauge the outcome of the treatments.

Test oxen used had a high proportion of indigenous blood, and weighed an average of 340 kg (range 160-500). Treatment sessions occurred at intervals of one to two months, from January 2000 to April 2002. Each session involved four separate oxen, one of which was a control and three of which were treated. Each treatment was repeated on 3-5 separate oxen, in different sessions. Treatments were given at about 09.00 h, to the oxen restrained in individual crushes. The animals were then grazed separately during the day and held separately at night, in pens with cement floors and wire-fences that were scrubbed each day.

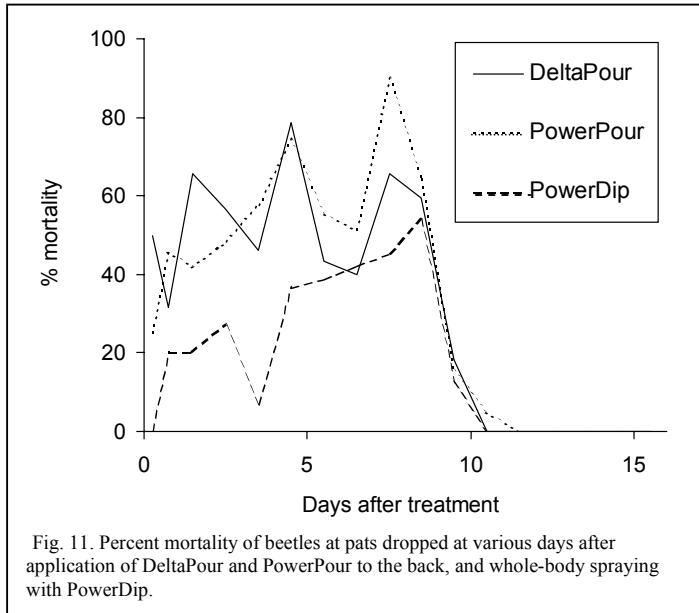
Recommended rates of Decatix and SpotOn.

Beetle assays associated with the treatments (fig. 10) show that mortality with SpotOn on the back was high in dung dropped on the first day after treatment, and declined gradually thereafter. For SpotOn applied to the flanks the mortality was initially low, peaking after a few days. With Decatix sprayed over the whole body the mortality was usually lower than with either of the SpotOn treatments, although by a week or so after treatment there was no clear difference between the low mortalities with any of the insecticides. The indications from these bioassays accord well with the chemical assays, allowing that the toxicity of deltamethrin per g. a.i.⁻¹ (equivalent concentration) can often be a little less in Decatix than in SpotOn.



DeltaPour, PowerPour and PowerDip. – In May and July 2001, oxen were sprayed all over with PowerDip or given the pour-ons along the back. As in above work with SpotOn and Decatix, the two pour-ons produced mortalities that were greater than with the spray in the first week, although the difference between the two types of application was now less marked; all produced peak mortalities several days after treatment, and

none produced kills after 10 days (fig. 11).

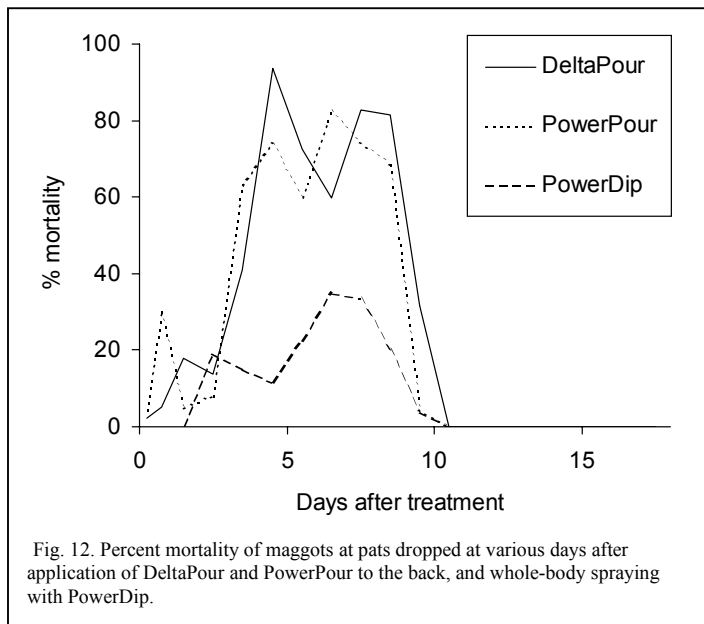


Deltapour: cheaper oil than in Spot-on

Powerdip: s.c of alphacypermethrin

Power pour: alphacypermethrin formulated in oil

The results with fly larvae (fig. 12) are somewhat similar to those of the beetle assays in that the mortality with PowerDip was less than with the pour-ons, and no mortality occurred beyond 10 days. Again there was a delay of a few days before the greatest mortalities occurred.

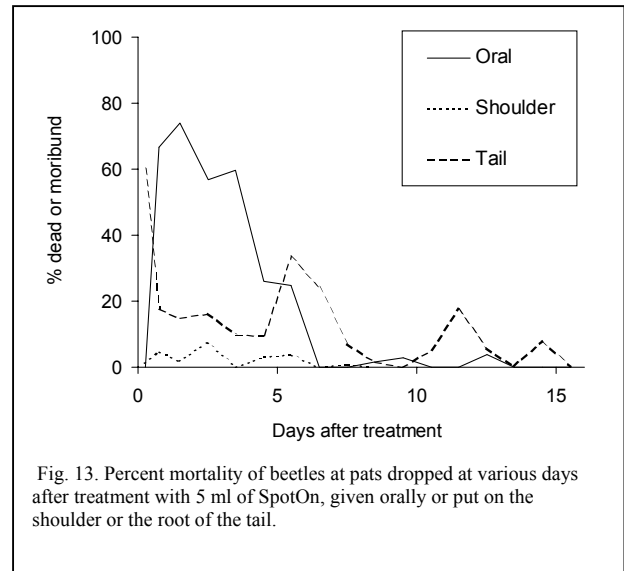


The next stage was to identify the route of contamination. Figures 1 and 10 showed distinctive patterns of dung contamination when insecticide was applied to the flanks and back of oxen, suggesting that further variations in application technique could help to elucidate the route by which insecticide enters the dung. For this work the SpotOn dose per animal was 5 ml, ie, about 10-15% of the normal dose that would

have been applied. Some oxen were given the 5 ml orally, to simulate licking the insecticide formulation off the coat. With other animals the 5 ml was applied to the root of the tail, close to where it could immediately contaminate the dung. With yet other animals the 5 ml was put on the top front of the shoulder, ie, where it could not be licked, and well away from the site of direct dung contamination. The tail and shoulder positions would each receive about 5 ml during normal treatment

(manufacturer's recommendations). The results of the 5 ml applications (fig. 13) should be compared with those for the full dose of SpotOn (fig. 10). The tail treatment, like the full dose applied to the back, produced quick contamination, whereas the oral treatment gave the delayed contamination typical of the full dose applied to the flanks. The shoulder treatment gave very little contamination.

The results suggest that the immediate contamination following normal application of SpotOn to the back is due to direct contact with dung at or near the anus, whereas the delayed contamination when the insecticide is applied to the flank, where it can be readily licked, is attributable to the time taken for the insecticide to pass through the digestive system. The fact that much of the deltamethrin in pour-ons spreads over the whole body surface in a few days (Stendel 1992, Vale *et al.*, 1999) could explain why the contamination levels due to back and flank treatments are similar after a week (fig. 10).

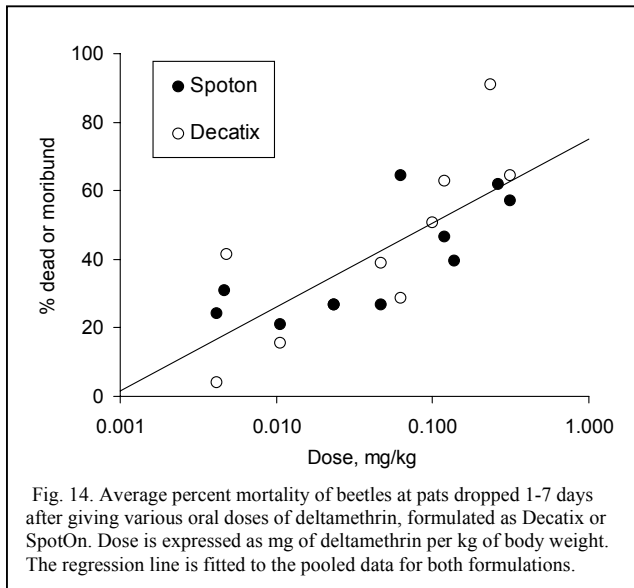


The importance of licking after the flank application of SpotOn was investigated by placing a plastic collar on the neck, extending outwards for 60 cm, and preventing the animal from licking its flanks, although it could still lick its front legs. The collar reduced by about two-thirds the degree of dung contamination during the 1-7 day period, so suggesting that licking could account for about half of all insecticide entering dung after normal flank treatment.

Oral dose

The importance of licking was investigated further by giving oral doses of 5 ml, 1 ml and 0.2 ml of SpotOn, each diluted to 20 ml with sunflower oil prior to administration. Other oxen were given the equivalent amount of deltamethrin (mg a.i. kg⁻¹) as Decatix, diluted to 20 ml with water. The results (fig. 14) were expressed as the mean mortality in pats dropped 1-7 days after treatment. The mortalities due to SpotOn and Decatix at any given level of active ingredient were similar (in contrast to when directly applied to dung, where deltamethrin in SpotOn is about twice as active against beetles). Hence, oral administration seems to destroy the distinction between formulations.

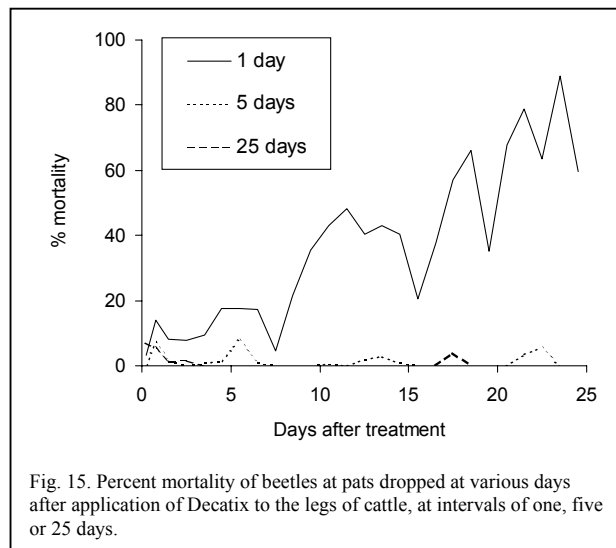
Given that about 1.6% of the topically applied deltamethrin gets into the dung it seems that the oxen would have to lick up 12% ($1.6 \times 100/13$) of the applied deltamethrin if all of the contamination were due to licking. Allowing that some of the contamination occurs directly, not orally, it is likely that somewhere around 5-10% of the topically applied deltamethrin is licked away.



Leg treatments
 Since the degree of dung contamination seems directly related to the amount of insecticide administered, the risk to dung fauna might be reduced substantially by avoiding pour-ons and using only the spray or dip formulations that involve the application of relatively little active ingredient. Moreover, the amount of insecticide applied might be reduced further by spraying or dipping only that part of the body surface most frequented by the target pests. A 90% reduction

in the treated area is achieved by treating only the legs. (Any loss of efficacy against the tsetse might be offset by applying the insecticide more frequently)

By treating oxen just once at the start of 25-day assay periods, or at intervals of one and five days within these periods, fig. 15 shows that dung contamination would be slight with legs-only treatments at intervals of five days or longer, but much more serious after a few weeks of daily applications.



Outputs

Chemical analyses of cattle dung confirmed the presence of insecticide residues in insecticide-treated cattle. The level of dung contamination with insecticides is around 0.01 to 0.1 ppm in dung dropped on most days up to about 1-2 weeks after cattle treatment, and persists in the pats for many weeks after dropping (more than 60 days).

Abnormally high levels of contamination were needed before the dispersal of experimental pats was much impeded. Bioassays of spiked dung indicated that the overall population of insects was not much affected: if some insects died during colonisation there were many others to take their place. Under operational conditions in which all or most of the pats are contaminated over large areas for many months, relatively low levels of contamination might impair dispersal by decreasing the population density of insects (ranches and widespread use of pour-ons in communal areas).

Impact at population level was gauged by focusing first on the indicators for mortality and breeding disruption. The indications from field and laboratory bioassays with adult beetles and muscid larvae were much the same. The suggestion is that the amounts of insecticide in pats dropped for a week or so after treatment approaches the LD₅₀ of deltamethrin and other commonly used pyrethroids (as recommended for application at higher doses). If deltamethrin or the other pyrethroids recommended for tsetse control are applied at the advised frequency of once or twice a month (Vale *et al.*, 1999) it seems that the average mortality among beetles and flies will be about 10–30% for much of the time. The rates will tend to be a little higher with pour-ons than with dips or sprays, since pour-ons usually involve relatively large doses of active ingredient. Furthermore, even at comparable levels of active ingredient there seems something about the pour-on formulation of deltamethrin (SpotOn) that sometimes makes it more toxic than the spray (Decatix), at least when the insecticides are introduced directly to the dung. Perhaps formulation with oils – in which they are bound (absorbed). It is unfortunate, therefore, that the convenience of pour-ons makes them highly popular. [*In presenting these conclusions on impact, it is recognised that beetles exposed to dung in the laboratory had no opportunity of escape; that some of the beetles visiting the pats must have departed before counting field assays of beetle mortality; it was not clear what percentage of these died after leaving, and that many of the dead beetles must have been removed by predators, and were not recorded. Deaths of predatory insects would have interfered with numbers of prey remaining.*]

Extensive modelling showed that toxicity of contaminated dung could reduce markedly the abundance of dung fauna, especially the slower breeding species (Warhaugh *et al.*, 1998; Vale & Grant, 2002). Admittedly, the toxicity assessments and modelling are not cogent proof of serious ecological risk, and even if they were they might be of little immediate concern to farmers with a pressing need for tsetse control. As there is clearly some risk, the project then focused on identifying types of cattle treatment that minimise contamination through economical, effective and convenient options - in order to increase their chances of adoption.

In addressing these tasks the project developed and tested quick and cheap means of measuring contamination. Chemical assays are expensive and anything but rapid

(one or more months). The simple laboratory and field bioassays with beetles delivered seemingly reliable answers in a day, and although their biological interpretation is somewhat vague they are no less sensitive and reliable than chemical methods. Compared with the refined bioassays of Wardhaugh et al. (1998), Kruger et al. (1999) and Sommer et al., (2001), which also slow and require holding facilities, these bioassays are rapid and reliable, although they may be restricted to seasonal use.

The dissipation of pyrethroid residues from dung to soil was so slight under simulated wet season conditions that adverse ecological reactions to soil fauna and processes are not expected. It is however recognised that these residues are persistent in the dung and will eventually be removed by fauna and later degraded, especially when soil microbial activity is high (moist conditions).

The indications for the route of contamination by which topically applied insecticide enters the dung are summarised in Table 2, with the direct anal route intended to include all other contamination via dung contact with the body.

Table 2 Suggested importance of anal (A) and oral (O) routes of dung contamination, at various times after applying different formulations of insecticide to various sites on the body surface of cattle.

Formulation	Site	Days after treatment		
		<1	1-7	8-16
Spray/dip	All over	AAA	AAOO	AO
Pour-on	Back	AAAAA	AAOO	AO
	Flanks	A	AAOOO	AO

The pyrethroids are irritating to the skin, especially when in the concentrated pour-on formulations intended to make good contact with the body. It is not clear whether the vegetable oil in pour-ons is itself a licking stimulus, nor whether other chemicals might be added to inhibit licking. The licking is not only a threat to dung fauna; it is also regrettable that up to a tenth of costly insecticides used extensively throughout the cattle industry can soon be removed from the point of contact with target pests.

The use of sprays or dips, as alternatives to the pour-ons, would reduce substantially the risks to dung fauna without affecting much the efficacy against tsetse, it being shown that SpotOn and other pour-ons are about as potent as Decatix and other sprays or dips against *G. pallidipes* (Vale et al., 1999). Moreover, the dips and sprays could also be more economical since they are usually about a quarter of the cost of pour-ons. The problem in some countries is that unlike pour-ons, the dips and sprays require the inconvenience and expense of dip tanks or spray races, and need plenty of water to operate.

The restriction of treatments to the legs promises to reduce yet further the insecticide costs and the risks to dung fauna. It also requires relatively little water and might be achieved by comparatively inexpensive leg-baths, small hand-sprayers or even

brushes. As legs-only treatments require so much less insecticide than the whole-body applications, it means that substantial savings in insecticide costs can be made, possibly at intervals of a fortnight or a month. Such increased frequency of application could well improve the efficacy against tsetse, it being known that the normal intervals are too long to produce a high and steady rate of kill at all seasons (Vale et al., 1999). However, the potential advantages of legs-only treatments against tsetse need to be demonstrated as real – a matter to be addressed by another article

The contamination of animal blood and milk may not be at a level that is deleterious to health. Other factors used to calculate daily intake are presently unknown. Further confirmation is required before giving the techniques the thumbs up: it was not possible to collect the information in the current political climate of Zimbabwe. The attached paper (Vale and Grant, 2002) models the impacts of contaminated dung on dung fauna in a wide variety of scenarios and in some ways acts as a surrogate for soil fertility projections, the opportunity for field determinations of soil status having been lost as a result of farm take-overs in Zimbabwe.

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