TABLE 2.—Insecticides with high mammalian toxicities evaluated only in preliminary screening tests.

Insecticides	Estimated LC_{90}	Acute oral toxicity to rats (LD_{50} mg./kg.)
Hercules 13462	0.004	11.6
Union Carbide UC-21149	.009	1.0
Schering 34615	.024	35
Hercules 9200	.025	Unknown
Bay 48772	.037	3
Azodrin®	.038	23
Murfotox®	.040	49
Bay 48792	.044	2.5

(Table 2). Union Carbide UC-21149 had an LC_{90} of 0.009; the other compounds were about as effective as malathion in the previous tests. The chemical composition of each of the test compounds listed in Table 2 is given in Table 2a.

TABLE 2a.—Chemical composition of compounds listed in Table 2.

Hercules 13462: (*O,O*-dimethyl phosphorodithioate *S*-ester with N-(1-mercaptoethyl)succinimide)
Union Carbide UC-21149: (2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl) oxime)
Schering 34615: (*m*-cym-5-yl methylcarbamate)
Hercules 9200: (*m*-(methoxymethoxy)phenyl methylcarbamate)
Bay 48772: (*O*-methyl *O*-[4-(methylsulfinyl)-*m*-tolyl] methylphosphonothioate)
Azodrin®: (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate)
Murfotox®: (65% E. C. of mecarbam)
Bay 48792: (*O*-ethyl (*O*-[*p*-(methylsulfinyl)-phenyl] ethylphosphonothioate)

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LABORATORY BIOASSAY OF PESTICIDE-IMPREGNATED RUBBER AS A MOSQUITO LARVICIDE [1]

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The use of rubber as a carrier for mosquito larvicides represents a new variation of a known technique. Raley and Davis (1949) combined casting plaster and sawdust with a DDT-lindane mixture to control mosquitoes in California. Evans and Fink (1960); Symes, Thompson, and Busvine (1962); and Laird (1967) similarly achieved control using various chlorinated hydrocarbons impregnated into plaster of paris or cement. Barnes *et al.* (1967 and 1968) obtained control of mosquito larvae in laboratory and field experiments using Abate® (o,o,o′,o′-tetramethyl o,o′-thiodi-*p*-phenylene phosphorothioate) formulated into plaster of paris briquettes. Whitlaw and Evans (1968) achieved laboratory control of larval mosquitoes using Abate®, Dursban®, Dibrom®, or malathion impregnated into plastic pellets of polyvinyl chloride, polyurethane or polyamide.

The United States Army Environmental Hygiene Agency is currently involved in the development of long lasting, slow release, polymer-larvicide formulations for seasonal mosquito control. The following characteristics should be considered in the

[1] Mention of a proprietary product is for identification purposes only and does not necessarily imply endorsement by the United States Army.

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development of such formulations: (1) Any toxicant considered should be specific for mosquitoes and thus harmless to non-target organisms, including man. (2) The polymeric carrier should protect the toxicant from hydrolysis in water while allowing a minimally toxic release rate over an extended period of time, preferably not less than one full mosquito-breeding season. (3) The geometric configuration of the perfected formulation should permit foliage penetration if applied by aircraft. (4) The finished product should be competitively priced with existing carriers when compared on the basis of cost per seasonal control.

The research described in this paper began as a search for new uses of tributyltin marine anti-fouling materials developed by the Aerospace and Defense Products Division of B. F. Goodrich Company, Akron, Ohio. The United States Army Environmental Hygiene Agency suggested the incorporation of other pesticides into elastomeric rubber sheets for mosquito larvicide testing.

METHODS AND MATERIALS. Studies were conducted to determine the relative potentials of thirteen different pesticide-impregnated rubbers selected from a larger group after a screening procedure in 1967. Identical disc-shaped pellets approximately 0.2 cm. thick and 0.6 cm. in diameter were stamped from test sheet samples for use in bioassays. Second instar larvae from a DDT and malathion susceptible colony of *Culex pipiens quinquefasciatus* Say were added to quart mason jars containing 800 ml. of distilled water and 100 mg. of rabbit chow. After a 24-hour period of stabilization, weighed pesticide-impregnated rubber pellets were added to the jars. Rubber "blanks" containing no toxicant were used as controls.

All candidate materials and controls were tested in replicates of three. Mortality rates were recorded and evaporation levels were adjusted after the stabilization period and after 24, 48 and 72 hours following the introduction of the pellets. Pellets were removed and air-dried for 24 hours following each 72-hour immersion. The mason jars were cycled through an acetone rinse, a 10-minute detergent washing at 185° F, a 5-minute tap water rinse and finally a 1-minute distilled water rinse before larvae, food and fresh water were again added. The larvae were stabilized for 24 hours before the air-dried pellets were replaced for each subsequent 72-hour test. Investigations were also conducted to determine if gravid mosquitoes would oviposit on 800 ml. replicates of water in which single pellets of 5.7 percent Abate in EPDM had been immersed for 72 hours.

RESULTS AND DISCUSSION. Table 1 presents summary data of the different biocidal rubbers tested. Mortality during the 24-hour stability periods averaged less than 0.2 percent for all mason jars. Only the formulations of Abate in EPDM retained their larvicidal characteristics through all 72-hour tests. The tributyltin compounds tested demonstrated little, if any, potential as mosquito larvicides under these test conditions.

Six different formulations, each containing an insecticide, had no lethal effect on the mosquito larvae tested by the methods described. The consulting chemists at B. F. Goodrich stated that curing temperatures required for formulating these insecticidal-rubber compounds did not reach the level required to decompose the various insecticides. The ineffectiveness of these formulations may possibly be explained by the formation of a strong chemical bond between the insecticide and the rubber substrate within the molecular matrix which renders the insecticide unavailable for dissolution into an aqueous media.

The 5.7 percent Abate in EPDM was the first formulation of its type prepared by B. F. Goodrich Company and as such received the most thorough testing. The average 24, 48, and 72-hour mortality readings through twenty-six, 72-hour tests were 60 percent, 90 percent and 100 percent, respectively. Since the predetermined LC_{95} of Abate to the mosquito colony

TABLE 1.—Effectiveness of control of various toxicants incorporated into several rubber materials.

Rubber base	Toxicant	% Toxicant	Av. wt. of pellets (mg.)	No. 72-hour tests	No. tests with 90–100% mortality in 72 hrs.
Neoprene	None (control)	0.0	73.0	26	0+
EPDM (floating rubber)	Abate	5.7	45.3	26*	26
EPDM (floating rubber)	Abate	7.0	48.9	26*	26
EPDM (floating rubber)	Abate	8.3	49.7	26*	26
Neoprene	Tributyltin chloride	5.8	72.5	7	1
Neoprene	Tributlytin fluoride	5.7	69.5	7	0
Neoprene	Tributyltin oxide	9.6	78.0	10	3
Neoprene	Tributlytin oxide + Tributlytin acetate	5.7 + 1.4	70.0	10	2
EPDM (floating rubber)	Chlordane	5.7	47.2	7	0
EPDM (floating rubber)	Bayluscide (Bay 73)	7.9	48.8	7	0
Hycar	Abate	5.1	59.1	7	0
Neoprene	Naled (Dibrom)	5.8	78.0	7	0
Neoprene	Abate	8.4	70.5	7	0
Neoprene	Abate + Tributyltin oxide	4.2 + 2.8	70.0	7	0

* Tests in progress.
† Mortality for all control tests averaged less than 1.0%.

was 0.0006 p.p.m. (Whitlaw and Evans, 1968), it is probable that one 45.3 mg. pellet of 5.7 percent Abate in EPDM (floating rubber) reached this toxic dosage in 800 ml. of water in approximately 48 hours. The average weight loss of the pellet after twenty-six, 72-hour tests was 3.2 percent. The true significance of this figure cannot be stated because curing additives may have been included in the material lost.

The concept of dissolving a larvicide into a rubber substrate seems to represent a possible improvement over older larvicidal granules and briquettes which rely on the simple principle of mechanical leaching. Conventional larvicidal granules and briquettes rely on adsorption onto or absorption into a porous substrate. The toxicant becomes actively larvicidal through mechanical interaction with water. The toxicity of rubber formulations tested appears to depend on the solubility and mobility of the toxic molecules within the polymeric substrate and their subsequent diffusion into the fluid medium.

It was observed that gravid mosquitoes would oviposit freely in 800 ml. of water in which a 5.7 percent Abate in EPDM pellet had been immersed for 72 hours. Larvae hatched from the *Culex* egg rafts, but died within an hour.

SUMMARY. The only formulations tested which seemed worthy of further consideration as potential mosquito larvicides were the three formulations of Abate in EPDM. Although some encouraging data have been presented from the laboratory bioassays of Abate in EPDM, higher toxic concentrations may be needed for field application. Furthermore, a real need exists for a thorough quantitative analysis of larvicide-polymer residues in water samples.

Improved larvicide-polymer formulations may permit seasonal control of larval

mosquitoes by a "one-shot" dosage of the aquatic environment. A cumulative seasonal larvicidal level might be consolidated into one early spring application of "metered" pellets with no accompanying danger to food-chain organisms. Such rubber or plastic formulations could bring about increase in payload efficiency with an accompanying decrease in labor and equipment costs for an efficient mosquito larviciding program.

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LABORATORY TESTS OF PROMISING MOSQUITO LARVICIDES

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Investigators at the Insects Affecting Man and Animals Research Laboratory of the Insects Affecting Man and Animals Research Branch, Entomology Research Division, Agricultural Research Services, U. S. Department of Agriculture continually develop and evaluate effective mosquito larvicides. Part of this program involves the primary screening of candidate compounds against larvae of *Anopheles quadrimaculatus* Say. Those that give 100 percent kill of larvae at a concentration of 0.1 p.p.m. in these tests and that are not highly toxic to mammals (oral LD_{50} to rats or mice of 50 mg/kg or higher) are then tested to determine the dose-mortality response. The present paper reports the results of tests made from 1965 to 1967 with 14 promising compounds. Screening data obtained for six other compounds that were toxic to larvae but had high mammalian toxicity are given.

Testing Technique. The chemicals were dissolved in acetone and added to 225 ml. of distilled water in glass jars. After about 1 hour, 25 fourth instar larvae

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