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Multi-Resistant Aedes aegypti in Puerto Rico and Virgin Islands

Abstract. The Isla Verde, Puerto Rico, laboratory colony, highly resistant to DDT and dieldrin in 1959, became even more so by 1960; resistance to organic phosphates was also greater than before. In laboratory tests Bayer 29493 was best for killing resistant larvae and adults. It is improved with piperonyl butoxide at the ratio of 10:1.

The theory that *Aedes aegypti* can be resistant to DDT or dieldrin but not to both (*1*) was disproved by the Isla Verde strain (*2*, *3*). This dual resistance occurs not only in the laboratory but along the whole north coast of Puerto Rico (*4*). When there is danger of the spread of yellow fever in the Caribbean (*5*), the presence of vigorous races recalcitrant to control has ominous implications for public health. To find an insecticide for overcoming resistant strains is imperative and for this purpose the Isla Verde strain is obviously more useful than the susceptible strains of most laboratories (*6*). The original toxicological tests (*2*) on this strain were made from March through July 1959 on about the F_{10} generation of a colony started in November 1958. By July 1960 the colony was estimated to be at the F_{50} generation after continuous inbreeding. At that time, in the field not far from the original site, more specimens were collected (*7*), and the F_1 and F_2 generations were obtained for tests to compare with the original data. In 1959, 2.5 parts of malathion, diazinon, and dipterex per million gave 100 percent mortality; therefore, this concentration was used as a standard (*8*). Table 1 shows that the colony became even more resistant to DDT, lindane, dieldrin, and chlordane; in the field, except for lindane, the same high resistance occurs. As larval resistance to the chlorinated hydrocarbons increased in the colony, so did the resistance to the organic phosphates, but field resistance to the latter was not so marked. Resistance developed also to Bayer 21/199, the best larvicide in 1959. For specific use in mosquito control, Bayer 21/199 has been replaced by the manufacturers with Bayer 29493 (*9*), which is more effective against adult mosquitoes. Bayer 29493 was the most toxic in our tests and was made more so by synergism with piperonyl butoxide (*10*) at the ratio of 10:1 rather than the reverse ratio (*11*).

To prove further the resistance to organic phosphates, tests were made on the Isla Verde colony larvae at 0.5 part per million (ppm). In 1959 (*2*) dipterex, diazinon, and malathion only gave 72, 74, and 85 percent dead, and the same for dead plus moribund mortality; Bayer 21/199 gave 87 and 100 percent mortality. In 1960 the results were as follows (average of two replicates, dead and dead plus moribund mortality): Bayer 21/199, 65 and 100 percent; dipterex, 5 and 37 percent; malathion, 41 and 68 percent; diazinon, 34 and 73 percent; Bayer 21/199 plus piperonyl butoxide (10:1), 64 and 100 percent; Bayer 29493, 80 and 100 percent; Bayer 29493 plus piperonyl butoxide (10:1), 92 and 100 percent.

Females of the Isla Verde colony, which were exposed for 1 hour to bond paper impregnated with the following concentrations of insecticides at the rate of 3.6 mg/cm^2, gave the following percentage mortalities after 24 hours (average of two replicates): 4 percent DDT, 81 percent; 4 percent dieldrin, 47 percent; 3 percent Bayer 21/199 alone and with piperonyl butoxide (10:1 and 1:10), 18 to 22 percent; 0.4 percent malathion, 100 percent; 0.1 percent diazinon, 100 percent; 0.1 percent Bayer 29493, 100 percent; 0.05 percent Bayer 29493 plus piperonyl butoxide (10:1), 100 percent.

Table 1. Percentage mortality of *Aedes aegypti* fourth instar larvae, Isla Verde, P.R., strain after 24 hours of exposure to insecticides at 2.5 ppm (average of four replicates, dead only). The effect of 18 months of colonization is compared with data from field material.

Insecticides	Laboratory colony		Field
	1959 ($F_{10\pm}$)	1960 ($F_{50\pm}$)	1960 (F_{1-2})
DDT	62*	25†	20†
Lindane	77*	30*	67*
Dieldrin	77*	0†	6†
Chlordane	25†	0†	2†
Dipterex	100‡	55§	94‡
Malathion	100‡	64§	94§
Diazinon	100‡	62‡	96‡
Bayer 21/199	100‡	58‡	97‡
Bayer 21/199 + piperonyl butoxide (10:1)		90‡	96‡
Bayer 21/199 + piperonyl butoxide (1:10)		97‡	55‡
Bayer 29493		91‡	94‡
Bayer 29493 + piperonyl butoxide (10:1)		100‡	100‡
Bayer 29493 + piperonyl butoxide (1:10)		95‡	36‡

Dead plus moribund: *79–89 percent; †0–44 percent; ‡100 percent; §98–99 percent.

Table 2. Percentage mortality of *Aedes aegypti* fourth instar larvae of a colony (F3–4) from material collected at Christiansted, St. Croix, in October 1959 (average of two replicates, dead and dead plus moribund).

Insecticide	0.5 ppm		2.5 ppm
	Dead	Dead plus moribund	Dead*
DDT	77	88	90
Lindane	45	77	95
Dieldrin	33	45	88
Malathion	66	88	95
Diazinon	60	78	95
Bayer 29493	92	100	85

*Dead plus moribund, 100 percent mortality for all.

That DDT-dieldrin resistance already occurs in another island of the Caribbean was evident from tests on a St. Croix strain (*12*). Table 2 indicates moderate resistance to all insecticides listed except Bayer 29493. The lack of complete mortality with DDT and dieldrin at 0.5 ppm with this strain must be considered in relation to 100 percent mortality of susceptible strains at 0.02 ppm of these insecticides. It is likely that in about a year's time the same high resistance of Puerto Rico strains will occur in St. Croix, if DDT continues to be used there.

In conclusion, the following points are emphasized: (i) DDT-dieldrin resistance is not simply a laboratory phenomenon but is general in Puerto Rico and perhaps in other islands of the Caribbean where DDT resistance has been reported. (ii) A DDT-dieldrin resistant strain under the conditions of our laboratory did not lose its resistance after 18 months' colonization (*13*), but in fact became more resistant. (iii) The evidence is against the idea that genetic differences separate resistance to insecticides into distinct types.

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6. This work was supported partly by research

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7. Larvae and pupae were taken by Michel Guion from a boat on 22 July 1960 at the Yacht Club, Boca de Cangrejos, Isla Verde, P.R.
8. As in previous tests 20 fourth instar larvae in 200 ml of solution in cardboard disposable containers were used (*4*). To obtain dilutions a previous scheme (*2*) was modified to involve only two tubes. Thus for Bayer 29493: tube 1, 1 gram 25 percent wettable powder plus 24 ml distilled water; therefore each milliliter contains 0.01 g. Bayer 29493. Tube 2, 1 ml of tube 1 plus 99 ml water; therefore each milliliter contains 0.0001 g. To obtain 2.5 ppm (0.00025 part per 100), add 5 ml of tube 2 to 195 ml water; and for 0.5 ppm (0.00005 part per 100) add 1 ml of tube 2 to 199 ml water.
9. Bayer 29493 was obtained from the Chemagro Corporation, P.O. Box 4913, Hawthorn Road, Kansas City 20, Mo. It is a 25 percent wettable powder, O-O- dimethyl O- [4-(methylthio)-*m*-tolyl] phosphorothioate, 25 percent; inert ingredients, 75 percent.
10. We thank Dr. W. E. Dove, Fairfield Chemical Division, P.O. Box 1616, Baltimore, Md., for piperonyl butoxide.
11. The value of a low ratio of synergist to toxicant has also been indicated in the case of Sevin and Sesoxane against resistant houseflies by M. E. Eldefrawi, R. Miskus, and W. M. Hoskins [*Science* **129**, 899 (1959)].
12. Larvae and pupae were collected by Arthur H. Boike, Jr., from rain barrels in Christiansted, St. Croix, V.I., 1 Oct. 1959.
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Uterotrophic Action of the Insecticide Methoxychlor

Abstract. Dusting of rats and mice, as well as oral treatment with the insecticide methoxychlor, produced uterine weight increase in these rodents. Ablation of the ovaries, adrenals, or pituitary did not alter this effect, thus indicating a direct trophic action of this agent on the uterus. It is apparent that the application of this insecticide to animals used for hormonal experiments introduces an additional variable.

During the past several months, marked uterine stimulation of unknown origin in immature female mice which had been obtained for use in hormone bioassay has been observed in this laboratory. After extensive examination and bioassay of diets, bedding, and several types of insecticide used in the animal production area, it was determined that one of the insecticide powders used for ectoparasite control contained the agent responsible for the uterotrophic effect. That this effect was not mediated through the ovaries was demonstrated by the marked uterine stimulation and concomitant vaginal opening induced in ovariectomized, immature mice and rats 3 days after these animals had received a single dusting with the insecticide. Each of the components of the insecticide was then tested by the dusting procedure and it was established that technical methoxychlor, an ingredient present at a level of 1.91 percent, produced the effects observed in the female genital tract.

In a series of quantitative studies that followed, graded doses of technical methoxychlor (*1*), in sesame oil solution, were administered orally to groups of ovariectomized mice (Table 1). The data indicate that technical methoxychlor stimulates uterine weight increase in the mouse. Similar data were obtained in the rat at higher dose levels. An unusual effect was noted in the vaginal cytology of methoxychlor-treated mice. Instead of the typical cornified vaginal smear induced by 3 days of estrogen treatment, the smear was characterized by large numbers of leukocytes. Continued treatment resulted in vaginal cornification. However, in the rat, treatment with the compound resulted in vaginal cornification although occasional leukocytes were seen. Because of the extremely weak activity of methoxychlor when compared with estrone on the basis of weight increase in the uterus of the ovariectomized mouse (0.02 percent or less), the possibility that the insecticide might act indirectly through the metabolic pathways of the adrenal cortex was investigated. However, uterotrophic effects produced by oral treatment with technical methoxychlor in adrenalectomized, ovariectomized rats (Table 1) did not differ from those observed previously in ovariectomized animals. Experiments carried out in female hypophysectomized rats further demonstrated that methoxychlor acts directly on the uterus rather than by way of anterior pituitary hormones (Table 1).

In order to compare the pituitary depressant with the uterotrophic potency of methoxychlor, its action on the anterior pituitary gonadotrophic response of the rat ovary was also tested in parabiotic rats. In castrated female and intact female rats placed in parabiosis at 28 days of age, a 30-mg total dose of technical methoxychlor, given daily by stomach tube for 9 days, resulted in a mean ovarian weight of 16 mg (six parabiotic pairs) as compared with a mean of 104 mg for untreated controls (five parabiotic pairs). Thus, in common with the estrogens and related compounds, this substance blocks the gonad-stimulating action of the anterior pituitary. This inhibitory effect may clarify earlier unexplained findings (*2*) of extraordinarily small tests in experimental rats (pair-fed) maintained on a diet containing 1-percent methoxychlor.

The known adrenocorticolytic effects of related insecticides such as DDD (*3*) suggested the possibility that methoxychlor might alter adrenocorticoid secretion. In two adult mongrel dogs treated with this technical material in daily oral doses of 200 mg/kg for 15 days, there was no unusual deviation from the normal range of values for adrenal venous 17-hydroxycorticoid secretion as measured by Porter-Silber chromogens. Moreover, no distinct morphological changes in the adrenal cortex were noted.

Highly purified *p,p'*-methoxychlor was also assayed for uterotrophic effects in the rat and mouse. This isomer was only half as active as the technical product in the rat and was much less potent in the mouse. Anisole, one of the starting materials in the synthesis of technical methoxychlor has been reported to have estrogenic activity (*4*). In view of this report, anisole was considered as a possible contaminant of the technical material. However, when anisole was administered orally to immature, ovariectomized mice in total doses of 15 mg (5 mg daily for 3 days), there was no effect on uterine weight.

These findings indicate that an unidentified isomer or contaminant of technical methoxychlor is responsible in part for the uterotrophic effect (*5*).

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Table 1. Uterotrophic effects of methoxychlor in the mouse and rat. Technical methoxychlor in sesame oil solution was administered daily for 3 days by stomach tube. Controls received sesame oil only. Body and uterus weight: mean and standard deviation. NIH general-purpose strain mice and Sprague-Dawley rats were used in these studies.

Animals (No.)	Total dose (mg)	Operative procedure	Final body wt. (g)	Uterine wt. (mg)
		Mouse		
9	0	Ovariectomy	9 ± 1	5.2 ± 0.6
10	0.5	Ovariectomy	10 ± 2	11.3 ± 3.3
9	1.0	Ovariectomy	9 ± 1	19.4 ± 3.7
10	5.0	Ovariectomy	10 ± 2	34.5 ± 6.7
		Rat		
10	0	Ovariectomy plus adrenalectomy	55 ± 4	24 ± 5
10	20.0	Ovariectomy plus adrenalectomy	54 ± 6	70 ± 5
10	0	Hypophysectomy	73 ± 4	17 ± 2
10	20.0	Hypophysectomy	70 ± 5	58 ± 4