ESTABLISHMENT OF BASELINE DATA ON THE INSECTICIDE SUSCEPTIBILITIES OF THE CHAGAS' DISEASE VECTOR *RHODNIUS PROLIXUS* IN VENEZUELA[1]

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INTRODUCTION

Following a meeting of the WHO Expert Committee on Insecticides held at Geneva in September 1975, Dr. J. R. Busvine[4] suggested determining the susceptibility of triatomid bugs to a specific insecticide by exposing them continuously to filter-paper strips impregnated with a single concentration of test insecticide. In this way, he felt, it might be possible to simplify the existing evaluation process, which involved brief exposure to between four and six different concentrations of the insecticide being tested *(1)*.

In addition, this simplified approach as it was later employed in Venezuela enabled us to reduce the number of test insects required for complete testing of a particular insecticide *(2)*. It was thus considered highly useful in our country, where triatomid control efforts had achieved sufficient success to make field collection of a large number of stage IV or V nymphs of the predominant vector (*Rhodnius prolixus*) difficult. These large numbers of vector insects, which had been required by the old procedure, were especially hard to procure where systematic spraying had been conducted, and where the vector bugs nevertheless still persisted, but where the number of available specimens was so small that breeding and rearing was required in order to obtain enough to test.

Preliminary trials of Dr. Busvine's concept were carried out by the Service for the Biological Evaluation of Pesticides, and an effort was subsequently begun to determine the baseline susceptibility of *R. prolixus* in Venezuela to various pesticides.

These new baseline data proved extremely useful for evaluating the susceptibility of *R. prolixus* strains in different parts of the country. The susceptibilities of these strains were com-

[1] This article has previously been published in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*, vol. 98, no.3, 1985.

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pared to those of the standard laboratory strain established by the service more than 20 years before. Until the time of these evaluations, neither Venezuela nor any other country in the Americas had a basic standard of comparison for determining whether triatomid strains from any given area were susceptible, tolerant, or resistant to a given product.

INITIAL BASELINE DATA

During 1976 and 1977, breeding colony strains of *R. prolixus* and *Triatoma maculata* were given continuous exposure to filter-paper strips impregnated with test insecticides, and a record was kept of the times at which half the test insects and all the test insects were found to have been killed. In this way the data needed to determine the baseline susceptibilities of these insects were obtained, and it became possible to develop a technical instrument for evaluating or cataloging their responses *(3)*.

The results of these tests are shown in Table 1. This table has been used many times to assess the insecticide susceptibility of *R. prolixus* strains from many parts of Venezuela. Among other things, because these baseline standards were available, we were able to identify dieldrin-resistant strains of *R. prolixus* in several states of Venezuela. In particular, the test response to dieldrin by *R. prolixus* specimens from the state of Trujillo (including specimens from the town of Santo Domingo and other localities) led to their classification as dieldrin-resistant. This finding was later confirmed at the London School of Hygiene and Tropical Medicine by Dr. Busvine, and was reconfirmed by subsequent work performed on specimens sent to WHO Headquarters in Geneva and to the University of California at Riverside, California, in the United States. The classic plateaus appearing on mortality charts over the course of prolonged exposures provided clear evidence of resistance *(4–6)*.

The table has also been used in other Latin American countries where different triatomid vectors of Chagas' disease are encountered *(7)*. In such cases, where other vector species were involved, our data provided only general guidance, since susceptibility to a given insecticide tended to differ considerably from one species to another and from one environment to another. This circumstance underlined the need for researchers in each affected country to produce their own baseline data, indicating the susceptibility of prevalent vector species, and to have such data available for use in testing collected vectors for susceptibility to the insecticides currently employed in local vector control efforts. It also pointed up the importance of reviewing and updating this baseline data from time to time, so as to accommodate changes in insect behavior and incorporate new criteria for gauging insect responses to insecticides as research in this field continues to advance.

TEST MODIFICATIONS

The times shown in Table 1 are the approximate times when 50% and 100% of the test bugs died. However, the dead bugs presumably accumulated a lethal dose of test insecticide some time before they actually perished. The exposure time needed to produce

Table 1. Baseline data obtained in 1976 and 1977 indicating the exposure times at which 50% and 100% mortality were observed among *R. prolixus* and *T. maculata* specimens continuously exposed to strips of filter paper impregnated with the indicated insecticides at the specified concentrations.[a]

Insecticide and concentration	Species exposed	No. of insects exposed	Time at which the indicated mortality was observed	
			50% mortality	100% mortality
Dieldrin (4%)	*R. prolixus*	100	12 hours	32 hours
	T. maculata	80	16 hours	38 hours
Lindane (0.4%)	*R. prolixus*	100	8 hours	28 hours
	T. maculata	70	16 hours	24 hours
Propoxur (0.4%)	*R. prolixus*	100	8 hours	16 hours
	T. maculata	80	4 hours	12 hours
Dioxacarb (0.4%)	*R. prolixus*	100	36 hours	62 hours
	T. maculata	60	48 hours	96 hours
Fenthion (0.5%)	*R. prolixus*	100	36 hours	96 hours
	T. maculata	100	48 hours	84 hours
Fenitrothion (0.5%)	*R. prolixus*	100	14 hours	24 hours
	T. maculata	100	16 hours	24 hours
Iodofenphos (0.5%)	*R. prolixus*	100	70 hours	8 days
	T. maculata	100	70 hours	5 days
Malathion (2.5%)	*R. prolixus*	100	70 hours	8 days
	T. maculata	100	14 hours	2 days
Bromophos (0.5%)	*R. prolixus*	100	50 hours	5 days
	T. maculata	100	70 hours	4 days

[a] This table has previously appeared in WHO Document VBC/EC/80-40, p. 2.

heavy mortality is thus somewhat shorter than the exposure time indicated in the table; and indeed, postexposure evaluations have shown that much shorter exposures than those specified were able to produce 100% mortality.

This implies that the table does not really reflect what happens in the field, a situation that causes difficulties when field strains are classified as susceptible, tolerant, or resistant in accordance with parameters established for the breeding colony strain.[5] To reduce that problem, we sought to develop a testing method that would expose the vectors to a single concentration of insecticide for three specified time periods, and that would enable us to plot the observed percentages of dead bugs on probit-log paper. In this way, using the projections generated, we sought to determine the exposure times needed to produce 50% and 95% mortality. This

[5] For our purposes, we define susceptible, tolerant, and resistant bugs as follows: susceptible bugs yield mortality data comparable to those obtained with their laboratory-bred counterparts; insecticide-tolerant bugs are killed by the test insecticide but take longer to die than the laboratory-bred insects (i.e., more time is needed to achieve 50% and 95% mortality); and resistant bugs are not killed by prolonged exposure to the test insecticide.

method was used in subsequent (1982 and 1983) tests that were conducted to update our previous baseline data, and that can be summarized as follows:

The equipment used for all these tests, the same as that recommended by WHO in 1964, was very simple. It consisted of grids for holding groups of 24 test tubes, test tubes 20 cm in length and 2.5 cm in diameter, strips of filter paper impregnated with oil-based insecticide solutions, a holding chamber for the bugs, a hygrometer, a thermometer, and various minor items such as markers, tweezers, scissors, rubber bands, gauze, and so forth.

Ten stage V *R. prolixus* nymphs fed on chicken blood 48 hours previously were inserted into each test tube. The tube, which contained a strip of insecticide-impregnated filter paper, was then covered with a strip of gauze, and this was secured with a rubber band near the mouth of the tube to keep the insects from escaping. All of the insects used were well-developed stage V nymphs that had been preselected before the test began. No *T. maculata* specimens were tested because of breeding difficulties and evidence that this species does not feed on humans.

A total of 240 stage V nymphs fed on chicken blood were used to make the determination for each product tested, so as to ensure there would be enough to permit accurate assessment of both the appropriate insecticide concentration and the exposure times to employ. However, once this information had been obtained, subsequent tests exposing collected insects for one predetermined time could be performed using only 40 nymphs as test insects and 10 as controls. (It should be noted that in cases where sufficient numbers are not available, it becomes necessary to breed the insects until suitable numbers of test specimens are obtained. The purpose of the control insects is to determine whether those tested have been properly handled and to ensure that the materials used are free of contamination.)

The laboratory's temperature and relative humidity were recorded with each entry. The temperature in the laboratory was generally between 20°C and 30°C, and the relative humidity was generally between 50% and 65%. These ranges of temperature and relative humidity are suitable for triatomid bugs.

Also, it is worth mentioning that the insecticide-impregnated strips of filter paper, which the triatomids grasped with their legs inside the test tubes, had to be discarded at the end of each test. When it was necessary to perform a new test with the same insecticide a new paper strip was used, the old one being invariably covered with triatomid excrement.

In addition, during the course of the test run it was found advisable to place the triatomids being tested in a dark chamber, in some area where they would not be excited by the presence of laboratory workers and would therefore tend to remain in contact with the paper strips.

We employed only the insecticides shown in Table 2; we did not test any pyrethroids. The three exposure times indicated in the table were intended to produce relatively low, intermediate, and high *R. prolixus* mortality, so as to assist probit-log estimation of exposure times that would produce 50% and 95% mortality. A single reading was then taken 24 hours after the exposure

Table 2. Baseline data obtained in 1982 and 1983 indicating the estimated exposure time needed to produce 50% and 95% mortality among *R. prolixus* specimens continuously exposed to insecticides at the specified concentrations.

Insecticide	Concentration	No. of *R. prolixus* tested	Exposure time (hours) / % mortality	Estimated exposure time (hours) needed to produce 50% mortality	Estimated exposure time (hours) needed to produce 95% mortality
Dieldrin	4%	240	4h/2.5%; 6h/20%; 16h/65%	11.5	30
Lindane	0.4%	240	6h/15%; 12h/57.5%; 24h/85%	12	41.5
Fenthion	1%	240	1h/15%; 6h/72.5%; 8h/97.5%	2.1	10
Fenitrothion	1%	240	1h/15%; 2h/90%; 4h/98.5%	1.24	2.45
Malathion	5%	240	4h/5%; 8h/40%; 16h/97.5%	8	14
Bromophos	5%	240	4h/47.5%; 8h/90%; 16h/95%	5.5	12
Iodofenphos	2.5%	240	4h/2.5%; 8h/42.5%; 16h/90%	9.5	22
Methylpyrimiphos	1%	240	6h/47.5%; 8h/90%; 12h/95%	5.5	11
Propoxur	0.4%	240	1h/18%; 2h/52.5%; 4h/87.5%	2	6.5
Bendiocarb	2%	240	3h/2.5%; 6h/17.5%; 12h/90%	8	16

began, and in cases where the original insecticide concentration seemed weak or ineffective (several days of exposure being needed to produce 100% mortality) the test was repeated using a stronger concentration capable of producing heavy mortality within 24 hours.

As indicated in Table 2, the degree of mortality achieved by each of the three preselected exposure times was recorded. The data obtained were then entered onto a probit-log sheet for the purpose of drawing a regression line and estimating the exposure times needed to produce 50% and 95% mortality. These estimates are included at the right of the table, which presents the 1982–1983 baseline data for *R. prolixus* susceptibility to insecticides in Venezuela. These are the data that were established for use by the Service for Biological Evaluation of Pesticides during the ensuing five-year period.

CONCLUDING REMARKS

As a comparison of Tables 1 and 2 suggests, increasing the test concentrations of certain insecticides helped to markedly reduce the exposure times required to produce high mortality.

The doses of dieldrin and lindane remained the same as previously in the second test, and the response of *R. prolixus* to these long-stored products did not differ significantly from that observed five years previously. This showed that these insecticides remained stable and effective despite the long storage period involved.

The previously tested phosphate insecticides (bromophos, fenthion, fenitrothion, iodofenphos, and malathion) were used at two to five times greater strengths. This was presumably responsible for drastic reductions in the times needed to achieve heavy mortality. The change thus yielded an important benefit, making it possible to obtain results within 24 hours of the initial ex-

posure and eliminating any possible technical difficulties connected with keeping the bugs in contact with insecticide-impregnated paper for days on end.

Methylpyrimiphos, a phosphate insecticide that had not been tested earlier, yielded results at a concentration of 1% that were similar to those obtained with the other phosphate insecticides included in these tests.

It is also worth noting that of the carbamates, propoxur showed itself the most toxic to the test insects, a 0.4% concentration producing 52.5% mortality in two hours and 87.5% mortality in four.

In general, the laboratory times for LD_{50} and LD_{95} that were recorded are the shortest times that could reasonably be expected to produce these mortalities with the test insecticide at the concentration used. When working in the field, and in dealing with bugs from areas where insecticides have been used heavily, longer times can be expected because some or all of the bugs may tolerate or be resistant to the insecticide employed.

Summary

An important obstacle to determining the susceptibility or resistance of Chagas' disease vectors to insecticides is lack of baseline data obtained by testing standard laboratory strains of these vectors. Since 1976, such baseline data have been obtained in Venezuela by exposing vector insects to various test insecticides (one particular concentration of each) and estimating the time required to produce moderate (50%) and heavy (95 or 100%) mortality with that concentration.

Initial baseline tests, performed in 1976 and 1977, yielded data on two insects, *Rhodnius prolixus* and *Triatoma maculata*, exposed to bromophos, dieldrin, dioxacarb, fenthion, fenitrothion, iodofenphos, lindane, malathion, and propoxur. The results, used in subsequent field tests, have helped to detect dieldrin-resistant *R. prolixus* in various parts of Venezuela.

More tests were conducted in 1982 and 1983 to obtain updated information. Only *R. prolixus* was tested. The test procedures and insecticide concentrations were modified so as to permit each test run to be completed in 24 hours. The procedures described here have proved especially useful in cases where limited numbers of insects are available for testing, because they permit procurement of both baseline and subsequent field data with a relatively small number of bugs.

References

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World Public Health Federation to Meet in Mexico City

The Fifth International Congress of the World Federation of Public Health Associations (WFPHA) will be held in Mexico City, Mexico, on 22–27 March 1987. The triennial event, bringing together public health professionals from many countries, will take as its theme "International Public Health in an Era of Economic Constraint: The Challenge." Specific topics to be discussed within this context include primary health care and child survival, various types of cost containment efforts, international economic cooperation in strengthening health programs, health and economic development, women and health, and the special problems posed by population growth, natural disasters, famine, and AIDS. Among the speakers scheduled to address the meeting are the current Director of PAHO, Dr. Carlyle Guerra de Macedo, and Director-Emeritus Dr. Abraham Horwitz.

The WFPHA is a nongovernmental international organization comprised of public health associations from 45 countries. Enjoying formal and active relations with WHO and UNICEF, it seeks to strengthen public and primary health care worldwide and to help bridge the gap between governmental and private voluntary organizations. Further information about the forthcoming meeting may be obtained by contacting the WFPHA Secretariat, c/o American Public Health Association, 1015 15th St., N.W., Washington, D.C. 20005, U.S.A., telephone (202)789-5697.