LABORATORY EVALUATION OF CERTAIN LARVICIDES AGAINST *CULEX PIPIENS*, LINN., *ANOPHELES ALBIMANUS* WIED. AND *ANOPHELES QUADRIMACULATUS* SAY.

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Knowledge of the relative toxicity of new larvicides may be valuable in the choice of chemicals needed to replace those made ineffective by physiological resistance. Such data would also serve as valuable references in following the development of resistance and cross-resistances in mosquito populations. In a previous paper (Klassen, Keppler, Kitzmiller, 1964) we presented the dosage-mortality relationships of 30 larvicides to two strains of *Anopheles quadrimaculatus* Say. This paper presents similar data for three important disease vectors, *Culex pipiens* Linn., *Anopheles albimanus* Wied. and *Anopheles quadrimaculatus.*

MATERIALS AND METHODS. The following strains were cultured in the laboratory and utilized in this study.

Allerton: A strain of *Culex pipiens* collected in June, 1963 Allerton Park in central Illinois.

El Salvador: A strain of *Anopheles albimanus* derived in 1962 as a sub-colony of the dieldrin-resistant colony maintained at Johns Hopkins University, which had been field-collected at El Salvador. While in our laboratory the dieldrin resistance of this strain reverted to susceptibility (Keppler, Klassen and Kitzmiller, 1964).

Dothan: A strain of *Anopheles quadrimaculatus* field-collected in January, 1964 at Dothan, Alabama. This strain possessed incipient resistances to DDT and dieldrin.

Anopheles larvae were reared at a density of 75–100 in white enamel pans 10 x 12 inches with shallow water and fed daily on a coarsely ground mixture of equal parts of Kellogg's Concentrate, wheat germ and live yeast. This rearing method provided vigorous larvae of uniform size. The adults and larvae were maintained in an insectary with a constant temperature at 80° F. and 80 percent relative humidity. As a source of blood a guinea pig with a shaven back was taped to a pan and offered every day.

Culex pipiens larvae at a density of 100–200 per pan were reared in deep water, which was changed at least three times during the larval period. Larvae were fed the above mentioned diet. The insectary was maintained at 72° F. and 50 percent relative humidity. As a source of blood a pigeon whose breast feathers had been plucked was taped on its back in a pan and offered at least twice a week.

Adults of both strains were maintained in 12 x 12 x 12-inch cages. A wet sponge placed on the top of the cage provided water while a honey-saturated ball of cotton-wool provided energy. Oviposition was accomplished in a water-filled petri dish lined with a strip of filter paper.

Technical or purified insecticide was dissolved in 95 percent ethanol or acetone to provide the desired concentration of actual toxicant. The dosage mortality relationships were determined with four replicates, representing 100 larvae, at each concentration by the WHO standard method (World Health Organization, 1960) using tap water at 21–23° C. Percentage mortalities were plotted on a probability scale against concentration of actual toxicant on the logarithmic scale. Some of the relatively unknown compounds are listed as follows:

Abate (AC-52160): o, o, o′, o′—tetramethyl o, o′ thiodi-*p*-phenylene phosphorothioate

Stauffer N-2404: o-isopropyl o-(2-chloro-4-nitrophenyl) ethylphosphonothioate

Bayer 52957: 0, 0-diethyl 0-(5-chlorobenzisoxazolyl-(3)-) phosphorothioate
Bayer 37289: 0-ethyl, 0-2, 4, 5-trichlorophenyl ethylphosphonothioate
Bayer 64995: 4,4′-Bis (0,0-dimethylthiophosphoryl-oxy) diphenyldisulfide
Baygon Bayer 39007: 2-isopropoxyphenyl methylcarbamate
Bayer 37344: 4-(methylthio)-3, 5-xylyl methylcarbamate
SD7438: Toluene-a,a-dithiol bis (0,0- dimethyl) phosphorodithioate
SD7587: 1-(p-chlorophenylthio) vinyl dimethyl phosphate
GC6506: 0, 0-dimethyl-0-(4-methylmercaptophenyl) phosphate
Dimethrin: 2, 4-dimethylbenzyl chrysanthemumate
Zytron: 0(2-4-dichlorophenyl) 0-methyl isopropylphosphoramidothiate
Deutero-DDT: 2, 2-bis (p-chlorophenyl)-1, 1, 1-trichloroethane-2-d
WARF: N, N-di-n-butyl-p-chlorobenzenesulfonamide
DTT: 1,1,1-trichloro-2,2-di-(p-tolyl) ethane
Prolan: 1,1-bis(p-chlorophenyl)-2-nitropropane
Bulan: 1,1-bis(p-chlorophenyl)-2-nitrobutane
Chlorobenzilate: Ethyl 4,4-dichlorobenzilate
Strobane: Terpene polychlorinates (65% chlorine)
Kepone: Decachlorooctahydro-1,2,4-metheno-2H-cyclobuta(c,d) pentalen-2-one
Mirex: Dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta (c,d) pentalene
GC-9160: Delta-(5-hydroxyl-1,2,3,4,6,7,8,9, 10,10-decachloropentacyclo [5.3.9.0^{2,6}.0^{8,9}.0.4.8] decyl) ethyl levulinate
Telodrin: 1,3,4,5,6,7,8,8-octachloro-1,3,3a, 4,7,7a-hexahydro-4,7-methanoisobenzofuran
Pentac: Decachloro-1,1-bi-2,4-cyclopentadienyl
Thiodan (endosulfan): 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide (Mixture of the alpha and beta isomers; melting range 70°–100° C.).

RESULTS AND DISCUSSION. Of the cyclodienes (Table 1) Telodrin and endrin are seen to be the most toxic for all strains. Lindane, aldrin, GC-9160, heptachlor and chlordane are seen to be more toxic to the anopheline strains than to the dieldrin-tolerant *Culex* strain.

Of the DDT-type compounds (Table 2) deutero-DDT is the most effective; yet its effectiveness may be exceeded by that of DDT when potentiated by WARF against anopheline strains. Methoxychlor, Perthane, Bulan, Prolan and Rhothane (TDE) proved to be more effective than DDT against *C. pipiens* and *A. quadrimaculatus;* while Rhothane proved to be more effec-

TABLE 1.—Toxicities of cyclodienes to fourth instar larvae of *Culex pipiens, Anopheles albimanus* and *Anopheles quadrimaculatus.* Values are in parts per million.

Material	*C. pipiens*		*A. albimanus*		*A. quadrimaculatus*	
	LC_{50}	LC_{100}	LC_{50}	LC_{100}	LC_{50}	LC_{100}
Endrin	.0086	.08	.013	.08	.0028	.08
Telodrin	.02	.04	.02	.04	.004	.04
Dieldrin	.017	.16	.02	.04	.0098	.32
Aldrin	.01	.32	.015	.08	.041	.08
Lindane	.042	1.28	.05	.08	.048	.08
GC 9160	.082	.16	.012	.32	.031	.16
Heptachlor	.17	.64	.0027	.08	.018	.16
Chlordane	.38	1.28	.088	.16	.064	.16
Thiodan	.05	.32	.22	.64	.47	1.28
Strobane	.17	.32	.48	1.28	.64	2.56
Toxaphene	.053	.16	.76	1.28	.64	2.56
Kepone	.37	.64	.66	1.28	.74	1.28
Mirex	4.2	10.2	21.5	41.	23.5	..

TABLE 2.—Toxicities of DDT-type compounds to fourth instar larvae of *Culex pipiens*, *Anopheles albimanus* and *Anopheles quadrimaculatus*. Values are in parts per million.

	C. pipiens		*A. albimanus*		*A. quadrimaculatus*	
Material	LC_{50}	LC_{100}	LC_{50}	LC_{100}	LC_{50}	LC_{100}
Deutero-DDT	.0076	.04	.0020	.04	.0024	.02
DDT in WARF (1:1)	.028	.16	.0011	.01	.0020	.01
Methoxychlor	.024	.08	.035	.08	.039	.16
Perthane	.015	.04	.035	.16	.028	.16
Rhothane	.041	.08	.011	.02	.0066	.01
Prolan	.045	.08	.032	.08	.0098	.04
DDT	.048	.32	.02	.04	.0074	.32
Bulan	.011	.04	.07	.16	.022	.04
DTT	.096	.32	.063	.32	.096	.32
Kelthane	1.45	2.56	1.9	5.12	1.45	2.56
Chlorobenzilate	.72	2.56	6.2	10.24	1.2	5.12

tive than DDT against *A. albimanus*. DTT proved slightly less effective than DDT. Kelthane and Chlorobenzilate possess a low order of toxicity.

The toxicities of 25 organophosphorus compounds to the three strains are seen in Table 3 to show fairly wide variations. Yet in broad terms there appears to be a correlation between the responses of the three strains to nearly every compound. The most notable exception to this occurs with regard to Bayer 64995 which is 1000 times more toxic to *Culex* than to *Anopheles*. Generally the *Culex* strain is the

TABLE 3.—Toxicities of organo-phosphorus larvicides to fourth instar larvae or *Culex pipiens*, *Anopheles albimanus* and *Anopheles quadrimaculatus*. Values are in parts per million.

	C. pipiens		*A. albimanus*		*A. quadrimaculatus*	
Material	LC_{50}	LC_{100}	LC_{50}	LC_{100}	LC_{50}	LC_{100}
Bayer 64995	.00058	.00125	.47	1.28	.59	2.56
AC 52160 (Phenatox)	.0007	.0025	.0047	.04	.0068	.02
Methyl Parathion	.0028	.005	.0056	.02	.0085	.04
N-2404	.0047	.01	.0072	.04	.005	.01
Bayer 52957	.0037	.01	.008	.04	.006	.02
SD 7438	.0025	.02	.0095	.08	.015	.16
Dicapthon	.009	.02	.022	.16	.012	.02
Parathion	.0032	.01	.029	.16	.007	.02
EPN	.0030	.01	.0068	.02	.0078	.04
Bayer 37289	.0058	.04	.0086	.04	.013	.08
Folithion (Sumithion)	.0041	.01	.032	.08	.02	.04
Fenthion (Baytex)	.0038	.01	.026	.08	.072	.16
GC 6506	.024	.08	..	..	..	..
Trithion	.021	.08	.058	.16	..	..
SD 75787	.014	.04	.032	.16	.05	.16
Ronnel	.012	.02	.029	.16	.058	.16
Guthion	.015	.08	.12	.64	.089	.32
Ethyl Guthion	.028	.08	.18	.32	.11	.32
Dylox (trichlorfon)	.18	.64	.11	.32	.063	.16
DDVP (dichlorvos)	.042	.16	.23	.64	.048	.32
Malathion	.2	.16	.26	.64	.24	.64
Diazinon	.023	.08	.043	.08	.024	.08
Dibrom	.019	.04	.23	.64	.18	1.28
Zytron	.062	.32	.52	2.56	.18	.32
Bomyl	1.05	10.2	26.0	..	22.0	..

most susceptible excepting its responses to Dylox (trichlorfon). The compounds most toxic to *Culex* are Bayer 64995, Phenatox (AC-52160), methyl parathion, Stauffer N-2404, Bayer 52957, SD 7438, dicapthon, parathion, Bayer 37289, Folithion, fenthion and ronnel.

The toxicity of the carbamate, Bayer, 39007, is seen in Table 4 to be potentiated by piperonyl butoxide against *A. albimanus,* but not for *C. pipiens;* while the toxicity of Sevin to both species could be potentiated by piperonyl butoxide. Dimethrin proved to be effective against the three species, and it could be potentiated against *A. albimanus* at the LC_{100} level with piperonyl butoxide. The toxicities of Lethane 60 and paris green showed little interstrain variation.

Telodrin has been shown to be effective against dieldrin-susceptible *Culex pipiens* and dieldrin-resistant *Anopheles albimanus* (Metcalf and Georghiou, 1962) and against dieldrin-resistant and susceptible *Aedes aegypti* (L.) (Klassen, Keppler and Kitzmiller, in press). In *Aedes aegypti* dieldrin-resistance does not extend to GC-9160; therefore the structural analogues, Kepone, Mirex and GC-9160 probably do not belong to the cyclodiene cross-resistance category.

The effectiveness of deutero-DDT to mosquitoes is due to the low rate at which this compound is dehydrochlorinated (Pillai, Hennessey and Brown, 1963). The mode of potentiation of DDT by WARF has been shown to be due to the inhibition of dehydrochlorination of DDT (Pillai, Abedi and Brown, 1963).

Among the organophosphates Abate (AC 52160) was shown by Mulla, Metcalf and Kats (1964) to be the most effective compound against *Culex pipiens quinquefasciatus, Culex tarsalis* and *Aedes nigromaculis.* Dimethrin was found by Schoof and Jakob (1964) to be effective against DDT-susceptible *Aedes aegypti* but ineffective against a DDT-resistant strain; we have unpublished observations to substantiate this finding.

TABLE 4.—Toxicities of various larvicides to fourth instar larvae of *Culex pipiens, Anopheles albimanus,* and *Anopheles quadrimaculatus.* Mixtures of compounds are in equal parts. PBO designates piperonyl butoxide. Values are in parts per million.

Material	*C. pipiens*		*A. albimanus*		*A. quadrimaculatus*	
	LC_{50}	LC_{100}	LC_{50}	LC_{100}	LC_{50}	LC_{100}
Bayer 39007	.25	.64	.67	2.56	.02	1.28
Bayer 39007+PBO	.22	.64	.078	.16	..	..
Bayer 37344	.60	1.28	1.6	2.56	.77	1.28
Sevin	2.2	5.12	1.4	2.56	1.72	2.56
Sevin & PBO	.47	1.28	.046	.16	..	..
Dimethrin	.031	.08	.036	.64	.029	.32
Dimethrin & PBO	.019	.08	.03	.08	..	..
Lethane 60	2.1	5.12	2.7	5.12	.92	5.12
Paris Green (Hg/larva)	.11	.40	.16	.80	.094	.80
Pentac	>82	..	>82	..	>82	..

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THREE TECHNIQUES FOR LABELING *CULICOIDES* (DIPTERA: HELEIDAE) WITH RADIOACTIVE TRACERS BOTH IN THE LABORATORY AND IN THE FIELD

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The use of radioactive substances for labeling insects for dispersal studies is becoming more widespread. Techniques have been developed for many insects, including mosquitoes (Provost, 1952) and blackflies (Fredeen *et al.*, 1953). A two-isotope technique was used by Lewis and Warloff (1964) to study the dispersal of mirids in plots of broom.

The smaller the insect, the more attractive does the possibility of using radioactive tracers become, since other labeling techniques involving dusts and dyes are impracticable.

As a preliminary to investigating the dispersal of *Culicoides furens* Poey, and *C. barbosai* Wirth and Blanton, from Jamaican swamps, the following three techniques have been developed. The larvae are introduced to sufficient concentration of P-32 for them to metabolize enough radioactive phosphorus to be detectable in the adult, from 9 days to 9 weeks later.

The three techniques, with examples, are described below:

DIRECT APPLICATION TO LARVAE IN THE LABORATORY. Larvae placed directly into solutions containing from 0.5 to 5 microcuries of P-32 per ml. will be adequately labeled after 24 hours. At the end of that time they should be removed and rinsed in distilled water to remove any active ions adhering to the cuticle.

The concentrated radiophosphorus, when received from the suppliers, usually has an activity of the order of 1 millicurie/ml. This should be diluted with a carrier solution of distilled water containing about 0.1 percent of inactive orthophosphate. Otherwise, a significant portion of the active ions will become adsorbed on the walls of vessels and pipettes.

Larvae to be checked are placed on pieces of card and fixed in place with adhesive cellulose tape. The card is then placed face down on a sheet of X-ray film (Kodak Industrial type AA serves well) and left in a cassette to expose for 6 days. When the film is developed a radioactive larva will be indicated by an exposed spot on the film.

Table 1 gives an example of *C. furens* larvae which were treated with 4 concentrations of P-32 in 3 batches. Batch 1 was exposed on film immediately, batch 2 was rinsed in water and then placed in