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Investigation of the Possible Vapour Action of α-Endosulfan on *Glossina morsitans morsitans*

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Abstract: An all-glass apparatus was set up whereby insects were brought into contact with α-endosulfan vaporized from a heated bulb. The attribution of the initial high mortality to vapour action was not substantiated by subsequent chemical analysis. Use of reduced amounts of α-endosulfan to provide more nearly the calculated saturated vapour concentration resulted in little kill. The α-endosulfan vaporized transiently then condensed onto the surrounding surfaces; any mortality resulted from contact action. These observations do not support the contention that α-endosulfan acts via the vapour phase at long range in the field.

INTRODUCTION

Aerial spraying of endosulfan using the 'sequential aerosol technique' has become an established method for the control of tsetse flies over large areas of central southern Africa.[1] Application takes place at night under conditions of temperature stability or inversion, resulting in a cloud of small droplets (10–30 μm diameter) dispersing into the tsetse environment.

It has generally been assumed that mortality results from direct impaction of droplets onto the insects and considerable effort has been made in the past to understand the processes involved, in order to optimize parameters such as drop size, insecticide concentration and volume rate.[2] One aspect that remains to be explained is the effect of insecticide drift downwind from treated areas. The distance over which this effect has been observed is considerable in some cases. For example, in Botswana in 1977, the tsetse population was reduced by 98·3% along the whole length of fly rounds positioned up to 12 km from the spray block. Marked reductions were also reported in areas 20 km and 27 km from the edge of the spray area and drift effects were noted within the spray block itself at a distance of up to 40 km. It may be significant that in 1977 the endosulfan was not diluted with its carrier ('Shellsol AB') to such a great extent and the formulation was therefore more volatile than in previous years.[3]

This downwind effect raised the question as to whether endosulfan could be active in the vapour phase, rather than just as discrete droplets, and whether vapour can contribute to tsetse mortality.

During field operations, the rate of endosulfan used for tsetse control varies widely, e.g. in Zambia 28 g ha^{-1} has been used,[4] whereas a lower rate of 14–20 g ha^{-1} in Zimbabwe was applied for more rugged terrain.[5] If we assume that 20 g ha^{-1} is evenly distributed in the air up to a height of 10 m, there is 0·2 μg of insecticide per litre of air. The LD_{95} for tsetse by topical application is approximately 8 ng per insect.[6] Therefore, in one litre of air there is the equivalent of 25 lethal doses (200 ng). Assuming that a tsetse fly occupies a volume of 0·5 ml, it appears unlikely that it would be able to absorb 8 ng (the lethal dose by topical application), so, unless the LD_{95} by inhalation is much lower, field mortality from vapour would not be expected. Careful monitoring over many years has demonstrated that initial deposition is in the form of droplets which enter the canopy of vegetation and impact on resting tsetse (or vegetation) before evaporation takes place.[7] It is possible that the component filtered by the vegetation forms a more concentrated sump and focus for further evaporation. If this process is efficient, endosulfan vapour may be concentrated locally in the tsetse habitat, not over the volume of 100000 m^3 postulated in the volumetric model. The lethal effect may thus be a combination of contact and vapour action.

This investigation was carried out to determine tsetse mortality resulting from vapour action. The report describes the methods used and the results obtained.

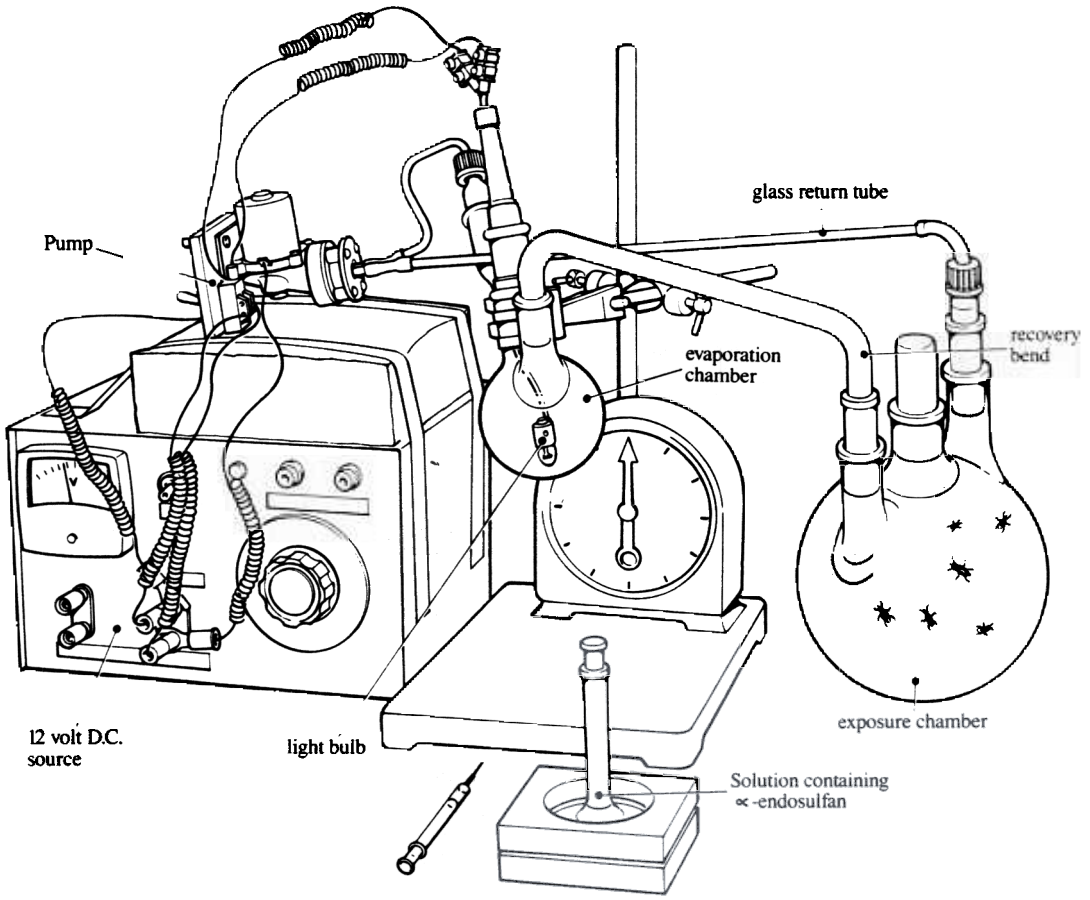

Fig. 1. Diagram of the apparatus used to study the potential vapour effect of α-endosulfan on *Glossina morsitans*.

2 METHOD AND MATERIALS

In order to study the action of endosulfan as a vapour an essentially all-glass apparatus was used because of its low porosity and ability to be thoroughly cleaned (Fig. 1). This was subsequently modified to incorporate a sintered-glass gas wash-bottle and an airflow meter. Later the design was simplified by removal of the circulatory apparatus. An aquarium pump was used to circulate the air in a closed system (1400 ml min^{-1}) and heat from a 4-Watt (12-V) light bulb vaporized the insecticide. The pump and light bulb were operated from a variable DC power supply. Evaporation of the α-isomer of endosulfan took place in a 100-ml flask and the air + vapour was channelled through tubing into a 1-litre flask which contained the test insects, and then returned, through another glass tube, to be recirculated by the pump.

For the purpose of measuring vapour levels, a sintered-glass gas wash-bottle containing toluene (5 ml) was incorporated into the system, between the 100-ml and the 1-litre flasks. Insecticide in the toluene was measured using a gas-liquid chromatograph (GLC).

An airflow meter was also incorporated between the 1-litre flask and the 'return' glass tube. As the closed system occupied a volume of approximately 1200 ml and the flow rate was 1400 ml min^{-1}, it was possible to flush the system through in less than a minute, although in practice circulation was continued for longer.

2.1 Biological testing procedure

All the glassware was very carefully cleaned by rinsing internal surfaces of the flasks and tubes with commercial acetone, then washing in hot water containing detergent, before rinsing with distilled water and drying in a hot-air oven at 120°C.

When cool, the apparatus was assembled as illustrated (Fig. 1). Ten 0 to 1-day-old teneral insects, *Glossina morsitans morsitans* Westwood, were anaesthetized with carbon dioxide and transferred to the 1-litre flask, taking care not to introduce any of the gas into the flask. The sexes were treated separately. The flies were then allowed to recover (requiring about 5 min). When they were resting on the glassware the test exposure commenced. The lamp and pump were switched on for 15 min (this being the average exposure time thought to occur in the field). Any activity during this time was noted. The insects were then anaesthetized again and transferred to paper cups which were held in a cabinet at 25°C and 70% relative humidity. Mortality was recorded after 24 and 48 h. These insects acted as the controls for the subsequent tests with the active ingredient. Any

remaining carbon dioxide was evacuated by a pump before the test run.

In order to examine the effect of the vapour, only the α-isomer, which is the more volatile and active of the two endosulfan isomers, was used. The same procedure was used as when exposing control flies, but this time 5 μl of hexane containing the required amount of α-endosulfan (e.g. 10 μg, 0·1 μg) was placed on the glass of the light bulb using a 10-μl syringe. The bulb was then placed in the 100-ml flask and switched on. Evaporation took place and the vapour was circulated around the apparatus, subjecting the test insects to exposure for the required time. All the connections in the apparatus were checked to prevent any of the vapour leaking and lowering the concentration. The insects were then transferred to paper cups and held in the recovery cabinet. A further batch of *G. morsitans* was introduced into the same flask. It was then connected to a pump for another 15 min so that any remaining vapour was evacuated and the residual effect of the α-endosulfan could be compared.

Subsequently, the apparatus was considerably revised. The bulb was placed directly into the 1-litre flask containing the test insects, thereby eliminating circulation and allowing the insects to be in the chamber while evaporation was taking place. Evaporation time was kept at 15 min as before. For the control, insects were allowed to sit in the chamber with the bulb lit.

2.2 Chemical analysis

In order to relate the results to the mass of α-endosulfan, as both vapour and condensed solid on the vessels, chemical analysis was carried out as follows.

2.2.1 Surface deposits

After each run, all glass surfaces were rinsed thoroughly on the inside (the glass bulb was rinsed on the outside) with hexane. Samples were then analysed using a gas-liquid chromatograph (Perkin Elmer 8300) with ^{63}Ni electron-capture detector and glass column (1 m × 2 mm o.d.) packed with 0·36% OV 17 + 2·64% OV 210 on Gas Chrom Q 80–100 mesh. Carrier gas was nitrogen at 60 ml min^{-1}; temperatures were: column 200°C, detector 350°C, injector 200°C; on-column injection.

2.2.2 Vapour collection

In order to establish the presence of vapour within the system, a sample of the toluene from the sintered-glass gas wash-bottle (which removed vapour from the circulating air) was passed through the GLC.

After the method and apparatus had been modified in order to dispense with circulation, the presence of endosulfan between the light bulb and the flask was detected by the use of woollen strands. Five strands, approximately 30 cm in length, with an effective volume of 75 ml, were suspended between the two outer ground glass joints of the flask. Care was taken not to touch the sides of the flask whilst removing the strands in order to avoid contamination. The wool was washed in hexane (2 × 20 ml) and a sample of each washing was analysed.

2.3 Theoretical considerations

Elliot and Wilson provide the following relation between saturation vapour concentration (SVC) and vapour pressure (p).[8]

$$\text{SVC} = K\,p\,M$$

where M is the molecular mass (g) and the constant $K = 5{\cdot}575 \times 10^{-5}$ at 25°C.

SVC is expressed in g litre^{-1}, units of p are in mm Hg.

Noting that 1 mm Hg ≡ 1 torr ≡ 133 N m^{-2} (Pa) and that 1 Pa ≡ 0·00752 mm Hg; for the α-isomer of endosulfan,[9] $p = 0{\cdot}59$ mPa ≡ $4{\cdot}45 \times 10^{-6}$ mm Hg at 25°C.

Taking M = 400 g, then at 25°C,

$$\text{SVC for α-endosulfan} = 5{\cdot}575 \times 10^{-5} \times 4{\cdot}45 \times 10^{-6} \times 400 \text{ g litre}^{-1} = 0{\cdot}099\ \mu\text{g litre}^{-1} \text{ at } 25°\text{C}.$$

3 RESULTS AND DISCUSSION

An outline of the strategy of all this work is presented in Fig. 2, so that the rationale behind the various experiments and the order in which they were carried out can be clearly seen.

Initial results for the biological tests are shown in Table 1, together with the chemical analysis carried out on the 1-litre flask. In each case the application rate was 10 μg and only the time of insect exposure was varied. Results are taken as 48-h mortality. No control mortality was obtained when the insects were held in the exposure chamber without any airflow for 15 min and only 1·5% mortality occurred at the 15-min exposure time when the system was running.

These results demonstrate that the mortality of *G. morsitans* was a consequence of contact with the condensed α-endosulfan, rather than by vapour action. The total mass of insecticide left on the flask after 15 and 10 min circulation was above the LD_{95} for *G. morsitans*. Indeed the values of 0·89 μg and 0·51 μg for 15 and 10 min circulation could produce 111 and 64 lethal doses respectively. Unfortunately, it was not possible to measure individual fly doses due to the unclean nature of the fly extracts.

Having identified the residue left in the 1-litre flask as being a potential cause of mortality, it was felt necessary to demonstrate the presence of vapour which might be a contributory factor. In order to establish this, the application rate was reduced to a level whereby it was thought that there could be no residual contact action.

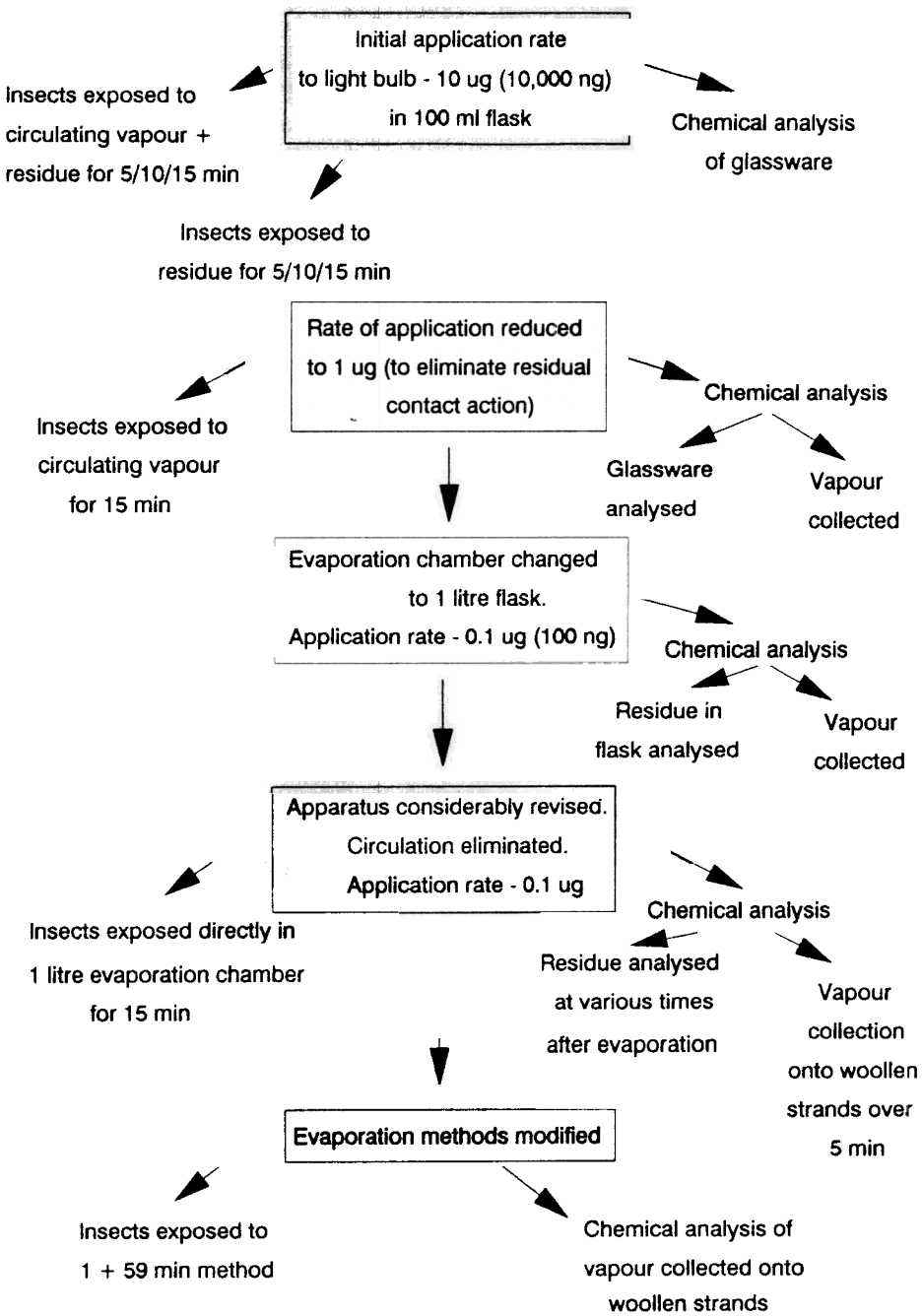

Fig. 2. Diagram outlining the strategy of the work.

Table 2 shows the biological and chemical results obtained.

It was felt that the low mortality could still be due to the presence of a small amount of residual endosulfan in the 1-litre flask, an interpretation supported by the very small amount of endosulfan vapour collected.

The saturated vapour concentration of α-endosulfan had been calculated earlier as being 0·099 μg litre^{-1} and it was decided to reduce the amount of insecticide applied to the bulb one-hundred-fold commensurate with initial evaporation taking place in the limited volume of a 100-ml flask, i.e. applying 0·01 μg. At this stage some problems of contamination were noted. These were overcome by rinsing various parts of the apparatus not normally subject to washing. The glass bulb-holder was shown to be the source of contamination.

TABLE 1
Mortality of *Glossina morsitans* after Exposure to α-Endosulfan at an Application Rate of 10 μg

Test	*Time of exposure (min)*	*Analysis of residue in flask (μg)*	*48-h Mortality (%)*		
			Female	*Male*	*Mixed*
Initial vapour + residual exposure	15	1·10			
		0·84	100	100	100
		0·72			
		$\bar{x}$ = 0·89			
	10	0·45			
		0·49	95	95	95
		0·58			
		$\bar{x}$ = 0·51			
	5	—	30	0	20
Residual exposure only	15	0·26			
		0·65	95	100	97
		0·36			
		0·78			
		$\bar{x}$ = 0·51			
	10	—	86	95	90
	5	—	25	0	17

TABLE 2
Chemical Analyses and Mortality of *Glossina morsitans* at 1 μg Application Rate

Residue in 1-litre flask (μg)	*Endosulfan vapour collected (μg)*	*Mortality (%)*		
		Female	*Male*	*Mixed*
0·16	0·0195	—	18·2	—

TABLE 3
Chemical Analyses at 0·1 μg Application Rate, using 1-Litre Evaporation Chamber

Residue in flask (ng)	*Vapour collection (ng)*
73·0	4·8
80·0	1·6
90·0	2·0
82·0	—

Due to the small amounts of endosulfan involved, the evaporation chamber was at this stage replaced by a 1-litre flask in order that proportionally larger amounts of α-endosulfan could be dealt with, improving the sensitivity of the chemical analysis and overcoming the interference of contamination. As can be seen from Table 3, the amount of endosulfan vapour collected was still very low, while the greater proportion was collected as residue on the 1-litre flask.

TABLE 4
Residue of α-Endosulfan left on the 1-Litre Flask after Application of 0·1 μg

Period	*Residue (ng)*
5 min	62·0
15 min	77·2
15 min + 2 h flask stoppered	80·8
15 min + 4 h flask stoppered	74·8

Subsequently the procedure was revised considerably in order to confirm that α-endosulfan was present as a vapour, rather than merely adsorbed onto the glassware. It was decided to place the insects directly into the evaporation chamber and eliminate the pumped circuit. The aim was to guarantee exposure to the α-endosulfan immediately it was vaporized from the heated bulb. No mortality occurred with insects exposed for 15 min at an application rate of 0·1 μg.

Subsequent tests showed that the majority of α-endosulfan evaporated and then condensed onto the glassware within 5 min and remained bound to the glassware over a period of several hours when the flask was left stoppered after the initial evaporation period. These results are shown in Table 4. It appeared, therefore, that the insects were only effectively exposed to the vaporizing endosulfan over the initial period of 5 min or less.

In order to demonstrate the presence of endosulfan between the bulb and the 1-litre flask, woollen strands

TABLE 5
Vapour Collection on Woollen Strands in 1-Litre Flask, with Corresponding Biological Test, at an Application Rate of 0·1 μg

Evaporation schedule	*Vapour collected (ng)*	*Mortality (%)*		
		Female	*Male*	*Mixed*
Continuous	6·6			
5 min	17·0			
	8·0			
	20·0			
	$\bar{x}$ = 12·9			
Intermittent	17·6			
5 × 1 min	16·0			
over 1 h	20·0			
	$\bar{x}$ = 17·9			
Initial 1 min	13·2			
+ 59 min	18·7			
exposure	21·7			
	17·4			
	$\bar{x}$ = 17·8	6·8	0·0	5·6

a = Not assayed.

were suspended in the flask as previously described and evaporation was allowed to take place over a continuous 5-min period. Results showed that up to 20% of the α-endosulfan collected on the wool and that the insecticide was, therefore, in a vapour state between the glass bulb and the 1-litre flask (Table 5). In order to try to increase the amount of endosulfan vapour, it was decided to evaporate the α-endosulfan in two slightly different ways. In the first instance, forced evaporation took place intermittently over one hour, with five periods consisting of 1 min evaporation followed by 11 min with the lamp off. With the second set of tests, evaporation took place for 1 min and the flask was then left stoppered for a further 59 min before analysis was carried out. A biological test was also carried out in conjunction with the latter method. The results for all these tests are shown in Table 5.

4 CONCLUSIONS

In the first instance, biological results appeared very positive, with high mortalities resulting from the circulation of α-endosulfan vapour around the glass apparatus. However, subsequent chemical analysis revealed that the residual amounts of α-endosulfan left in the exposure chamber were greatly in excess of the lethal dose required for *G. morsitans*, thus indicating that mortality was most probably caused by contact with the residue rather than by vapour action. Even with the suction pump operating to remove any vapour from the exposure chamber, a significant amount of α-endosulfan remained on the walls of the flask, resulting in mortality by contact action, e.g. 0·51 μg constitutes a lethal dose for 64 insects. Reduction of the exposure time to 5 min resulted in reduced mortality, but virtually 100% mortality was obtained with the exposure time of 10 min and again chemical analysis showed that sufficient residue was left in the glass chamber to account for this (Table 1).

Initial attempts to show the presence of α-endosulfan as a vapour achieved only limited success. Reducing the amount of insecticide applied to the bulb, to provide the theoretical saturated vapour concentration did not reduce the proportion of condensation on the surface of the exposure chamber, or increase the proportion of endosulfan vapour present. Little mortality occurred with the test flies and this could probably be attributed to the reduced amount of α-endosulfan deposited on the flask: i.e. 0·16 μg gives only 20 lethal doses (Table 2).

Altering the apparatus, by using a larger evaporation flask, whilst overcoming the noted problems of contamination, did not increase the amount of endosulfan vapour present in the exposure chamber (Table 3). No mortality resulted when the apparatus was rearranged to ensure exposure to any α-endosulfan vapour present and chemical analysis showed that approximately 80 ng of insecticide remained on the flask, or the equivalent of 10 lethal doses (Table 3). This amount was obviously insufficient for the flies to be able to pick up a lethal dose by contact action and they were unaffected by the presence of any α-endosulfan vapour.

As shown by the results in Table 4, the majority of α-endosulfan evaporates within the first 5 min of the evaporation period, thus exposing the insects to vapour for that short time only. Once adsorbed onto the glassware the insecticide remains bound and does not re-

evaporate to enhance the atmosphere of endosulfan vapour. Whilst the presence of vapour between the heated bulb and the surface of the 1-litre flask was verified by adsorption on suspended woollen strands (Table 5), it was not possible to increase the amount of vapour present by alternative evaporation methods intended to expose the tsetse to the vapour for longer periods. Only a very low mortality was obtained with *G. morsitans* under these conditions, again indicating the lack of effect of endosulfan vapour.

These simple experiments show the difficulty experienced in keeping endosulfan in the vapour phase. Rather than remaining as a discrete vapour, the α-endosulfan merely vaporizes for a short time from one surface, i.e. the heated glass bulb, and subsequently condenses on the nearest surrounding surface, whether this is the cooler exposure chamber or the suspended woollen strands. The insects only exhibit an effect as a result of acquiring the insecticide by residual contact action. When insufficient insecticide is present to allow this, virtually no mortality occurs. Any effect from α-endosulfan vapour is negligible under these conditions.

It should be pointed out that these experiments were not wholly representative of conditions in the field, where a wide variety of meteorological factors can dictate the efficiency of spraying. A further point is the use of the formulated product (which contains both the $\alpha+\beta$ isomers) in the field, whereas in the laboratory only the more volatile and toxic *alpha* component of endosulfan was used. While a vapour effect cannot be categorically ruled out, the difficulty experienced in these experiments in creating an atmosphere of vapour for a sufficient length of time to be effective against tsetse indicates that it is most unlikely that vapour action could explain the mortality associated with drift observed during monitored control operations in the field. Perhaps a more feasible hypothesis could be that meteorological conditions sometimes allow the emitted cloud of droplets to disperse only slowly in the environment and to drift downwind cohesively until changing conditions enable the droplets to be more rapidly deposited, giving rise to enhanced localized mortality. Further investigation might involve the storage of drops under various conditions and for varying lengths of time in order to assess their potential for longer-term effectiveness against *G. morsitans*. Some work in this area has been carried out involving the storage of drops at 25°C and their subsequent analysis,[10] but storage at lower temperatures more relevant to night-time spraying could be investigated.

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