DURSBAN PREMISES APPLICATIONS AND THEIR EFFECT ON THE CHOLINESTERASE LEVELS OF SPRAYMEN

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Dursban [5], 0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate, has been demonstrated to be effective against numerous mosquito species in diverse habitats. Ludwig and McNeil (1966) reported a high degree of biological activity against larvae and adults of *Aedes aegypti* and *Aedes sollicitans* and larvae of *Culex quinquefasciatus* when it was applied as a granular formulation, emulsion, or thermal fog. Sjogren and Mulla (1968) obtained good results with Dursban emulsifiable concentrate applied by the drip method for the control of mosquitoes in sewage ponds. Brooks *et al.* (1967) demonstrated 19 weeks of effective kills of *Ae. aegypti* larvae in cans and tires with emulsifiable Dursban. Mathis and Schoof (1968) reported Dursban residues on clay surfaces to be highly effective against *Anopheles quadrimaculatus*.

Because of its high level of effectiveness as a mosquito larvicide, field trials with Dursban against *Ae. aegypti* were started in Perrine, Florida, in 1966. However, the adverse effect of these treatments upon the cholinesterase level of the spraymen led to the termination of the study within 2 weeks after spraying was begun. Since the application rates and the amount of exposure in these tests were extreme, a second study was conducted in 1967. These results indicated no adverse effects on the four spraymen, but the limited exposure period and amount of Dursban applied necessitated a further and more intensive evaluation designed primarily to measure the hazard of the compound to exposed spray personnel. Consequently, in 1968 a closely monitored field test was set up to determine what effect Dursban would exert on spraymen applying treatment at the rates normally encountered on operational programs for eradication of *Ae. aegypti*. The present paper describes the three studies with the major reference given to the 1968 investigations.

METHODS

INSECTICIDE TREATMENT PROCEDURES —1966 TEST. The area sprayed consisted of premises and vacant lots normally encountered in a subtropical, lower socioeconomic neighborhood. Lush and heavy vegetative growth was common. Dursban as a 0.5 percent emulsion was applied by means of power spray equipment using an application pressure of 250 pounds per square inch. All types of premises and vacant lots were treated, some of which contained an extremely heavy plant growth. The high pressure resulted in micronization and considerable splash-back of the finished spray. The average number of gallons applied per premises was 29.1. Five spraymen and one foreman were involved in the 9-day spraying period.

1967 TEST. This test was carried on

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[5] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U. S. Department of Health, Education, and Welfare.

in an area which had a low *Ae. aegypti* positive container index. Dursban was applied as a 0.25 percent suspension and as a 0.5 percent emulsion. This test was run for only 5 days during which 177 gallons of suspension and 145 gallons of emulsion were applied to two 9-block areas containing 182 premises. The average number of gallons sprayed per premises was 0.5. Four spraymen, one foreman, and two controls were involved.

1968 TEST. These studies were conducted in an area similar to that used for the 1966 tests with the exception that vacant lots, premises heavily overgrown with vegetation, and large piles of containers were eliminated from the spray schedule. Dursban was applied as a 0.5 percent suspension or emulsion with a separate crew spraying each type of formulation. Treatment was made with truck-mounted power equipment with the working spray pressure at 125 pounds per square inch. A pistol-type spray gun was used in preference to the orchard type employed in 1966. The pistol-type made it possible to produce a coarse cone spray without having to adjust through a fine particulate spray pattern as was necessary with the orchard type unit. The average number of gallons applied per premises was 4.6.

Individual containers were treated and blanket spraying avoided. The seven spraymen and hosemen were instructed to stay upwind of the spray gun wherever possible to minimize exposure to spray drift. They were cautioned to spray only in open areas and to avoid heavy vegetative undergrowth where they might be more subject to inhalation of airborne spray particles. Clean, uncontaminated clothing was provided daily. Protective masks and gloves were worn by men that handled the concentrate when mixing the formulation in the sprayers. The foreman was cautioned to have as little contact with the spray as possible. This individual and three persons who would have no contact with cholinesterase inhibiting compounds during the test period served as controls.

CHOLINESTERASE DETERMINATION. Cholinesterase measurements were made by the pH stat method as described by Nabb and Whitfield (1967). In 1966 and 1967 the weekly venous blood samples were drawn locally, packed in ice, and shipped to the NCDC Pesticides Program, Toxicology Section, for cholinesterase value determinations. In 1968 all cholinesterase values were determined locally by the Pesticides Research Laboratory. Determinations were made on the day following the withdrawal of the blood samples from the spraymen and were immediately available to us. Values reported (Table 1) are expressed in micromoles of acetylcholine hydrolized per minute per milliliter of plasma or packed red blood cells.

In each test it was decided to terminate the exposure of any individual if a depression of 50 percent of his normal baseline activity was observed. This level was chosen as the criterion, since symptoms of organophosphate poisoning rarely appear until this level is reached and, therefore, the chances of deleterious effects are still negligible (Goltz and Shaffer, 1960).

RESULTS

Since the 1966 study was primarily done to test the larvicidal activity of Dursban against *Ae. aegypti* and cholinesterase activity in the spraymen was a secondary consideration, the study was not designed to monitor closely the output of the individual sprayman. However, it was readily evident that three spraymen showed a marked depression of their plasma cholinesterase, the values ranging from 68 to 82 percent reduction of the normal pretreatment exposure levels. Two other spraymen for whom pretreatment values were not available showed 52 to 56 percent reduction in their plasma values over a 9-day spraying period. Because of the significant cholinesterase inhibition that occurred in these spraymen, the study was discontinued after 2 weeks of spraying.

The data for the 1967 application indicated that none of the four spraymen showed any significant difference between the pretreatment and the posttreatment cholinesterase value determined on three separate occasions during the 1 week of spraying. However, the plasma cholinesterase level for the foreman did fall from a level of 4.5 to 2.1 over the 7-day period. Since this individual was not involved in actual spraying, the reason for this depression was not apparent. Although no preexposure level was available for the second foreman, his plasma values for the same period ranged between 4.5 and 4.8. The small sample and the limited time and amount of exposure were such that the above findings on the spraymen permit only a limited interpretation. These data required validation and confirmation

TABLE 1.—Cholinesterase levels[1] in spraymen applying known amounts of 0.5 percent Dursban as a larvicide for *Aedes aegypti* control (1968 test).

			Exposure			Postexposure	
		Preexposure[2]	April 25	May 2	May 6	May 23	June 12
Suspension							
Sprayman 1	RBC	14.8	16.0	16.6	18.8	13.2	16.7
	Plasma	4.6	1.1	0.4	1.0	2.4	3.2
	Gal. sprayed[3]	0	141	301	65	20	0
Sprayman 2	RBC	12.3	16.9	13.2	19.2	12.3	13.9
	Plasma	4.0	1.9	1.1	1.2	2.8	4.0
	Gal. sprayed[3]	0	109	260	85	10	0
Sprayman 3	RBC	14.0	16.5	..	22.5	..	16.5
	Plasma	4.7	5.0	..	2.2	..	4.9
	Gal. sprayed[3]	0	90	..	355	..	32
Emulsion							
Sprayman 4	RBC	15.3	18.0	17.0	21.0	13.0	16.2
	Plasma	5.0	3.5	2.4	2.3	3.7	5.5
	Gal. sprayed[3]	0	70	165	35	20	0
Sprayman 5	RBC	12.2	15.0	16.0	..	13.4	13.9
	Plasma	4.3	3.8	4.0	..	4.4	5.5
	Gal. sprayed[3]	0	55	47	..	115	0
Sprayman 6	RBC	15.0	17.9	15.4	..	..	15.2
	Plasma	5.6	4.5	3.3	..	..	4.9
	Gal. sprayed[3]	0	59	210	..	..	110
Sprayman 7	RBC	12.8	13.0	14.8	..	..	14.1
	Plasma	5.2	5.8	4.4	..	..	5.3
	Gal. sprayed[3]	0	51	65	..	..	85
Foreman	RBC	15.9	14.7	18.0	..	13.8	
	Plasma	4.3	4.9	4.3	..	4.0	
Control 1	RBC	15.1	16.9	14.0	..	12.0	
	Plasma	3.7	3.2	3.8	..	3.9	
Control 2	RBC	10.5	13.1	13.0	..	11.2	
	Plasma	4.2	4.0	4.0	..	4.4	
Control 3	RBC	11.1	14.1	13.6	..	12.0	
	Plasma	5.6	5.9	5.7	..	6.1	

[1] Values reported are expressed in micromoles acetylcholine hydrolized/minute/ml. plasma or packed red blood cells.

[2] Average for analyses done prior to commencement of spraying.

[3] Gallons of finished spray applied between consecutive blood analysis dates.

in a study over a much longer period during which a much higher degree of exposure would be involved.

In the 1968 study five of the seven spraymen showed more than 50 percent reduction of plasma cholinesterase values within 2 weeks after treatment was begun, thus necessitating a termination of the test. The data on the exposure and the cholinesterase levels for the spray personnel, foreman, and controls are shown in Table 1. Analyses of the data for these individuals show that the quantity of Dursban sprayed proportionately inhibits plasma cholinesterase. No clinical manifestations of organophosphate poisoning were noted in any of the spraymen although in the case of Sprayman No. 1, there was over a 90 percent decrease in his plasma enzyme level on May 2 as compared to his preexposure level. No inhibition of red cell cholinesterase activity was observed at any time.

A cholinesterase level for an individual may be considered abnormal if it shows excessive deviation from his own normal values, or if it falls outside the range of values observed in a normal population. There is a reasonably large range in plasma (23 percent) and red cell (25 percent) cholinesterase determinations taken periodically from the same normal, unexposed individual (Ganelin *et al.*, 1964). Nabb and Whitfield (1967) found a range of 3.6–6.8 μM of acetylcholine hydrolyzed per minute per milliliter of plasma. Judged either on the basis of their individual normal values, or on the basis of the values for a normal population, all of the spraymen, with the exception of numbers 5 and 7, exceeded the expected deviation for plasma cholinesterase activity, although the values for the foreman and the controls remained within the expected range.

DISCUSSION

In general, the expected results of exposure to most organophosphate insecticides, as measured by blood cholinesterase activity, is the initial depression of plasma cholinesterase followed by a decrease of red blood cell cholinesterase (Goltz and Shaffer, 1960). The rate and extent of the decrease in activity relate to the amount of organophosphate absorbed. Dursban did produce the expected effect that the amount and rate of depression were related to the gallonage sprayed. The question of whether or not a decrease in red cell cholinesterase would have been observed if a longer exposure period at the same average daily rate had been used is not answerable from the data obtained.

However, in laboratory tests (Gaines, T. B., unpublished data) the dosages of Dursban (e.g., 1.0 mg./kg.) that decreased the plasma cholinesterase levels in rats usually exerted a similar but lesser degree of lowering of the red blood cell cholinesterase. A critical dosage that had only a slight depressive effect on the plasma cholinesterase was associated with a small rise in the red blood cell cholinesterase. In the 1968 spray trial, a similar slight increase in red cell cholinesterase was observed in the men during the exposure period, and it persisted irregularly into the postexposure period. No increase was seen during the 1966 study. The increase in red cell cholinesterase activity associated with dosages just adequate to inhibit plasma enzyme to some degree is not fully understood, but it has been observed occasionally in animals in connection with some other compounds. It is thought to represent an overcompensation without clinical significance.

These findings for Dursban when used as a larvicide treatment under outdoor conditions were not anticipated when the original study commenced. Although Dursban, in common with all organophosphate insecticides, inhibits cholinesterase (Gray, 1965) the oral LD-50 value for it with female rats is 82 (Gaines, 1969) to 135 mg./kg., a value range in which a compound is generally considered as only moderately toxic. While the results of the initial tests were at first thought to be due to the high gallonage applied per premises, the amount of material applied during the 1968 study was at

a level considered to be in the range of the normal amount of spray applied in the *Ae. aegypti* operational program. Although the results for 1967 showed no effect on the cholinesterase of the spraymen, the gallonage applied was below that normally sprayed on operational programs and, consequently, the primary interpretation of the suitability of Dursban as a premises larvicide against *Ae. aegypti* must be based on the data obtained in 1968. The findings for the last test confirm the results obtained from the original work in 1966 even though there was an 84 percent reduction in the average amount of Dursban spray applied per premises. The application of a suspension formulation apparently had no advantage over the use of an emulsion nor was there any obvious beneficial effect of reducing the spray pressure from 250 to 125 pounds per square inch.

Inasmuch as a compound that produces a measurable decrease in plasma or red blood cell cholinesterase level in spraymen is not considered to be a suitable material for use in public health work in the absence of an epidemic or the immediate threat of one, the conclusion is that Dursban emulsion or suspension formulations are not acceptable for use as a premises larvicide treatment in the control of *Ae. aegypti* under the conditions now present in the United States. It is conceivable that Dursban could be used as a granular formulation, at a much lower dosage as described for a nonresidual type of larviciding or at an intermittent or less frequent interval without any effect on cholinesterase levels. The findings also suggest that the use of Dursban in any operation similar to that described previously might produce a depression of cholinesterase levels. Therefore, on all such control programs, careful monitoring of the cholinesterase levels of spraymen should be included in the operation.

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References

BROOKS, G. D., SMITH, E. A., and SCHOOF, H. F. 1967. Residual effectiveness of eleven insecticides under weathering conditions against *Aedes aegypti*. Mosq. News 27(1):93–97.

GAINES, T. B. 1969. The acute toxicity of pesticides. Tox. and Appl. Pharm., 14(3):515–534.

GANELIN, R. S., CUETO, C., JR., and MAIL, G. A. 1964. Exposure to parathion. J. Am. Med. Assoc. 188:807–810.

GOLTZ, H. H., and SHAFFER, C. B. 1960. Toxicological information on Cyanamid insecticides. American Cyanamid Company.

GRAY, H. E. 1965. Dursban . . . a new organo-phosphorus insecticide. Down to Earth 21(3):2, 26, 27. (A publication of The Dow Chemical Company, Midland, Michigan.)

LUDWIG, P. D., and MCNEIL, J. C., IV. 1966. Results of laboratory and field tests with Dursban insecticide for mosquito control. Mosq. News 26(3):344–351.

MATHIS, W., and SCHOOF, H. F. 1968. Residual activity of several new insecticides to adult *Anopheles quadrimaculatus* in adobe-lined experimental huts. Mosq. News 28(3):412–415.

NABB, D. P., and WHITFIELD, F. 1967. Determination of cholinesterase by an automated pH stat method. Arch. Environ. Health 15:147–154.

STOCKEN, R. D., and MULLA, M. S. 1968. Drip application of three organophosphorus insecticides for mosquito control. Mosq. News 28 (2):172–177.