THE EFFECTIVENESS OF EPN AND SOME OTHER ORGANIC PHOSPHORUS INSECTICIDES AGAINST RESISTANT MOSQUITOES [1]

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The partial or complete failure of DDT and other chlorinated hydrocarbon insecticides against mosquitoes in California, Florida, and some other states has become a serious handicap to mosquito-control workers in recent years. In California resistance has developed rapidly in the San Joaquin Valley, and in 1952 field applications of larvicides at 10 to 15 times the normal dosages failed to control *Culex tarsalis* Coq. in three mosquito abatement districts and *Aedes nigromaculis* (Lud.) in one district. The high level of resistance found by Gjullin and Peters (1952) in these species and also in *Aedes dorsalis* (Meig.) and *Culex quinquefasciatus* Say suggests that with continued use chlorinated hydrocarbon insecticides will soon be ineffective against all these species in the San Joaquin Valley.

In 1952 the California Bureau of Vector Control, the U. S. Bureau of Entomology and Plant Quarantine, the California Mosquito Control Association, and the Kern Mosquito Abatement District carried on a cooperative project in the Kern District to determine the effectiveness of several organic phosphorus insecticides against resistant mosquitoes. Preliminary tests in the Kern District in the fall of 1951 indicated that EPN might be very effective against resistant mosquitoes. In laboratory tests at Corvallis, Ore., Yates and Lindquist (1952) found it to be the most toxic material they had ever tested against mosquito larvae.

LABORATORY TESTS—The comparative effectiveness of DDT and the three organic phosphorus insecticides is shown in table 1. On *Aedes nigromaculis* EPN was 67 times as toxic as DDT, while on *Culex tarsalis* it was 171 times as toxic. The LD-50 of EPN on *A. nigromaculis* was 0.000862 p.p.m. Yates and Lindquist (1952) found it to be 0.00135 p.p.m. on non-resistant floodwater *Aedes*. On *C. tarsalis* both tetra-*n*-propyl dithionopyrophosphate and malathon were more effective than DDT, but on *A. nigromaculis* tetra-*n*-propyl dithionopyrophosphate was slightly less effective than DDT.

The toxicity of the three phosphorus insecticides at different temperatures was tested on *C. tarsalis* larvae (table 2). EPN was about twice as effective, malathon about four times, and tetra-*n*-propyl dithionopyrophosphate five times as effective at 90° as at 70° F. Yates (1950) found DDT more effective at 63° than at 93° on several species of mosquito larvae, as did Lindquist *et al.* (1946) on adult *Anopheles quadrimaculatus* Say and *Culex quinquefasciatus*.

TABLE 1.—Parts per million of some organic-phosphorus and DDT larvicides required to give a 24-hour LD-50 of resistant fourth-instar mosquitoes collected in Kern County, Calif.

Insecticide	*Aedes nigromaculis*	*Culex tarsalis*
EPN	0.000862	0.000649
Tetra-*n*-propyl dithionopyrophosphate	.0625	.0178
Malathon	.025	.0185
DDT	.0588	.111

FIELD TESTS—The tests against *C. tarsalis* larvae were made in areas inundated by the Kern River. The water ranged from a few inches to 2 feet in depth, and usually was partly covered with grass. Most applications on *A. nigromaculis* were made in irrigated pastures in open water

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TABLE 2.—Toxicity of organic phosphorus larvicides to fourth instars of *Culex tarsalis* at 70° and 90° F. Average of six replications

Insecticide, p.p.m	Percent Mortality in 24 Hours 70° F.	90° F.
EPN		
0.00067	57	91
.0005	46	79
.00025	16	48
.0000125	4	24
Tetra-*n*-propyl dithionopyrophosphate		
0.033	44	100
.025	17	100
.0167	15	85
Malathon		
0.033	24	85
.025	19	75
.0167	8	40

from 1 to 10 inches deep, but in some plots the water was nearly covered with grass.

The sprays were applied from a jeep with the hose that is standard equipment on these vehicles. The spray head of the hose was equipped with a No. 3 disc, and the pressure ranged from 150 to 200 pounds per square inch. The diluted larvicides were applied at the rate of 10 gallons per acre in all tests. A few applications were made with a 3-gallon hand sprayer.

One series of tests was made with a Stearman plane, flying 90 miles an hour at a height of 25 to 50 feet. Plane applications were made at the rate of 1.2 gallons per acre.

EPN emulsions were made from a 45 per cent concentrate and EPN suspensions from a 25 per cent wettable powder. The tetra - *n* - propyl dithionopyrophosphate emulsion was made from an 85 per cent concentrate, and the malathon emulsion from a concentrate containing 5.5 pounds of malathon per gallon.

Jeep applications of EPN at 0.035 pound per acre gave kills of 99 and 100 per cent, but higher dosages were required for comparable kills in plane applications (table 3). This was probably due to the smaller volume of more concentrated spray applied by the plane, to the adverse effects

TABLE 3.—Field tests of organic phosphorus larvicides on fourth instars of *Aedes nigromaculis* and *Culex tarsalis*. Average of 2 to 5 replications

Insecticide	*A. nigromaculis* Pounds of Active Ingredient per Acre	Percent Mortality (24 Hr.)	*C. tarsalis* Pounds of Active Ingredient per Acre	Percent Mortality (24 Hr.)
	Airplane Applications			
EPN emulsion	0.045	98	0.045	96
	.035	89	.035	89
	.025	45	.025	57
	Jeep Applications			
EPN:				
Emulsion	0.035	99	0.035	100
	.025	95	.025	98
	.01	89	.01	97
			.005	57
Suspension	.035	99	.035	100
	.025	98	.025	100
	.01	55	.01	97
			.005	70
Malathon emulsion	.4	99	.3	83
	.3	92	.2	97
	.2	83	.1	67
Tetra-*n*-proply dithionopyrophosphate emulsion	.4	93	.3	99
	.3	89	.2	77
	.2	87	.1	76

of light winds in some tests, and to the evaporation of the smaller spray droplets. Malathon and tetra-*n*-propyl dithionopyrophosphate were about one-tenth as effective as EPN.

ROUTINE SPRAYING OPERATIONS — The Kern Mosquito Abatement District began routine application of EPN emulsion at 0.075 pound of EPN per acre with a plane and three jeeps in June after a permit for the experimental use of this insecticide had been granted by the State. Sixteen more jeep operators began using this insecticide in July and during the rest of the season for all control operations except in cities and towns where its use was not covered by State permit. It has given excellent control of larvae in all situations in which it has been applied.

The EPN emulsifiable concentrate is issued in small screw-top bottles, which are carried in compartments of a wooden box in the jeep. One bottle contains sufficient EPN for one tank of diluted emulsion. The operator wears rubber gloves when emptying one of these bottles into the tank, which has been partially filled with water. More water is then added to fill the tank. Instructions for handling the EPN and procedures to be followed in case the skin or clothing is contaminated have been issued to all operators.

TOXICITY HAZARDS—The toxicity of EPN and other phosphorus insecticides both to insects and warm-blooded animals is due to their ability to inhibit cholinesterase activity in the body. In man the cholinesterase activity of the red cells and the plasma of the blood can be used as a measure of this activity. The critical level is considered to be 60 per cent of the normal level for the individual.

The plane pilot and the three jeep operators who began spraying with EPN in the Kern District in June were given cholinesterase-activity tests before they used EPN and at 2-week intervals during the season in order to obtain information on the hazards of these spraying operations. The tests have shown no significant reduction in the cholinesterase level of the men.

Several agencies are investigating the toxicity hazard of EPN to warm-blooded animals. Tests by the Food and Drug Administration and others indicate that EPN has about one-fifth the acute oral toxicity of parathion and that the dermal toxicity of the two materials is about the same. Both malathon and tetra-*n*-propyl dithionopyrophosphate have low acute oral and dermal toxicities in comparison with EPN.

Determination of spray residues on grass has not yet been made, because there is no satisfactory method for recovery of EPN under these conditions. However, no adverse effects have been noted in cattle that have grazed in a pasture sprayed six times at 6- to 10-day intervals with EPN at 0.075 pound per acre.

CONCLUSION—These tests and routine applications indicate that EPN is an effective substitute for chlorinated hydrocarbon insecticides in areas where mosquitoe larvae have become resistant to these materials. Malathon and tetra-*n*-propyl dithionopyrophosphate are also effective against resistant mosquito larvae. They are less dangerous to handle than EPN, but they may not be so economical to use because of the large dosages required.

SUMMARY

DDT and other chlorinated hydrocarbon larvicides have failed to control *Culex tarsalis* Coq. and *Aedes nigromaculis* (Lud.) in several mosquito abatement districts in the San Joaquin Valley of California.

Laboratory tests were made to compare the effectiveness of three organic phosphorus insecticides and DDT. EPN was found to be the most toxic. The LD-50's of EPN for resistant larvae of *C. tarsalis* and *A. nigromaculis* from the Kern District were 0.000649 p.p.m. and 0.000862, respectively. It is 67 and 171 times as toxic, respectively, as DDT. EPN emulsion was about twice as effective against *C. tarsalis* larvae, and malathon and tetra-*n*-propyl dithionopyrophosphate about five times as effective at 90° as at 70° F.

In field tests in the Kern Mosquito Abatement District EPN killed 99–100 per cent of these mosquitoes when applied from a jeep at 0.035 pound per acre in emulsion or suspension. Malathon and tetra-*n*-propyl dithionopyrophosphate were about one-tenth as effective as EPN.

Routine applications of an emulsion at 0.075 pound of EPN per acre with 19 jeeps and 1 plane in the summer of 1952 were completely effective against these larvae.

Periodic blood tests of four of the men carrying on these spraying operations have shown no significant reduction in the cholinesterase level of their red cells or plasma.

No adverse effect has been noted in cattle that have grazed in one pasture after six successive applications of this emulsion.

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SOME TECHNIQUES FOR MOUNTING MOSQUITO EGGS, LARVAE, PUPAE AND ADULTS ON SLIDES

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The methods described in this paper have been used by the writer for years in making clear, permanent slides of all stages of mosquitoes.* Good slides do not always result merely by following certain steps. Manual dexterity and care in handling specimens are of utmost importance, and must be acquired. The loss of hairs or scales results in imperfect specimens which may not be identifiable after being mounted.

The mounting media preferred by the writer include (1) xylene-soluble resins such as Clarite, Piccolyte, Permount, or army issue synthetic resin, and (2) alcohol-soluble gums such as euparal and diaphane. These are preferred because there is no yellowing of preparations with age, and crystallization never occurs. Clarite is colorless, whereas all the other media mentioned have a slight tinge of yellow which is not readily apparent unless the mount is unusually thick. The synthetic resins in the first group are sometimes sold as crystals or nuggets which must be dissolved in xylene to make a mounting medium of desired viscosity. Permount is also available with toluene as the solvent, but xylene is preferred because it does not have the harsh effect that toluene sometimes has on insect tissues. Euparal and diaphane exert a clearing action on the specimen; xylene-soluble media also have some clearing action, as xylene is often used as a clearing agent by itself.

Certain types of equipment are very useful for manipulating specimens, but are not mandatory. The following items have been very useful: minuten nadeln;

* The material presented here in no way constitutes an endorsement by the Department of Defense.