EVALUATION OF DURSBAN[R] AND DOWCO 214 AS MOSQUITO LARVICIDES IN RICE-FIELDS

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INTRODUCTION

Among the mosquito-borne diseases, malaria and filariasis have been the most important diseases of mankind for centuries. This is true in many parts of the tropics and subtropics (Kessel, 1966; Johnson, 1973). In Malaysia, malaria is the most common mosquito-borne disease, especially so in the rural areas. Several species of *Anopheles* including *Anopheless campestris* are the main vectors of malaria in West Malaysia (Cheong, 1972). Filariasis caused by *Brugia malayi* is endemic in Malaysia (Ramachandran, 1972), the main vectors being the *Mansonia* species and several species of *Anopheles* particularly *Anopheles campestris* (Reid *et al.*, 1962; Ramachandran and Dondero, 1973). In Malaysia, antibodies to Japanese B encephalitis, another mosquito-borne disease which is epidemic in Japan, Korea and Taiwan, is widespread. A few cases involving children are seen throughout the year although the number of deaths are not known. *Culex tritaeniorhynchus* and *Culex gelidus* are the main vectors of Japanese B encephalitis (W.H.O., 1969; Reisen *et al.*, 1976).

Rice fields, especially those in Asia, are common breeding grounds for vector mosquitoes (Bram, 1967; Reid, 1968; Reisen *et al.*, 1976). On Penang Island, *Anopheles campestris*, *Culex tritaeniorhynchus* and several other species of *Anopheles* and *Culex* are commonly found in rice-fields. The control of these mosquitoes have so far largely depended upon the use of adulticides such as DDT (Cheong, 1972). No serious attempt was made to control the mosquitoes at the immature stages simply because of the dirth of information on larvicide field trials.

More recently, a few organophosphorous compounds have been found to be selective and effective as mosquito control agents (Schoof, 1966; W.H.O., 1972). Among the newer insecticides, Dursban[R] and Dowco 214 have been found to be effective mosquito larvicides and adulticides (Ludwig and McNeill, 1966; Kitagaki *et al.*, 1973).

In earlier laboratory tests, Dursban[R] (0, 0 diethyl 0-3, 5, 6-trichloro-2-pyridyl phosphorothioate) has been found to be a broad-spectrum insecticide against many agricultural and stored-product insect pests (Kenaga *et al.*, 1965). Its effectiveness as a larvicide against mosquitoes has been confirmed in laboratory tests (Ludwig and McNeill, 1966; Schoof, 1966; Yap *et al.*, 1968; Kitagaki *et al.*, 1973; Yap and Sulaiman, 1976) as well as in field trials (aerial and ULV applications) in many subtropical regions (Ludwig *et al.*, 1968; Mulla *et al.*, 1970; Washino *et al.*, 1972; Cooney and Pickard, 1974). Dursban has been found to be particular effective in controlling mosquito immatures in polluted water (Schaefer and Dupras, 1970b; Lowe and Parakarn, 1971). Various polymer control-release formulations of chlorpyrifos (Dursban) have been tested as mosquito larvicides (Miller *et al.*, 1973; Nelson *et al.*, 1973; Roberts *et al.*, 1973; Evans *et al.*, 1975; Nelson *et al.*, 1976). Adulticides effects of Dursban have also been investigated (Ludwig *et al.*, 1968; Husted *et al.*, 1975). At the dosages used for mosquito control, the biological effects of Dursban appear to be minimum (Ludwig *et al.*, 1968; Hurlbert *et al.*, 1970).

The insecticidal effects of Dowco 214 (0, 0 dimethyl 0-3, 5, 6-trichloro-2-pyridyl phosphorothioate), a dimethyl analog of Dursban currently known commercially as Reldan^R, are less studied. Laboratory tests indicate that Dowco 214 is promising as larvicides and adulticides against *Aedes*, *Anopheles* and *Culex* mosquitoes (Kitagaki *et al.*, 1973) and as larvicide against *Mansonia* mosquitoes (Yap and Sulaiman, 1976).

The present investigation concerns the field evaluation of Dursban and Dowco 214 as mosquito larvicides in some rice-fields of Penang. The physico-chemical conditions of the study sites were determined.

MATERIALS AND METHODS

Field study was carried out in the rice-fields adjacent to Jalan Kampung Masjid in the township of Telok Kumbar, situated on the southern coastal alluvial plain of Penang Island, Malaysia. Two rice-field sites were chosen for study from May to July, 1975. In the first site, fifteen plots totalling 0.284 hectare were used. The plot sizes range between 0.008 and 0.049 hectares. The second site lies adjacent to the first one, and consists of six plots totalling 0.162 hectare (ranging from 0.028 to 0.045 hectares). Air and water temperature and daily rainfall were recorded at these two sites for the entire study period.

Samples of mosquito larvae and adults were collected from the various plots prior to the larvicidal treatment and subsequently identified (Bram, 1967; Reid, 1968).

The chemistry of the semi-lotic water from all the plots within these two rice-field sites was studied. Water analysis was carried out on filtered samples taccording to standard methods (A.P.H.A., 1965; Golterman and Clymo, 1969). pH was measured at 25° ± 1°C with a pH-meter reading to ± 0.02 pH units. Alkalinity was determined using B.D.H. 4.5 as indicator. Total and calcium hardness were determined by means of sodium versenate (EDTA) technique. Specific conductance was measured with a conductivity meter. Orthophosphate was determined colorimetrically using stannous chloride-molybdate method.

The insecticides tested were in the forms of emulsifiable concentrates of Dursban (75 E.C., 7.5% chlorpyrifos, a.i. w/v) and Dowco 214 (22% chlorpyrifos-methyl, a.i. w/v). Dursban was tested at dosages of 14, 28 and 56 gm a.i. per surface hectare (i.e. 0.0125, 0.0250, 0.0500 lb a.i. per surface acre), while Dowco 214 was tested at 56 gm a.i. per hectare only. Two plots were used for each insecticide concentration in the first trial but in the second trial, one plot was used. The rest of the plots were used as control (untreated). The plots were chosen randomly.

Insecticides were diluted with tap-water and sprayed using a 2-gallon Hudson knapsack sprayer equipped with lance and nozzle (TEE JET, HSS 8002E). Two to four liters of insecticide - water mixture were sprayed per plot depending on the size of the plot. A dipper constructed of a light-colour enamelled plate (diameter 15.5 cm) with a 30-cm long handle was used to sample the mosquito larval population along the levees of the plots. Samples were taken immediately before and after 1, 2, 3, 7, 15, 30, 45 and 60 days after insecticide applications. Both the field trials were initiated immediately after the planting of rice on 2 May and 28 May 1975 respectively.

RESULTS

Physico-chemical conditions: The mean air temperature for Penang Island is 27.2°C (range: 23.3 - 31.1°C). At the study sites, the rice plots are totally exposed to direct insolation and as such the mean surface water temperature is high with mean values ranging between 29.1°C in the morning and 32.3°C in the afternoon. The lowest and highest water temperature recorded during this study are 25.5°C and 42.0°C respectively. Water depth varies between 7 and 15 cm.

The average annual rainfall for the last seventy five years is about 330 cm (130 in.). The daily rainfall record for the test sites over the study period (May-July 1975) is shown in Table 1. The total monthly rainfall for May, June and July are 31.2, 24.6 and 25.1 cm respectively. Spraying of insecticides was carried out on the relatively dry first and last week of May.

Some aspects of the chemical composition of the rice-field water are shown in Table 2. As a whole, the water is characteristically soft, weakly acidic and weakly buffered as indicated by the low bicarbonate alkalinity values. The ionic content is moderately high with mean specific conductance of 45.4 and 33.2 μmho cm^{-1} for Sites I and II respectively. Orthophosphate concentration varies between 0.015 and 0.034 ppm with means of 0.027 and 0.022 ppm for Sites I and II respectively.

Mosquito species: From the samples of mosquitoes collected from the rice-fields, at least three species of *Anopheles*, namely *An. campestris*, *An. jamesii* and *An. sinensis*, were encountered. *An. campestris*, the dominant species, is also a vector of malaria and filariasis in Penang. *Culex tritaeniorhynchus* is the most common *Culex* mosquito found here, the other being *Cu. bitaeniorhynchus*. *Cu. tritaeniorhynchus* is associated with the transmission of Japanese B encephalitis while *Cu. bitaeniorhynchus* is known to be naturally infected with *Brugia malayi* (Carter, 1948).

Larvicidal effects of Dursban and Dowco 214: The results of the field evaluation of the larvicidal effects of Dursban and Dowco 214 on

Table 1

Rainfall over the study area in Telok Kumbar, Penang.*

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
May	O	O	S	O	O	O	O	S	S	M	S	S	S	M	H	M	M	O	O	O	H	M	H	M	H	S	O	M	O	O	O
June	M	O	O	S	O	O	O	O	O	M	O	H	O	O	H	M	O	O	O	O	S	O	O	O	O	O	O	O	O	H	-
July	O	O	O	O	O	S	S	O	M	O	O	O	O	O	O	O	O	O	H	O	O	O	O	O	O	H	H	H	O	O	O

* O = No rain; S = Slight rain (less than 1.27 cm); M = Moderate rain (1.27-2.54 cm); H = Heavy rain (more than 2.54 cm).

Table 2

Chemical content of the rice-field water in experimental areas.
All values are mean ± S.E. from seven determinations of water samples collected from various plots throughout the experimental period.

Site No.	pH	Specific Conductance (μmho. cm^{-1})	Alkalinity (ppm HCO_3)	Total Hardness (ppm $CaCO_3$)	Calcium Hardness (ppm $CaCO_3$)	Orthophosphate (ppm)
I	6.8 ± 0.9	45.4 ± 6.8	13.9 ± 2.0	5.4 ± 0.8	3.7 ± 0.5	0.027 ± 0.004
II	6.9 ± 1.0	33.2 ± 4.9	11.4 ± 1.8	4.7 ± 0.7	3.6 ± 0.5	0.022 ± 0.003

larvae of *Anopheles* and *Culex* are shown in Table 3. The *Anopheles* larvae appear to be more sensitive to Dursban and Dowco 214 than the *Culex* larvae. At the concentrations used, both achieved 100% control of the *Anopheles* larvae for at least two days. The dosage-mortality relationship of Dursban to *Anopheles* is well defined in Site I. Dursban, at concentrations of 14, 28 and 56 gm hectare^{-1}, kept the paddy plots free from *Anopheles* for at least two, three and seven days respectively (Table 3). Mosquito control by Dursban at Site II appears to be less effective even at a concentration of 56 gm hectare^{-1}. With respect to *Culex*, the results are more erratic (Table 3). The few *Culex* larvae that reappeared in the first three days following larvicide application are of the fourth instar stage, which have apparently survived the insecticide treatment. From the seventh post-treatment day onwards, mosquito larvae were found in the treated plots but at densities generally lower than those of the untreated plots. The gradual reduction in larval densities in both the treated and untreated plots towards the end of the study period is attributable to the gradual drying up of the rice plots. Dowco 214, applied at a dosage of 56 gm hectare^{-1}, appear to be less effective against *Anopheles* larvae than Dursban at the same dosage.

DISCUSSION

As stated earlier, the mosquito larviciding and adulticiding effects of Dursban have been investigated using aerial and ultra low volume (U.L.V.) applications as well as various other types of polymer formulations involving large-scale study sites. The present study on

Table 3

Larvicidal effects of Dursban and Dowco 214 against *Anopheles* and *Culex* mosquitoes in rice-fields at Site I and Site II.

Number of days after treatment			0*	1	2	3	7	15	30	45	60
Treatment received (gm/hectare)	Plots	Mosquitoes	Mosquito larvae per twenty five dips per plot								
SITE I.											
Control (untreated)	7	*Anopheles*	23.7	11.6	13.1	18.9	10.9	12.3	14.1	4.4	2.7
Dursban (14)	2	,,	24	0	0	0.5	19	9	2.5	5	0
Dursban (28)	2	,,	18	0	0	0	0.5	7.5	6.5	0.5	0
Dursban (56)	2	,,	11.5	0	0	0	0	5	2.5	4	4
Dowco-214 (56)	2	,,	13	0	0	0	6	6	6.5	19	10.5
Control (untreated)	7	*Culex*	17.4	32	46.7	15.7	8.4	15	55.4	2.1	1.2
Dursban (14)	2	,,	21.5	4	0.5	0.5	5	7.5	4.5	3.5	0
Dursban (28)	2	,,	15	1	0	0	6.5	2	12	14.5	1
Dursban (56)	2	,,	18.5	0.5	0	0	5.5	7.5	13	5	1.5
Dowco-214 (56)	2	,,	7.0	0	0	0.5	0.5	7	9	20.5	31.5
SITE II.											
Control (untreated)	2	*Anopheles*	13.8	20.5	29	28.5	24.5	6.5	5	7.5	D**
Dursban (14)	1	,,	17	0	0	2	0	3	0	3	D
Dursban (28)	1	,,	16	0	0	0	9	22	3	12	D
Dursban (56)	1	,,	46.5	0	4	12	68	13	8	11	D
Dowco-214 (56)	1	,,	2.5	0	0	7	20	5	3	4	D
Control (untreated)	2	*Culex*	9.3	22	38	40.5	20	9.5	4.5	8.5	D
Dursban (14)	1	,,	9	0	0	0	0	0	4	0	D
Dursban (28)	1	,,	21.5	0	0	0	29	1	3	2	D
Dursban (56)	1	,,	20	0	5	0	6	0	0	0	D
Dowco-214 (56)	1	,,	27.5	0	0	3	33	0	0	0	D

* Mosquito larval count before spraying. ** Paddy field dried.

the larviciding effects of Dursban and Dowco 214 was carried out on small plots using emulsifiable concentrates and Hudson knapsack sprayer. The dosages (i.e. 14, 28 and 56 gm hectare^{-1}) tested in this study are below the normal application rates (112 gm hectare^{-1}) commonly used for organophosphorous larvicides (Schaefer and Dupras, 1970a). The results indicate that both Dursban and Dowco 214 have good potentials as mosquito larvicides in tropical rice-fields.

Direct sunlight and temperature fluctuation may enhance the hydrolysis of organophosphorous insecticides such as Dursban and Dowco 214. It is probable that these two factors are operational in this study and thus affect the results to some extent. Since rainfall was negligible during the first week following each treatment (Table 1), overflowing or washing away of insecticides did not occur. Under the existing chemical conditions of the rice-field water, the breakdown rates of Dursban and Dowco 214 should be moderate.

Dursban at the dosages used for mosquito control has been reported to be safe against non-target freshwater organisms. When applied at rates below 0.1 lb acre^{-1} (112 gm hectare^{-1}) it causes less than 10% mortality to the caged mosquito fish *Gambusia affinis* and does not affect fish reproduction (Hurlbert *et al.*, 1970). However, application of Dursban at the same dosage in combination with the herbicide propanil in rice fields has been shown to trigger photo-toxic response in the rice plants (Craven and Steelman, 1968). The persistence of Dursban in pond waters has been shown to be relatively short, but its residues in the mud layer have been shown to increase gradually after application (Hurlbert *et al.*, 1970). The longer persistence of Dursban in polluted water is attributable to the fact that Dursban is absorbed onto the organic matter and thus is protected from hydrolysis (Schaefer and Dupras, 1970b).

Like most organophosphorous insecticides, Dursban and Dowco 214 are blood cholinesterase inhibitors. Repeated exposure to these compounds, even at low dosages, may cause cholinesterase depression. The oral toxicity values (LD_{50}s) of chlorpyrifos (Dursban) and chlorpyrifos-methyl (Dowco 214) to white rats are 97-276 and 941-2,140 mg kg^{-1} respectively. However, the dermal toxicity values for both are 2,000 mg kg^{-1}. This relatively low dermal toxicity makes both the safer organophosphorous compounds to be used for mosquito control (W.H.O., 1972; Kenaga and End, 1974).

With the tremendous increase in the prices of petroleum products of which the anti-malaria oil is no exception, alternatives are being sought to replace the often-used anti-malaria oil. Abate appears to be a good alternative with its good larviciding property and also from the point of view of safety (Cyanamid, 1969; Yap, 1976). From this present study, Dursban and Dowco 214 appear to be promising as mosquito larvicides. They could be used as substitutes for the anti-mosquito oil particularly so in polluted waters.

SUMMARY

Emulsifiable concentrates of Dursban^{R} (chlorpyrifos) and Dowco 214 (chlorpyrifos-methyl) were tested as mosquito larvicides using Hudson knapsack sprayers on small plots of rice-fields on Penang Island. The mosquitoes found in these rice-fields are predominantly *Anopheles campestris* and *Culex tritaeniorhynchus*. At dosages of 14, 28 and 56 gm hectare^{-1}, Dursban is effective in maintaining the rice-fields free of *Anopheles* larvae for at least 2, 3 and 7 days respectively. Dowco 214 at 56 gm hectare^{-1} is able to keep the fields free of all mosquito larvae for at least 2 days.

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