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STUDIES OF TAIWAN LEECHES

I. Insecticide Susceptibility-Resistance Tests[1]

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ABSTRACT

Tests were conducted to determine susceptibility or resistance of Japanese medicinal leeches, *Hirudo nipponia,* and specimens of an undetermined species of *Hirudo* from southern Taiwan to several chlorinated hydrocarbon and organophosphorous insecticides.

Insecticides used were: DDT, chlordane, dieldrin, BHC, malathion, diazinon, Baytex and Sumithion.

Insecticide concentrations were prepared according to WHO instructions for determination of susceptibility of resistance of mosquito larvae to insecticides.

Leeches were exposed to the various insecticide concentrations in pint jars. Two replicates of 10 leeches each were exposed to each concentration of insecticide. Total continuous exposure time was 72 hours. Per cent deaths were recorded at 24, 48 and 72 hours. Tests were conducted at room temperature (21-24 C).

Under test conditions, chlordane was the most effective material tested and DDT was least effective. Minimum concentration of each insecticide giving 100 per cent kill of specimens of *Hirudo* sp. from Taiwan was: chlordane, 1.0 ppm; dieldrin, 10.0 ppm; lindane, 5.0 ppm; malathion, 15.0 ppm; Sumithion, 15.0 ppm; diazinon, 15 ppm; and Baytex, 10.0 ppm. Only 40 per cent of test leeches died after 72 hours continuous exposure to 50.0 ppm of DDT. Thirty per cent died after 72 hours exposure to a concentration of 2.5 ppm of BHC.

It is probably that leeches in farming areas of both Taiwan and Japan have been exposed to a variety of insecticides. Farmers in both areas have used chlorinated hydrocarbon and organophosphorous insecticides for several years. At the present time parathion, malathion and Follidol-E605 are popular among farmers in the Cha'o Chow area of Taiwan, where most of the test leeches were collected.

Although several workers have reported reactions of land and aquatic blood sucking leeches to various chemicals, including insect repellents and molluscacides, no published information is available concerning susceptibility or resistance of leeches to insecticides, or feasibility of

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area control of leeches by use of insecticides. Results of a series of laboratory tests conducted as first steps toward accumulation of needed information on these topics are reported in this paper. These investigations commenced with a single test, conducted through curiosity, with leeches purchased from a drug store in Tokyo, and were later conducted in greater detail with specimens collected on Taiwan, where leeches are active throughout the year, and where climate and other environmental factors more closely approximate those of Southeast Asia.

MATERIALS AND METHODS

Leeches of two species were used in these tests. Examples of the common Japanese medicinal leech, *Hirudo nipponia* Whitman, 1886, were purchased from a drug store in Tokyo. Although leeches are available in drug stores throughout the year, all such specimens are collected during the warm months, as leeches are not active during the winter in Japan. Specimens of an as yet undetermined species of *Hirudo*, possibly a variant of *nipponia*, were collected by two of the authors (HLK and REW), assisted by local field helpers, in the vicinity of Cha'o Chow, Pingtung, Taiwan, during October 1963 and January and April 1964.

Leech colonies were kept in the laboratory in cylindrical glass jars, each 8¼ inches (20.9 cm) in diameter and 8 inches (20.3 cm) in height. Each jar contained tap water to a depth of three or four inches. The top of the jar was closed with a muslin cover secured to the jar with masking tape. Water in each jar was changed weekly, but specimens were not fed, as they do very well for long periods of time without blood meals. (Many specimens now in the laboratory have survived for over nine months without blood meals without observable ill effects.) As many as 300 adult leeches per jar have been maintained successfully under these conditions.

Insecticides tested were: chlordane, DDT, lindane, dieldrin, BHC, Baytex, malathion, diazinon and Sumithion. The diazinon, Baytex and BHC used in leech tests were obtained from WHO test kits. The remaining insecticides, except Sumithion, were of technical grade and were products of the Nutritional Biochemicals Corporation, Cleveland, Ohio. Technical grade Sumithion was obtained from the Institute for Infectious Diseases of Tokyo University. Sumithion, an organophosphorous insecticide, is a recent (1959) product of the Sumitomo Chemical Co., Ltd., Osaka, Japan. Insecticide test concentrations were prepared in the manner specified in the WHO publication "Instructions for Determining the Susceptibility or Resistance of Mosquito Larvae to Insecticides" (1960).

Leeches were exposed to insecticide concentrations in pint jars. The top of each jar was covered with gauze, secured with a rubber band, to prevent escape of leeches. Two replicates of 10 leeches each were exposed to each insecticide concentration. Controls also consisted of two replicates of 10 leeches each. In conducting the tests, leeches were placed in jars containing insecticide concentrations and were exposed continuously for 72 hours at room temperature (21–24 C). During the test behavior of exposed leeches was observed, and deaths were recorded at 24 hours, 48 hours and at 72 hours when the test was terminated. Results were expressed as per cent mortality after 24, 48 and 72 hours exposure.

RESULTS AND DISCUSSION

Results obtained in tests with specimen *Hirudo nipponia* are shown in TABLE I. Results of tests with Taiwan leeches, *Hirudo* sp., are given in TABLE II.

As no standard technique exists for testing susceptibility or resistance of leeches to insecticides, selection of the technique followed in these tests was arbitrary. It was thought best to modify

TABLE I

Cumulative mortality in leeches, ***Hirudo nipponia,*** *exposed to insecticides in the laboratory*

Insecticide	Concentration*		Per cent deaths at indicated intervals		
			24 hours	48 hours	72 hours
Chlordane	0.5 ppm	Replicate I	0	0	80
		Replicate II	0	0	80
	2.5 ppm	Replicate I	80	100	—
		Replicate II	80	100	—
Malathion	0.5 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	2.5 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
Dieldrin	0.5 ppm	Replicate I	0	0	30
		Replicate II	0	0	10
	2.5 ppm	Replicate I	40	90	100
		Replicate II	40	80	100
DDT	0.5 ppm	Replicate I	0	0	20
		Replicate II	0	0	0
	2.5 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
Controls		Replicate I	0	0	0
		Replicate II	0	0	0

*Insecticide concentrations expressed as parts per million.

the technique employed in the larval mosquito test to provide for exposure for 72 hours rather than 24 hours because of the possibility that leeches might crawl up the side of the jar above the water line and, at least temporarily, escape contact with the insecticide. Actually, this problem did not materialize. Except at the highest concentrations of insecticides, the majority of leeches did not attempt to crawl completely above the water surface, but rested in a usual position with a third or more of the body above the water line, or remained on or near the bottom, clinging to the glass with one or both suckers.

Leeches affected by insecticides showed spasmodic contractions and were unable to normally carry on the wave-like ventilatory movements. Seriously affected specimens often formed tight coils, or spiral coils, with or without attachment of one or both suckers to the substrate. Moribund leeches often hung flacidly, attached to the side of the jar by the anterior sucker, or lay unattached and twitching slightly on the bottom of the container.

Leeches surviving 72 hours exposure were removed to tap water in usual colony jars and observed for persisting ill effects and for further deaths. It has been noted that leeches not actually moribund at termination of the test recovered rapidly when transferred to tap water. Most of these have since lived for months with no apparent ill effects. Among specimens now doing well in this laboratory are leeches subjected to resistance tests nine months ago.

"Normal" strains of leeches were not available for comparison with strains which showed resistance to insecticides. It is likely that leeches throughout farming areas in Japan, and also on Taiwan, have a long history of exposure to both chlorinated hydrocarbon and organophosphorous insecticides. In April 1964 it was found that parathion, malathion, endrin and Follidol-E605 were particularly popular among

TABEL II

*Cumulative mortality in leeches, **Hirudo** sp, exposed to insecticides in the laboratory*

Insecticide	Concentration*		Per cent deaths at indicated intervals		
			24 hours	48 hours	72 hours
Chlordane	0.5 ppm	Replicate I	0	40	60
		Replicate II	0	40	50
	1.0 ppm	Replicate I	—	80	100
		Replicate II	—	90	100
	2.5 ppm	Replicate I	80	100	—
		Replicate II	80	90	100
	10.0 ppm	Replicate I	100	—	—
		Replicate II	100	—	—
Lindane	1.0 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	2.5 ppm	Replicate I	40	40	40
		Replicate II	10	10	10
	5.0 ppm	Replicate I	100	—	—
		Replicate II	100	—	—
Dieldrin	1.0 ppm	Replicate I	0	10	30
		Replicate II	0	0	0
	2.5 ppm	Replicate I	30	60	60
		Replicate II	50	80	80
	5.0 ppm	Replicate I	70	70	70
		Replicate II	90	90	90
	10.0 ppm	Replicate I	100	—	—
		Replicate II	100	—	—
Sumithion	2.5 ppm	Replicate I	10	10	10
		Replicate II	0	0	0
	5.0 ppm	Replicate I	0	10	10
		Replicate II	0	0	0
	10.0 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	15.0 ppm	Replicate I	100	—	—
		Replicate II	100	—	—
Malathion	2.5 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	5.0 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	10 0 ppm	Replicate I	0	0	20
		Replicate II	0	10	20
	15.0 ppm	Replicate I	100	—	—
		Replicate II	90	90	100
DDT	2.5 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	10.0 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	50.0 ppm	Replicate I	0	40	40
		Replicate II	0	40	40
BHC	1.0 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	2.5 ppm	Replicate I	0	20	30
		Replicate II	0	20	20
Baytex	2.5 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	5.0 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	10.0 ppm	Replicate I	70	100	—
		Replicate II	70	100	—
Diazinon	2.5 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	5.0 ppm	Replicate I	0	0	0
		Replicate II	0	0	0
	10.0 ppm	Replicate I	0	0	0
		Replicate II	0	0	40
	15.0 ppm	Replicate I	10	50	100
		Replicate II	0	10	70
Controls		Replicate I	0	0	0
		Replicate II	0	0	0

*Insecticide concentrations expressed as parts per million.

farmers in the Cha'o Chow area of Taiwan, where most of the test leeches were collected. Local shops contained a variety of chlorinated hydrocarbon and other insecticides for control of pests on rice and other crops. At present, the government is sponsoring an educational program to insure safe use of these toxic materials.

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