Insecticide studies in vector control of Guatemalan onchocerciasis

1. Short carry of temephos in minute streamlets*

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Abstract: Effective distance of application of temephos wdp against blackfly larvae or insecticidal carry in streams, was studied by decrease of larval population on substrates and by bioassay of water and soil in minute streamlets in and near an onchocerciasis endemic area in Guatemala. An application of temephos with the dose of 2 ppm (AI) for 10 min water discharge was effective up to 100 m downstream in a streamlet of 0.8 liters/sec discharge, but only 25 m in a smaller streamlet of 0.13 liters/sec discharge. In a similar small streamlet of 0.07 liters/sec discharge, the carry was the same 25 m, even when an extraordinarily high dose of 200 ppm was applied. It was found that, as far as temephos is concerned, the effective distance should depend on the amount of water discharge, resulting in an extremely short carry in streamlets with minimum water discharge, and that in minute streamlets of 0.07–0.13 liters/sec discharge, the carry could not be extended by increasing insecticidal dose. The probable reasons for such short carry in minute streamlets were discussed.

INTRODUCTION

In Guatemala, a pilot area for onchocerciasis vector control was established in 1976, by the Guatemala-Japan Cooperative Project on Onchocerciasis Research and Control, the activity of the Project having been reviewed by Ogata (1981) and Suzuki

(1981). Larval control of blackflies with the main target species of *Simulium ochraceum*, was initiated in 1979, using temephos (Abate®). Through preliminary studies on insecticides, it was found that application of temephos with the dose of 1 ppm for 10 min water discharge, either of slow releasing solid formulation or of water-dispersible powders (wdp) was effective up to 160 m downstream (Tabaru *et al.*, 1982). Based on this, vector control in the Barretal River basin was carried out with great success (Nakamura *et al.*, 1981; Takaoka, *et al.*, 1981). Extensive stream tests in the Guachipilin River basin of the same pilot area in 1980, however, revealed that effective distance of the application of temephos with 2 ppm/10 min was sometimes short, 100 m or even less, particularly in small streams (unpublished).

Obviously, the dose of temephos in our

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study is excessive, nevertheless the carry being very short, comparing with that reported in the other parts of the world. In Canada, temephos emulsion with the dose of 0.15 ppm/30 min gave excellent control (Swabey *et al.*, 1967). In Africa, application of temephos emulsion with 0.54 ppm/30 min was effective to 6–32 km downstream (Quéllenec, 1970). In the Onchocerciasis Control Programme (OCP) in West Africa, application of temephos emulsion with the dose of 0.05–0.1 ppm/10 min by aircrafts has been the standard control measure, which gives excellent control (Davies *et al.*, 1978; Walsh *et al.*, 1979; Philippon *et al.*, 1981).

A great difference in efficacy of temephos between two areas under onchocerciasis vector control is presumed due to the different characters in breeding streams of vector blackflies. *S. damnosum*, s.l., the vector in Africa, breeds in large rivers or medium-sized streams, while *S. ochraceum*, the principal vector in Guatemala, breeds in small streams of 0.1–30 liters/sec discharge, or sometimes even less (Takaoka, 1981). Lea and Dalmat (1955), working in Yepocapa of Guatemala, reported in their classic but valuable paper that flow volume of stream was an important factor to dominate carry of insecticides, when DDT or EPN was used.

In the present study, carry of temephos was compared in small-scale tests of the application of 2 ppm/10 min with that of 200 ppm/10 min, one-hundred-fold more than the standard dose. The results revealed that as high as 200 ppm/10 min was able to sweep away the larvae only up to 25 m downstream, if the test was done in a very small streamlet of 0.07 liters/sec discharge.

STUDY SITES AND DATE

Studies were carried out in three streams, i.e. Rincon (R-1), Jazmines (J-18-C) and Guachipilin (G-18-10), during the period from October to December 1981. Water discharge of R-1, J-18-C and G-18-10 was 0.8, 0.13 and 0.07 liters/sec, respectively. All the streams tested harboured blackfly larvae. The predominant species was *S. ochraceum* (65–75%), followed by *S. metallicum*, *S. callidum* and *S. horacioi*. Water temperature

during the tests ranged from 18.0 to 19.5°C.

METHODS

Fifty per-cent temephos water-dispersible powders (wdp) were diluted with stream water at the application site, and poured into the stream in a few seconds, or instantaneously, with the dose of 2 ppm/10 min (R-1 and J-18-C) or 200 ppm/min (G-18-10). Effect of larval control was assessed by counting number of blackfly larvae on artificial and natural substrates in the streams. A bundle of four silicon tubes devised by Nakamura *et al.* (1978), each 25 cm long and 0.2 cm in diameter, was used as the artificial substrate. Five bundles of the tubes were set at each observation site in the stream seven days prior to the application. Immediately before the application, number of larvae attached to the tubes was counted with care, keeping the tubes below the water surface. No species identification was made at that time. Mean number of larvae per bundle was regarded as the pre-control density level. On the designated day after the application, all bundles of the tubes at each site were changed for new ones, and transferred into a plastic vial filled with 70% alcohol solution. Species identification was done later in the laboratory. Larvae on natural substrates such as trailing vegetation and fallen leaves were searched around the observation sites. The larvae collected were kept in alcohol solution for later identification.

Bioassay of stream water and streambed samples was done, in order to measure the concentration of temephos in the material. The water at six sites downstream was taken at the appointed time after the application. The streambed samples such as soil and fallen leaves on the bed were also taken in the tests of J-18-C and G-18-10, after 2 hr, 1 day, 7 days and 14 days of the application in order to measure residues of the insecticide. The samples were brought back to the laboratory, and bioassay was done using larvae of a *Culex quinquefasciatus* laboratory colony. In case of streambed samples, tests were done after immersing 100 ml samples in 100 ml fresh water.

C. quinquefasciatus larvae were first col-

lected from ditches in Villa Lobos, southern suburb of Guatemala City in August 1981. Preliminary tests with the larvae revealed the population was fairly resistant to temephos, consisting of the resistant and susceptible sub-populations. Therefore, selection was done to separate temephos-susceptible population from the mixed one, by the similar procedure as done by Umino and Suzuki (1966) with a malathion-resistant *C. p. fatigans* (= *C. quinquefasciatus*) population. An almost homozygous temephos-susceptible colony was established after selection for several generations. This colony is being maintained in the laboratory for a bioassay tool of water and streambed materials.

RESULTS

The results on the effect of larval control are shown in Table 1. In R-1 stream with

0.8 liters/sec discharge, no larvae were found up to 100 m downstream, either on natural or artificial substrates on day 7, after application of temephos with 2 ppm/10 min. On day 14, no larvae have yet been found up to 80 m downstream on natural substrates, and up to 60 m downstream on artificial substrates; but on day 21, all the substrates were positive with larvae. On the other hand in J-18-C with 0.13 liters/sec discharge, although the same dose of 2 ppm was applied, the effect was apparently inferior to R-1. On day 7, no larvae were found at the 25 m-downstream site, but many at the 50 m-site, both on natural and artificial substrates. In G-18-10, which was characteristic in the smallest discharge, 0.07 liters/sec, extraordinarily high dose of 200 ppm was applied on an experimental basis. No larvae were found at the 25 m-downstream site, either on day 1, day 7 or day 14, but a number of larvae

Table 1 Population density of blackfly larvae before and after application of temephos in three streams

Distance from application site (m)	Before application		Days after application							
			1		7		14		21	
	N	A	N	A	N	A	N	A	N ¹⁾	A ²⁾
R-1 (2 ppm in 0.8 liters/sec)										
45	+	NO ⁴⁾	NO	NO	—	—	—	—	++	##
60	##	NO	NO	NO	—	—	—	—	++	###
80	+	NO	NO	NO	—	—	—	+	##	##
100	+	NO	NO	NO	—	—	++	+	++	###
135	###	NO	NO	NO	###	++	##	+	###	##
J-18-C (2 ppm in 0.13 liters/sec)										
25	+	##	###	++	—	—	NO	NO	NO	NO
50	###	###	###	###	###	###	NO	NO	NO	NO
75	###	###	+	+	+	###	NO	NO	NO	NO
100	##	+	—	++	++	++	NO	NO	NO	NO
125	##	++	##	##	##	##	NO	NO	NO	NO
150	###	+	###	###	###	+	NO	NO	NO	NO
G-18-10 (200 ppm in 0.07 liters/sec)										
25	+	—	—	—	—	—	—	—	NO	NO
50	++	##	—	###	+	###	++	++	NO	NO
70	##	###	++	##	++	++	+	—	NO	NO
100	++	##	+	+	+	+	—	+	NO	NO
125	+	+	+	+	+	+	—	+	NO	NO

¹⁾ Natural substrates.

²⁾ Artificial substrates.

³⁾ Marks in the table show following No. of all species larvae: — (0), + (1-3), ++ (4-6), ## (7-9) and ### (≥ 10).

⁴⁾ Not observed.

were found at the 50 m-site, both on natural and artificial substrates, except on day 1 on natural substrates.

The results of bioassay of stream water in R-1 test are shown in Fig. 1. The application of temephos with 2 ppm/10 min, the concentration at the 45 m-downstream site was as high as 0.1 ppm after 5 min of the application, but it declined sharply thereafter. At both 60 m- and 80 m-sites, the highest concentration of temephos of 0.1 ppm was observed after 10 min, and declined thereafter. At the 100 m-site, the peak was after 20 min, with the highest concentration of 0.007 ppm, and it declined gradually thereafter. At the 135 m- and 160 m-sites, the highest concentration ranged between 0.001 and 0.002 ppm during the period 20 to 60 min after the application. The decline thereafter was gradual. The results of bioassay of stream water in the J-18-C and G-18-10 tests are shown in Table 2. In the J-18-C test, low concentration of temephos (0.00036–0.003 ppm) was detected at the 25 m-downstream site after 10 to 30 min of the application, but no temephos was detected in the bioassay at the 50 m-site. In G-18-10, where temephos of 200 ppm was applied, 0.03 ppm was detected at the 25 m-site after 5 to 120 min of the application, but none at the 50 m-site.

The results of bioassay of soil and fallen leaves in the treated streams are shown in Table 3. In the J-18-C test with 2 ppm/10 min, 0.025 ppm was detected at the site immediately downstream of the application point up to 7 days after the application; but at the 5 m- and 25 m-sites, none was detected, except 5 m-site after 2 hr (0.008 ppm). In

the G-18-10 test with 200 ppm, within the range of 10 m downstream of the application point, more than 0.025 ppm was detected even 14 days after the application. At the 25 m-downstream site, more than 0.025 ppm was detected after one day of the application; it declined to 0.006 ppm on day 7, and none detected on day 14. At the 50 m-site, no

Fig. 1 Concentration of temephos in stream water after instantaneous application of temephos wdp with the dose of 2 ppm for 10 min discharge in Rincon (R-1)

Table 2 Concentration of temephos (ppm) in stream water after application

Distance from application site (m)	Time after application (min)					
	5	10	20	30	60	120
J-18-C (2 ppm in 0.13 liters/sec)						
25	ND ¹⁾	0.003	0.00085	0.00036	ND	ND
50	ND	ND	ND	ND	ND	ND
G-18-10 (200 ppm in 0.07 liters/sec)						
25	0.03	0.03	0.03	0.03	0.03	0.03
50	ND	ND	ND	ND	ND	ND

¹⁾ Not detected by bioassay.

Table 3 Residues of temephos in soil and on fallen leaves on the streambed after application

Distance from application site (m)	Time after application			
	2 hr	1 day	7 days	14 days
J-18-C (2 ppm in 0.13 liters/sec)				
0	0.025	0.025	0.025	—
3	0.025	—	—	—
5	0.008	ND	ND	ND
25	ND ¹⁾	ND	ND	ND
G-18-10 (200 ppm in 0.07 liters/sec)				
3	0.025	0.025	0.025	0.025
5	0.025	0.025	0.025	0.025
10	0.025	0.025	0.025	0.025
25	0.025	0.025	0.006	ND
50	ND	ND	ND	ND

¹⁾ Not detected by bioassay.

temephos was detected in any test.

DISCUSSION

The results in the present study revealed that application of temephos with 2 ppm/10 min was effective up to 100 m downstream in a stream of 0.8 liters/sec discharge, but only 25 m in a smaller stream of 0.13 liters/sec discharge. In a similar but slightly smaller stream of 0.07 liters/sec discharge, the carry was the same 25 m, even with the extraordinarily high dose of 200 ppm/10 min. From a practical control point of view, the information to be derived from the results is of considerable importance. First is that the carry should depend on the amount of water discharge, hence extremely short carry in streamlets with minimum water discharge. Second is that in minute streamlets like 0.07–0.13 liters/sec discharge, the carry could not be extended by increase of insecticide dose.

Up to the present, efficacy of larvicides applied for blackfly control has usually been compared with each other, based only on concentration of the toxicants together with application time, often ignoring water discharge of the river or stream. Only a few studies have been conducted so far on relative efficacy of insecticides with different water discharge. Of these, the study of Lea and Dalmat (1955) was comprehensive and conclusive. After extensive field studies in Guatemala, they concluded that, within some

range of the discharge, the greater the volume of the streams tested, the greater the distance of complete larval control by DDT. The streams under their study ranged 20 gal/min (1.3 liters/sec) to 10,000 gal/min (630 liters/sec) of water discharge, thus excluding minute streamlets with less than 1.0 liters/sec, where *S. ochraceum* larvae breed with high density (Takaoka, 1981), although the length of such streamlets is usually limited. The present study revealed the finding of Lea and Dalmat (1955) on the relationship between water discharge and carry could be applied even to such minute streamlets, when treated by temephos.

Based on the information mentioned above, a new system of larvicide application was recently introduced as a trial to vector control operation in the San Vicente Pacaya Pilot Area, Guatemala. In the system, fixed amount of temephos wdp is applied to each stream, instead of fixed concentration of temephos. Therefore, concentration of temephos is variable according to the water discharge; high in small streams, and low in large streams. The new system is simple in operation, and has given good control results so far (unpublished).

It should be mentioned here that most studies on larvicides and control operations of blackflies dealt with large rivers or streams, because either vector blackflies of onchocerciasis in West Africa or nuisance blackflies in Canada or USA breed exclusively in such

large rivers or streams. On the other hand, vector blackflies in the Mesoamerica, or in Guatemala and Mexico, have a special character of breeding in minute streamlets. Experiences and knowledges on blackfly control reported from the other parts of the world are, therefore, not applicable directly to Guatemalan blackflies. Short carry of larvicides is an example of the particular characters to be taken into account in Guatemala.

Our bioassay studies of water in the streams revealed the insecticidal wave passed in a short time with a high peak at the site near the application point; while at the 135–160 m-downstream sites, the wave passed in a longer time, but with a very low peak on concentration of 0.001–0.002 ppm. The whole picture of the insecticidal wave is similar to the pattern presented by Wallace *et al.* (1976), who measured the concentration of Rhodamin B dye in the Chalk River after pouring it in 15 min. In our studies, however, decrease of concentration of temephos in relation to the distance from the application point is quite remarkable. On the other hand, a considerable amount of temephos was found in the soil and natural substrates on streambeds within 10 m downstream after application with 200 ppm.

It was claimed by Fredeen *et al.* (1953, 1975) that adsorption of toxicants onto particulates suspended in streams is the main reason for long carry of insecticides applied in the stream. In the case of Guatemalan streamlets under our study, such adsorption of the larvicide onto movable particulates is unlikely to take place. Instead, temephos is presumed to be adsorbed to the static soil and substrates on the streambeds, which could reduce the toxicant available for the larvae to the minimum extent, probably because of extremely minute water discharge of the streamlets. Sedimentation of toxicants on the bottom of the stream, particularly in the section where current velocity is low, might be another reason responsible for the short carry. Laboratory studies on adsorption of temephos in soil are now underway, which will be presented in a separate article.

Throughout the present study, it was confirmed that bioassay using *C. quinquefasciatus* larvae is an excellent tool to trace the

toxicant existing in water or streambed samples after application of temephos. It has the advantages of easiness in handling, of inexpensiveness in running cost, of possibly reasonable accuracy, and, above all, of capability of repeated use of one stream in a short interval for studies on efficacy of insecticide application.

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摘 要

グアテマラにおけるオンコセルカ症 媒介ブユ防除の研究

1. 微少水流におけるテメホスの有効距離

グアテマラのオンコセルカ症流行地において、自然の基物（流水中の木葉、草葉茎、石等）および人工基物（直径 0.2 cm, 長さ 25 cm のシリコンチューブ）に付着したブユ幼虫数の減少および流水と底の土壌の bioassay によって、テメホス水和剤の有効距離を調べた。0.8 l/sec の水流では、2 ppm/10 min のテメホス水和剤の処理で 100 m 下流まで有効であったが、0.13 l/sec の水流では同量散布で 25 m しか有効でなかった。さらに、0.07 l/sec のより小さい水流では、100 倍にあたる 200 ppm/10 min 散布でも有効距離はやはり 25 m であった。テメホスに関する限り、ブユ幼虫に対する有効距離はその水流の流量量によってきまり、流量量のきわめて小さい水流では、その有効距離は極端に短く、またこの有効距離は薬剤の量を増加することでは延長できないことを知った。さらに、有効距離の短い理由について考察を加えた。