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Effect of temperature regime on the toxicity of endosulfan and deltamethrin to tsetse flies, *Glossina morsitans morsitans*

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Abstract *The effect of various temperature regimes on the toxicity of endosulfan and deltamethrin to tsetse flies was examined. The positive temperature coefficient of toxicity of endosulfan and the negative one for deltamethrin were confirmed. At 30°C deltamethrin was found to be ten times as effective as endosulfan, but this ratio increased to more than 300 at 10°C. Toxicity values for endosulfan were reduced when, after 48 h at 10°C, a 'warm-up' period of up to 24 h was allowed. The activity of endosulfan was merely delayed at cooler temperatures and continued once the temperature increased. With deltamethrin a long 'warm-up' period at 25°C increased the LD_{50} value by a factor of 1·5. Simulation of more natural field conditions using a cycled temperature showed that, on purely toxicological grounds, little difference occurred when application took place at dusk or dawn. The resulting LD_{50} values were a balance between the temperature at which each insecticide was most effective and the temperature at which its action was moderated. Thus, while deltamethrin remained the more toxic of the two insecticides, its potency ratio was reduced from >300 times to 80 times. The use of a cycled temperature regime gives a more realistic comparison between insecticides which have different temperature coefficients of toxicity.*

Keywords: tsetse, *Glossina morsitans morsitans*, toxicity, insecticide, temperature.

Introduction

Temperature is one of the many factors which affect the toxicity or the susceptibility of tsetse flies to insecticides (Allsopp 1984). Most work examining these effects of temperature has used a constant post-treatment temperature regime (Hadaway 1978; Harris *et al.* 1990). Researchers have compared the effectiveness of various insecticides under different, but constant, temperatures. Hadaway (1978) found that endosulfan, an organochlorine, has a positive temperature coefficient of toxicity but that of the pyrethroid deltamethrin is negative. In 1990 Harris *et al.* confirmed these findings using the 'Mature Aerosol Placement' technique of Johnstone *et al.* (1989).

During tsetse control operations it has been assumed that night-time spraying would

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enhance the effectiveness of negatively temperature-correlated pyrethroids such as deltamethrin but a positively temperature-correlated insecticide like endosulfan would be less effective under these conditions.

Harris *et al.* (1990), using post-treatment temperatures of 10°C and 25°C for periods of 48 h, concluded that deltamethrin was 26 times more effective than endosulfan at 25°C but as much as 64 times more toxic at 10°C. The 10°C toxicity figures were based on mortality assessments at a standard 1–2 h after removing the insects from the cool cabinet and allowing a period of 'warming-up' at 25°C so that any survivors were mobile enough to be recognized.

It was later observed (personal observations) that if the insects were allowed a longer 'warming-up' period at 25°C mortality with endosulfan increased further, while the controls remained unaffected. This suggested that the effect of endosulfan was halted at the low temperature (10°C), but continued once warmer conditions prevailed.

This observation was highly relevant to the assessment of the effect of temperature on the toxicity of insecticides as it might indicate that deltamethrin's apparent efficacy at low temperatures would be reduced during a 'warm' period.

The observation was also of particular importance in the understanding of how insecticides work under field conditions, where temperatures fluctuate during a 24 h period between 30°C by day and 10°C at night. Thus the toxicity of endosulfan might be increased during the daytime but reduced during the night; the reverse would be true for deltamethrin.

This investigation attempted to consolidate the various aspects of controlled temperature laboratory experiments on the relative toxicity of insecticides. The effects of a constant temperature regime were examined initially, followed by various lengths of 'warm-up' periods and finally a 'cycled-temperature' regime.

Materials and methods

Laboratory culture and maintenance of flies

The tsetse flies used for all bioassays were *Glossina morsitans morsitans* Westwood obtained as puparia from the Tsetse Research Laboratory, Langford, Bristol, UK.

The puparia were placed in 200 ml cups, approximately 50 per cup, mixed with vermiculite 2–3 cm in depth. A muslin square was secured over the top with two elastic bands. The cups were held at 25°C and 70–80% relative humidity. Emergence started 28–30 days after the puparium deposition date. Newly emerged flies were removed daily for use in the bioassays.

Experimental techniques

Unfed, 0–1 day old, male or female *G. morsitans* were treated in batches of ten (the sexes were kept separately). Each batch was gently anaesthetized under carbon dioxide and individual insects dosed according to the techniques described below.

Mature Aerosol Placement (MAP) technique. Aerosol drops (15 and 20 μm diameter) were applied to individual flies using the MAP technique (Johnstone *et al.* 1989). Using this

method it was possible to apply a specific number of drops to the eye of each insect to mimic application rates that occur in the field.

Microburette technique. A known concentration of the technical insecticide was made up in the solvent di-*iso*butyl ketone. This was delivered from a microburette in volumes of 5–40 nl, onto the dorsal thorax of each fly.

Microcapillary technique. Unlike the above technique, which used one insecticide concentration applied at different known volumes, this procedure involved the use of a standard 1 µl volume delivered from a 'microcap' microcapillary. A series of half dilutions were made up in 2-butanone. The insecticide was applied to the dorsal thorax as before.

Post-treatment regime. After treatment, each batch of flies was kept in paper cups secured with a muslin cover, in a temperature-controlled environment according to the requirements of the test. Mortality was recorded 24 h and 48 h after dosing. For the 'recovery' tests, insects at 10°C were transferred to a cabinet at 25°C and mortality recorded after a further 2–24 h (i.e. the final count was at 72 h). Assessments could not be carried out precisely at the end of the cool period because the flies were torpid, making it difficult to distinguish which were alive. For one set of tests insects were held at 10°C for 72 h and assessed after a short recovery period.

With the cycled temperature regime, insects were placed in a cabinet at the start of either a 12 h cycle at 10°C followed by 12 h at 25°C, representing night-time spraying (i.e. application at dusk), or a 12 h cycle at 25°C followed by 12 h at 10°C, representing daytime spraying (i.e. application at dawn).

Insecticide formulations

Commercial formulations were used for the MAP bioassays: deltamethrin – ULV formulation, supplied by Wellcome Foundation Ltd (now Roussel-Uclaf), Berkhamsted, Herts, UK; endosulfan – ULVT formulation, supplied by Hoechst, Frankfurt, Germany.

Technical materials from the same suppliers were used for the microburette and microcapillary bioassays.

Analysis of results

All results were analysed using a probit analysis computer program, Maximum Likelihood Program, MLP (Ross 1987). The 48 h LD_{50} values are quoted together with 95% confidence limits.

Results

Constant temperature regime

Table 1 shows the 48h LD_{50} values obtained for endosulfan and deltamethrin at three constant post-treatment temperatures. It confirms a negative temperature correlation for the pyrethroid deltamethrin and a positive one for the organochlorine endosulfan. The potency ratios of deltamethrin relative to endosulfan at each of the three temperatures are shown in Table 2. The microcapillary technique was used for these evaluations.

Table 1. Toxicity values of deltamethrin and endosulfan to *G. morsitans* at three temperatures

	Temperature (°C)	LD_{50} (ng)	95% Confidence limits	Potency ratio
Deltamethrin	18	0·032	0·030–0·035	1·00
	25	0·062	0·057–0·070	0·52
	30	0·184	0·162–0·208	0·17
Endosulfan	18	6·95	6·43–7·50	1·00
	25	2·77	2·57–2·99	2·50
	30	1·92	1·77–2·04	4·35

Table 2. Potency ratios of deltamethrin relative to endosulfan at three temperatures for *G. morsitans*

	Temperature (°C)	LD_{50} (ng)	Potency ratio
Endosulfan	18	6·95	1·00
Deltamethrin	18	0·032	217·19
Endosulfan	25	2·77	1·00
Deltamethrin	25	0·062	44·68
Endosulfan	30	1·92	1·00
Deltamethrin	30	0·184	10·43

Table 3. Toxicity values of endosulfan with various post-treatment temperature regimes against *G. morsitans*, using the MAP technique

Temperature regime	LD_{50} (ng)	95% Confidence limits
25 °C	3·90	3·43–4·32
10 °C + 1–2 h at 25 °C	17·17	15·29–19·78
10 °C + 4 h at 25 °C	13·99	10·03–16·45
10 °C + 24 h at 25 °C	5·32	1·36–7·38

Comparison of constant temperature regime with varying 'warm-up' periods

Table 3 shows the toxicity values for endosulfan when, in addition to the standard 48 h at the lower temperature, the 'warm-up' period was increased from 1–2 h (the usual time) to 24 h. The change in the LD_{50} values was threefold, from 17 ng per fly with a 1–2 h warm-up period to 5·3 ng with a 24 h period at 25°C. An intermediate value of 13·99 ng was recorded with a 4 h warm-up period. This evaluation was carried out by applying aerosol drops to the insects' eyes (MAP technique).

In order to confirm the results, additional data were collected using the much simpler microburette technique (Table 4). The same trend was evident, with a 24 h warm-up period producing an LD_{50} value of <2·75 ng, approximately one-third of the figure for the standard 1–2 h period at 25°C. It was not possible to compute the actual LD_{50} value as the lowest

Table 4. Toxicity values of endosulfan with various post-treatment regime, against *G. morsitans*, using the microburette technique

Temperature regime	$LD_{5($ (ng)	95% Confidence limits
25 °C	2·32	2·05–2·62
10 °C + 2 h at 25 °C	7·45	3·73–12·09
10 °C + 24 h at 25 °C	< 2·75	Not computable

dose of 2·75 ng/insect gave 74·1% mortality, while the LD_{50} figure of 7·45 ng resulted in 100% mortality when the warm-up period was extended from 2 h to 24 h.

For both the MAP and microburette techniques the lowest LD_{50} values were obtained at the constant 25°C post-treatment temperature (Tables 3 and 4), as would be expected with an insecticide with a positive temperature coefficient of toxicity.

Results obtained when insects were held for 72 h at 10°C, followed by a short recovery period, are shown in Table 5. The LD_{50} value of 7·48 ng is comparable with that obtained for a 48 h period at 10°C with 2 h recovery at 25°C (Table 4). The LD_{50} figures for 48 h and 72 h at 10°C with no recovery period were 19·63 ng and 10·90 ng respectively, 2·6 and 1·5 times greater than the value attained when a 2 h recovery period was allowed.

With deltamethrin, which has a negative temperature coefficient of toxicity, the data showed that a long 'recovery' period at 25°C increased the LD_{50} value from 0·055 ng to 0·081 ng, a factor of 1·5 (Table 6). A constant post-treatment temperature of 25°C produced an LD_{50} value of 0·110 ng, exactly twice the figure for the low temperature regime. Table 7 shows the data acquired using the microburette technique. Again, the value at 25°C was approximately 2·5 times greater than that obtained at 10°C with a short recovery time. A test with the longer (24 h) recovery period was not carried out.

The higher LD_{50} values obtained using the MAP technique when compared to those using the microburette method for both endosulfan (Tables 3 and 4) and deltamethrin (Tables 6 and 7) are attributed to the site of application. The cuticle overlying the eye, used for insecticide placement in the MAP technique, is less easily penetrated than the cuticle of the dorsal thorax, thereby giving rise to increased LD_{50} values. However, the MAP technique depends upon droplet application to the smooth surface of the eye due to the delicate nature of the threads employed (Johnstone *et al.* 1989).

Table 5. Toxicity values of endosulfan with a longer (72 h) period at 10 °C plus a short recovery time, using the microburette technique

Temperature regime	LD_{50} (ng)	95% Confidence limits
10 °C/48 h	19·63	16·37–26·42
10 °C/72 h	10·90	9·22–12·62
10 °C/72 h + 2 h at 25 °C	7·48	6·30–8·54

Table 6. Toxicity values for deltamethrin with various post-treatment temperature regimes against *G. morsitans*, using the MAP technique

Temperature regime	LD_{50} (ng)	95% Confidence limits
25 °C	0·110	0·097–0·124
10 °C + 2 h at 25 °C	0·055	0·048–0·061
10 °C + 24 h at 25 °C	0·081	0·074–0·089

Table 7. Toxicity values of deltamethrin with various post-treatment temperature regimes against *G. morsitans*, using the microburette technique

Temperature regime	LD_{50} (ng)	95% Confidence limits
25 °C	0·048	0·041–0·054
10 °C + 2 h at 25 °C	0·0195	0·018–0·021
10 °C + 24 h at 25 °C	–	–

Cycled temperature regime

The final element to study was the effect of 'cycled' temperature, alternating between 10°C and 25°C every 12 h. This reflected more nearly conditions in the field and, by exposing insects from the start of either a 12 h cool period or a 12 h warm period, it was possible to imitate dusk and dawn spraying conditions. The results are shown in Tables 8–10.

The data in Table 8 should be compared with those in Table 3 for endosulfan and Table 6 for deltamethrin. For the latter, the LD_{50} value of 0·073 ng obtained with a cycled temperature regime is similar to that at 10°C with a 24 h recovery period (0·081 ng) and between the standard 25°C and 10°C + 2 h warm-up figures. With endosulfan a similar trend occurs, with an LD_{50} value of 4·24 ng under the 'dusk' cycling regime comparable with the LD_{50} figure of 5·32 ng at 10°C + 24 h recovery period (Table 3). Only endosulfan was tested under both the 'dusk' and 'dawn' regimes using the MAP procedure and the potency ratio was shown to be close to unity (1·00:1·10).

Table 8. Toxicity values of endosulfan and deltamethrin to *G. morsitans*, with a cycled temperature regime, using the MAP technique

	Temperature cycle*	LD_{50} (ng)	95% Confidence limits	Potency dusk : dawn
Deltamethrin	Dusk	0·073	0·064–0·081	–
	Dawn	–	–	–
Endosulfan	Dusk	4·24	3·84–4·66	1·00
	Dawn	3·84	3·43–4·24	1·10

* Dusk = 12 h at 10 °C followed by 12 h at 25 °C
Dawn = 12 h at 25 °C followed by 12 h at 10 °C

Table 9. Toxicity values of endosulfan and deltamethrin to *G. morsitans*, with a cycled temperature regimen, using the microburette technique

	Temperature cycle	LD_{50} (ng)	95% Confidence limits
Deltamethrin	Dusk	0·040	0·036–0·045
Endosulfan	Dusk	3·50	3·06–3·99

Table 9 shows the results obtained using the simpler microburette technique and the 'dusk' temperature cycle. The LD_{50} value of 0·040 ng for deltamethrin is again intermediate between those obtained with the various post-treatment regimes shown in Table 7. Similarly the figure for endosulfan, 3·50 ng, is comparable with the data in Table 4.

The 'dusk' and 'dawn' cycles were examined for both insecticides using the micropipette application method and the data are shown in Table 10. No difference was found between the two cycles for deltamethrin. With endosulfan the dusk/dawn potency ratio was small, 1·00:0·87, and reversed to that found using the MAP technique.

The potency ratios of deltamethrin relative to endosulfan at the standard temperatures of 25°C and 10°C + 2 h at 25°C, compared with those where a cycled temperature regime was employed are given in Table 11.

Discussion

Constant temperature regime

This work confirmed the positive temperature coefficient of toxicity of endosulfan and the negative one of deltamethrin (Table 1) as found by Hadaway (1978). At 30°C deltamethrin was 10 times more toxic than endosulfan and this ratio increased to more than 200 times at 18°C (Table 2).

Varying post-teatment temperature regime

Given that constant post-treatment temperatures were used in previous bioassays (Hadway 1978; Harris *et al.* 1990) although the field temperature fluctuates diurnally, it seems likely that potency ratios observed in the laboratory would be modified under a cycled temperature

Table 10. Toxicity values of endosulfan and deltamethrin to *G. morsitans*, with a cycled temperature regime, using the micropipette technique

	Temperature cycle	LD_{50} (ng)	95% Confidence limits	Potency dusk : dawn
Deltamethrin	Dusk	0·041	0·037–0·047	1·03
	Dawn	0·040	0·035–0·046	1·00
Endosulfan	Dusk	3·195	2·961–3·446	1·00
	Dawn	3·681	3·377–4·020	0·87

Table 11. Potency ratios of deltamethrin relative to endosulfan with varying temperature regimens, against *G. morsitans*

	Temperature regime	LD_{50} (ng)	Potency ratio
MAP			
Endosulfan	25 °C	3·90	1·00
Deltamethrin	25 °C	0·110	35·45
Endosulfan	10 °C + 2 h at 25 °C	17·17	1·00
Deltamethrin	10 °C + 2 h at 25 °C	0·055	312·18
Endosulfan	Dusk	4·24	1·00
Deltamethrin	Dusk	0·073	58·08
Microburette			
Endosulfan	25 °C	2·32	1·00
Deltamethrin	25 °C	0·048	48·33
Endosulfan	10 °C + 2 h at 25 °C	7·45	1·00
Deltamethrin	10 °C + 2 h at 25 °C	0·0195	382·05
Endosulfan	Dusk	3·50	1·00
Deltamethrin	Dusk	0·040	87·50
Micropipette			
Endosulfan	25 °C	2·77	1·00
Deltamethrin	25 °C	0·062	44·68
Endosulfan	18 °C	6·95	1·00
Deltamethrin	18 °C	0·032	217·19
Endosulfan	Dusk	3·195	1·00
Deltamethrin	Dusk	0·041	77·93
Endosulfan	Dawn	3·681	1·00
Deltamethrin	Dawn	0·040	92·03

regime. The toxicity value of each insecticide would be modified by the temperature at which it was least effective.

Endosulfan

Tables 3–5 illustrate the effect that a lower temperature had on the action of endosulfan. The threefold decrease in the LD_{50} value from 17 ng with a short warm-up period down to 5·3 ng with a longer recovery time (Table 3) clearly illustrates that the toxicity of endosulfan was reduced at the lower temperature. However, at the lower temperature the insects' metabolic rate will be lower and any detoxification of the insecticide must also be inhibited, but once the temperature was increased the toxic action of endosulfan resumed, leading to a decrease in the LD_{50} value. This pattern, observed with the MAP technique, was repeated in further tests using the simpler microburette technique (Table 4). By holding the insects for the longer period of 72 h at 10°C it was possible to emphasize even more this delay in

endosulfan's toxic action with resulting high LD_{50} values, when no recovery period at increased temperature was allowed, decreasing once the recovery period temperature was increased (Table 5).

These observations lead to a better understanding of the way endosulfan works in the field. Although night-time spraying would appear to favour the negatively temperature correlated pyrethroid deltamethrin, in practice the toxicity of endosulfan is similar but its action is delayed until the warmer daytime period.

Deltamethrin

The toxic action of deltamethrin was shown to decrease by a factor of 1·5 (LD_{50} value increased from 0·055 ng to 0·081 ng) when a 24 h recovery period was allowed at 25°C (Table 6). Therefore the assumption that night-time spraying would enhance the effectiveness of this insecticide is only partially correct and must take into account the fact that warmer daytime conditions will reduce its toxic action.

Cycled temperature regime

The data presented in Tables 8–11 show the effect that a more natural cycled temperature regime had on the toxicity of each insecticide. Simulation of dusk and dawn cycles revealed very little difference in the toxicities of both insecticides overall. Indeed, for deltamethrin the dusk/dawn ratio was 1·03:1, a negligible difference (Table 10). With endosulfan the ratio was 1:1·1 when the MAP technique was employed (Table 8), but 1:0·87 when application was done by micropipette (Table 10), indicating very little overall difference.

It appears, therefore, that the time of treatment is not critical from a purely toxicological viewpoint. Practical reasons may necessitate night-time spraying. For example, in Botswana aerial spraying for tsetse control is carried out at night because atmospheric conditions are stable and most suitable then (Coutts 1980). Convection turbulence during the daytime would make it impossible to carry out this type of operation then (Allsopp, personal communication).

When potency ratios for both insecticides at the standard temperatures and recovery periods were compared with a cycled regime (Table 11), it was shown that the effective toxicity of both endosulfan and deltamethrin is a balance between the temperatures at which they work most efficiently and those which moderate their effect. Thus, for endosulfan with a positive temperature coefficient of toxicity, although the LD_{50} value for the dusk regime lies between those at the two standard temperatures it is closer to the value for 25°C at which it is most active. This trend was consistent for all three techniques used.

With deltamethrin, the LD_{50} value for the dusk regime was intermediate between the values for the two standard temperatures, suggesting that the higher temperature has a greater moderating effect on this insecticide.

Overall, deltamethrin is more toxic to tsetse flies than endosulfan. Even at a high temperature of 30°C it was 10 times more toxic (Table 2) and at 10°C the potency ratio increases to more than 300 times (Table 11). By using a cycled temperature regime it is possible to achieve a more realistic comparison of the two insecticides. The activity of deltamethrin is

shown to be moderated by the warmer 'daytime' period and the potency ratio is reduced from >300 times to approximately 80 times.

Conclusions

The laboratory evaluation of insecticides at a constant post-treatment temperature is useful for comparing their toxicity values, but where insecticides have differing temperature coefficients of toxicity, as in the case of endosulfan and deltamethrin, the use of a cycled temperature regime gives a more reliable and realistic comparison of how they are likely to work in the field.

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