

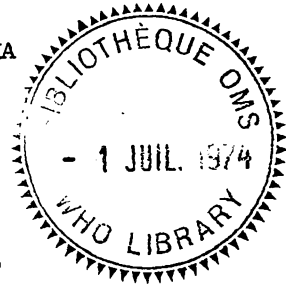


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STUDIES ON DDT RESISTANCE IN ANOPHELES CULICIFACIES IN SRI LANKA

by

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I. THE SUSCEPTIBILITY STATUS OF ANOPHELES CULICIFACIES TO DDT

The susceptibility status of Anopheles culicifacies has been very fully summarized up to 1971 (Brown & Pal, 1971).

In India the LC_{50} of DDT in both treated and untreated areas ranged from 0.2% to 0.6% even as late as 1957 (Rao & Bhatia, 1957). By 1959 the LC_{50} of DDT had risen to 2.2% in the Panch Mahals district of Gujarat State compared with the 0.5% observed in 1957 (Rahman, Roy & Singh, 1969) and had further risen to exceed 4% in 1961 (Luen & Shalaby, 1962).

DDT resistance has been found in seven States of India: Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Gujarat, Maharashtra and Mysore with, in some localities, mortalities to 4% DDT paper being as low as 5.6%.

DDT resistance in A. culicifacies also occurs in Afghanistan, Pakistan, Nepal and Burma.

In Sri Lanka where A. culicifacies is the only proven vector, DDT was used as a control measure from 1947-1955 supplemented with HCH. There was then a period without treatment, and in 1958 an eradication programme was instituted using DDT which was applied twice a year at 1 g/m² or three to four times per year at 0.5 g/m². At the completion of the operational phase of this programme in 1964 DDT coverage was withdrawn.

A serious resurgence of malaria in Sri Lanka commenced in 1967 and it was necessary to recommence DDT application in many areas. By the end of 1968 it had become necessary to bring all the previously malarious areas back under total coverage.

Prior to 1968 the number of susceptibility tests carried out was extremely limited, being confined to five localities in 1961-1962 and two localities in 1966. On the basis of the interpretation in use at that time all these tests indicated that the A. culicifacies population tested was susceptible to DDT.

Interpretation of the results of DDT susceptibility tests

A review of the available criteria for interpretation of the results of susceptibility tests is given by Zahar & Davidson (1973). The well-established discriminating dosage of DDT for most anophelines is 4.0% DDT for one hour. This use of discriminating dosages has necessitated the establishment of criteria to guide the interpretation of results.

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Due to the wide range of mortalities previously categorized as "intermediate" and the fact that subsequent experience has shown, particularly with DDT, that mortalities falling within the range of the "intermediate" condition were obtained when in fact the gene of resistance was present, Zahar & Davidson (loc. cit.) proposed the following arbitrary criteria for the different levels of mortality obtained using 4% DDT for one hour;

- (a) 99-100% mortality - susceptible
- (b) 80-98% mortality - verification required
- (c) < 80% mortality - resistant individuals present

When less than 80% mortality is obtained, then confirmatory tests are required and subsequent field observations should be intensified with parallel, periodical checking of resistance levels.

In this paper the criteria as proposed by Zahar & Davidson are followed.

Results of DDT susceptibility tests

- (a) At the discriminating dose (4.0% DDT with one hour exposure)

A summary of the susceptibility tests carried out in Sri Lanka on A. culicifacies using the DDT discriminating dosage of 4.0% with a one hour exposure is given in Table 1. Of the seven tests carried out prior to the present reversion to attack measures (1968) all populations tested were considered as susceptible. However, if the criteria as proposed by Zahar & Davidson are used, three of the tests indicated the need for further verification. Five tests were carried out in 1968 at the beginning of the present attack phase and on the criteria then existing were classified as: susceptible at Ambagaswewa (92% mortality) and intermediate at Kumbukwewa (89%), Passaradechena (87%), Kalundewa (77%) and Mirisgoniyawa (74%). These would now be regarded as "requiring verification" by repeated testing at Ambagaswewa, Kumbukwewa and Passaradechena, and indicating the "presence of resistant individuals" and the need for confirmatory tests at Kalundewa and Mirisgoniyawa.

The first indication of resistance (according to the old criteria) was recorded by one of us (PRJH) in April 1969 from Kadigawa in Maho Health Area when 46% mortality was recorded at the discriminating dose. An earlier test, carried out three weeks previously had given 64% mortality from the same locality. This was followed in May 1969 by only 43% mortality being obtained at Aralaganwila (Hingurakgoda Health Area).

When the susceptibility test results are considered according to the 1973 classification, it is seen that the presence of resistant individuals was indicated in all tests carried out since March 1969.

Consideration should be given to sample size in assessing the accuracy of observed mortalities, and ideally a minimum of 100 mosquitos should be exposed at the discriminating dose. In most cases the number of A. culicifacies exposed at the discriminating dose has been well below 100. This has been due to two main causes; firstly, in many cases exposures have been made at other concentrations and, secondly, although resistant individuals are present in the populations tested the actual number of A. culicifacies that could be collected has remained low. Additional collections have been carried out in numerous other localities in which the number of A. culicifacies has been insufficient to carry out a susceptibility test. The health areas in which DDT resistance is confirmed are indicated on the map.

A number of susceptibility tests carried out between May 1969 and October 1970 did not have controls (screening tests). As the results indicate the presence of resistant individuals they have been included in the test records. The only effect of any necessary

correction by Abbott's formula due to a control mortality above 5% would be to reduce the mortality obtained and thus indicate a still higher level of resistance.

During the period 1972-1973 some priority has been given to testing A. culicifacies at Tonigala (Puttalam Health Area) in the north and Mailagama (Hambantota Health Area) in the south. Tonigala is situated in the Northern Dry Belt with DDT at 1 g/m² applied on a four-monthly cycle whilst at Mailagama DDT at 1 g/m² is targetted to be applied on a two-monthly cycle. The corrected mortalities in the latest tests in these two areas have been Tonigala 50% (64 tested) and Mailagama 37% (100). The mortality obtained at Tonigala has remained approximately the same from January 1970, when the locality was first tested, until June 1973. At Mailagama there has been some variation in the mortalities obtained, the mortality in May - June 1973 being 31%, a higher mortality than obtained in May 1972 when, however, the sample size was small.

The lowest mortalities, 14% and 12% were obtained from one locality (Muthiyammegama, Hambantota Health Area) during 1970-1971.

(b) The LC₅₀ of DDT (Table 2)

Susceptibility tests undertaken in 1961-1962 utilized the full range of DDT-impregnated papers at one hour exposure. These tests indicated that the LC₅₀ range of A. culicifacies was 0.3-0.4% DDT. This agrees with the LC₅₀ range of 0.2-0.6% DDT observed in both untreated and DDT treated areas in India prior to the appearance of DDT resistance.

Tests carried out in three localities in February/March 1968 at the commencement of the present attack phase indicated LC₅₀s of above 2.0% DDT (approximately 2.2-2.4%). The LC₅₀ had thus increased by more than five times over the 1961-1962 figures.

(c) Tests using increased exposure times

The relationships between time and concentration in equitoxic exposures have been discussed by various authors for organo-chlorine and organo-phosphorus and carbamate impregnated papers (Busvine, 1958; Pennel et al., 1964; Ariaratnam & Brown, 1969; Hamon & Sales, 1970; Rongsriyam & Busvine, 1973). These show that, over a considerable range, the relations between concentration and exposure time for an equitoxic effect (e.g. to produce 50% mortality) are inverse, i.e. concentration x exposure time (Ct) = constant.

As Rongsriyam & Busvine (*loc. cit.*) point out, however, this relationship does not necessarily mean that future assessment of resistance should be based on a concentration-time product since this brings in an extra variable.

As a supplement to testing on the standard discriminating dose as mentioned above, and which now gives mortalities about or under the 50% level for all the populations tested, exposures were also made using 4.0% DDT papers with increasing exposure periods up to 24 hours (Table 3).

Although it is realized that while appreciable mortality is still occurring at the level of the discriminating dosage one is testing a heterogeneous population consisting of a mixture of the three genotypes, nevertheless, for the sake of crude comparison, some Ct values were calculated from the available data, with calculated LC₅₀ values ranging from 9.6% to 16% DDT for various localities (Table 4). This indicates levels roughly 24 to 40 times the 1961-1962 baseline. The 1972 figure for Gokarella (the only area for which records exist for both 1961-1962 and 1972) is in fact 35 times the 1961-1962 baseline, although the tests were performed in different ecological zones.

Summary and discussion

Details of susceptibility tests carried out in Sri Lanka between 1961 and June 1973 are given. The tests in 1961-1962 indicated a baseline LC₅₀ of 0.3% to 0.4% DDT. During the early stages of the present attack phase (1968) the LC₅₀ had risen to above 2.0% DDT.

During 1969 some susceptibility tests indicated LC₅₀ values above 4.0% DDT, the level at which the presence of resistant individuals was indicated by the then existing criteria. All susceptibility tests since March 1969 have indicated the presence of resistant individuals.

In 1972 tests were carried out using 4.0% DDT and increasing time, extending the time of exposure on a logarithmic basis up to 16 hours together with a 24 hour exposure. In all cases the mortality was recorded after a further 24 hours holding period. The LT₅₀ of DDT obtained has then been transformed into the equivalent LC₅₀ for a one hour exposure using the known relationship between concentration and exposure time for an equitoxic effect (i.e. $C \times t = \text{constant}$).

The LC₅₀s so calculated ranged from 9.6% to 16% DDT, thus the apparent LC₅₀ of DDT has increased from 0.4% in 1961-1962, to 2.0% in 1968 and up to 9.6% to 16% in 1972; an overall increase of between 24 and 40 times, depending on the area.

From 1970 to June 1973 there has been no indication of a significant increase in the resistance levels in areas subject to retesting using the discriminating dose of DDT, e.g. Mailagama, Hambantota.

Many countries are utilizing DDT residual spraying as the main attack measure even though some degree of resistance occurs in the local vector. As indicated by Zahar & Davidson (loc. cit.) the results of susceptibility tests cannot represent the actual reaction of A. culicifacies to DDT in the field. The aim of present and future susceptibility tests is to determine changes in the resistance level. In addition, activities are being commenced in two selected areas in which entomological, parasitological and operational observations will be made in parallel in order to evaluate the practical implications of DDT resistance in A. culicifacies in Sri Lanka.

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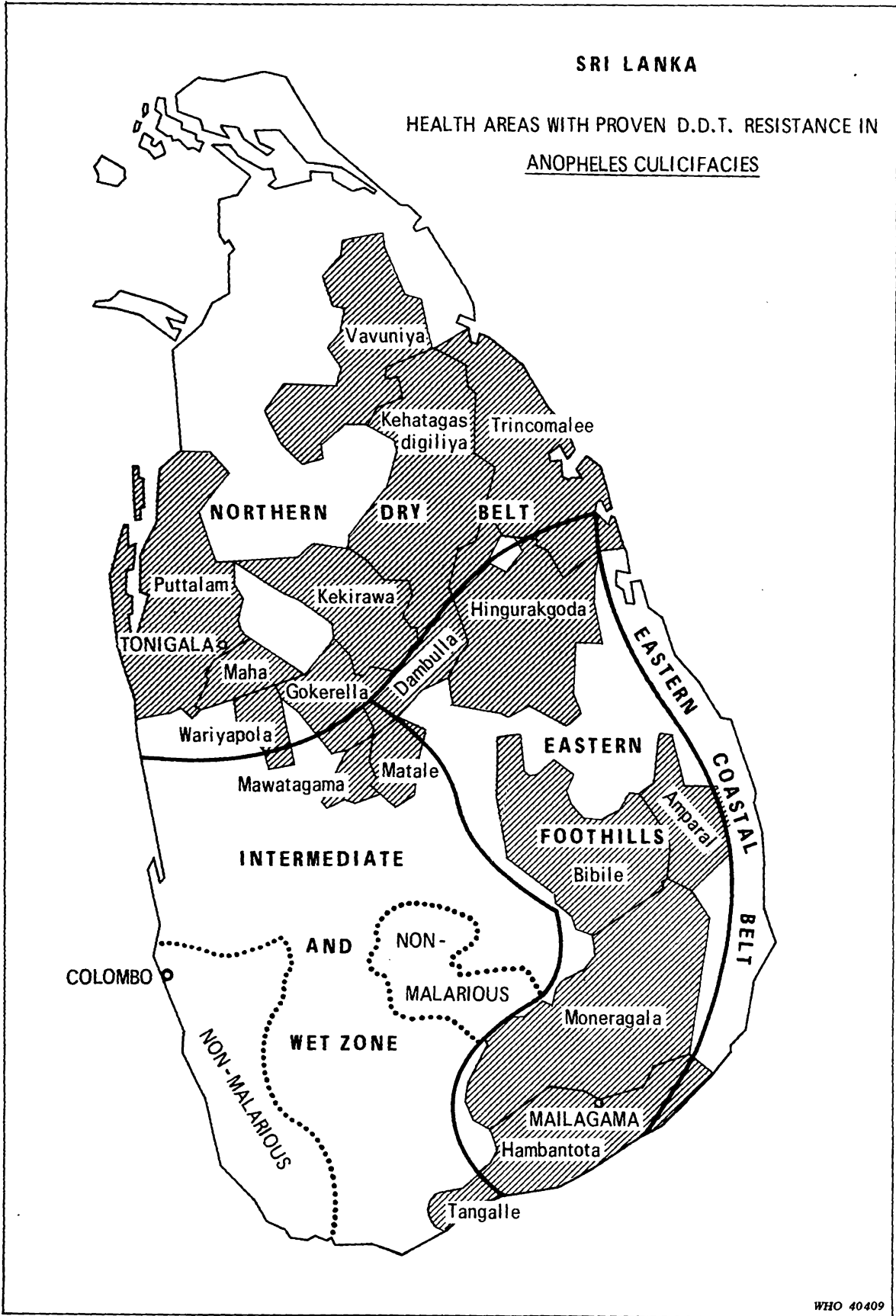


TABLE 1. SUMMARY OF DDT SUSCEPTIBILITY TESTS CARRIED OUT AT THE DISCRIMINATING DOSE (4.0% DDT FOR ONE HOUR).

Anopheles culicifacies - Sri Lanka

Date	Locality	Health area	Investigator	Mortality % (No. exposed)				Interpretation
				NDB ¹	EFH ²	I and WZ ³	ECB ⁴	
1961-1962	Ambagaswewa	Hingurakgoda	-	-	100 (102)	-	-	S
	Vavunikulam	Vavuniya	-	100 (115)	-	-	-	S
	Udugodagama	Gokarella	-	100 (100)	-	-	-	S
	Attaragollewa	Hingurakgoda	-	-	97.6 (25)	-	-	V
	Mannar	Mannar	-	94 (18)	-	-	-	V
1966	Mirisgonyawa	Dambulla	-	97 (75)	-	-	-	V
	Attaragollewa	Hingurakgoda	-	-	100 (49)	-	-	S
1968	Ambagaswewa	Hingurakgoda	-	-	92 (62)	-	-	V
	Mirisgonyawa	Dambulla	-	74 (131)	-	-	-	R
	Passaradechena	Amparai	-	-	-	-	87 (114)	V
	Kalundewa	Matale	-	-	77 (35)	-	-	R
	Kumbukwewa	Maho	-	89 (45)	-	-	-	V
March 1969	Kadigawa	Maho	RD	67 (33)	-	-	-	V
April 1969	Kadigawa	Maho	FH	46 (72)	-	-	-	R
May 1969	Aralaganwila	Hingurakgoda	RD/PH	-	43 (109)*	-	-	R
July 1969	Siyabalawewa	Gokarella	RD/PH	54 (37)*	-	-	-	R
December 1969	Kombuwachchikulam	Vavuniya	RD	65 (20)*	-	-	-	R
	Thambuttegama	Kekirawa	PS	66 (35)*	-	-	-	R
	Mankulam	Vavuniya	PS	32 (38)*	-	-	-	R
	Kepptigolloewa	Kahatagasdigiliya	PS	75 (57)*	-	-	-	R
	Nochikulam	Trincomalee	PS	60 (67)*	-	-	-	R
January 1970	Tonigala	Puttalam	PS	58 (80)*	-	-	-	R
	Tabbowa	Puttalam	PS	34 (41)	-	-	-	R
	Ethawa	Wariyapola	PS	42 (55)*	-	-	-	R
March 1970	Muthiyammegama	Hambantota	PS	-	26 (80)*	-	-	R
May 1970	Welawewatte	Hambantota	PS	-	74 (70)*	-	-	R
	Uduvila	Hambantota	PS	-	34 (59)*	-	-	R
June 1970	Aralaganwila	Hingurakgoda	-	-	36 (55)*	-	-	R
July 1970	Andaulpotha	Bibile	PS	-	47 (19)*	-	-	R
	Uduvila	Hambantota	RS	-	24 (67)*	-	-	R
August 1970	Mangala Oya	Amparai	PH/RS	-	42 (62)*	-	-	R
September 1970	Muthiyammegama	Hambantota	RS	-	14 (74)*	-	-	R
October 1970	Ibbankatuwa	Dambulla	PS	44 (57)*	-	-	-	R
	Tonigala	Puttalam	RS	51 (45)	-	-	-	R
January 1971	Tonigala	Puttalam	PH	51 (35)	-	-	-	R
	Aralaganwila	Hingurakgoda	PS	-	31 (16)	-	-	R
March 1971	Muthiyammegama	Hambantota	RS	-	43 (51)	-	-	R
August 1971	Mangala Oya	Amparai	RS/MW	-	30 (56)	-	-	R
September 1971	Muthiyammegama	Hambantota	RS	-	12 (75)	-	-	R
February 1972	Weliara	Tangalle	PS	-	32 (53)	-	-	R
March 1972	Kuda Oya	Moneragala	PS	-	32 (32)	-	-	R
May 1972	Mailagama	Hambantota	TS	-	25 (20)	-	-	R
	Kallady	Puttalam	MR	53 (18)	-	-	-	R
June 1972	Panjja Oya	Bibile	TS	-	36 (18)	-	-	R
July 1972	Ulukkatawewayawa	Dambulla	MR	25 (20)	-	-	-	R
August 1972	Mangala Oya	Amparai	PH	-	21 (16)	-	-	R
	Mailagama	Hambantota	TS	-	39 (20)	-	-	R
September 1972	Ridigama	Mawathagama	PS	-	-	23 (52)	-	R
	Kirindigolla	Gokarella	PS	-	-	30 (43)	-	R
	Mailagama	Hambantota	PH	-	29 (35)	-	-	R
October 1972	Okkampitiya	Moneragala	TS	-	33 (17)	-	-	R
November 1972	Gajanayakagama	Tangalle	TS	-	43 (18)	-	-	R
January 1973	Tonigala	Puttalam	PS	57 (86)	-	-	-	R
May 1973	Kallady	Puttalam	MR	56 (18)	-	-	-	R
	Tonigala	Puttalam	PS	50 (64)	-	-	-	R
	Mailagama	Hambantota	TS	-	37 (100)	-	-	R
June 1973	Mailagama	Hambantota	PS	-	39 (18)	-	-	R

¹ Northern Dry Belt.
² Eastern Foothills.
³ Intermediate and Wet Zone.
⁴ Eastern Coastal Belt.
* Test without controls.

S = susceptible; V = verification required; R = resistant individuals present.

Investigators: 1961-1962 - Rao & Ariaratnam
1966 - Muir
1968 - Badawi

RD - R. Darwish
PH - P. R. J. Herath
RS - R. Sloof
MW - M. B. Wickramasinghe
PS - P. S. M. Shihabdeen
TS - T. Shanmugarajah
MR - M. Rajathurai

TABLE 2. SUSCEPTIBILITY TESTS CARRIED OUT USING A RANGE OF
 DDT IMPREGNATED PAPERS WITH ONE HOUR EXPOSURE

Anopheles culicifacies - Sri Lanka

Year	Locality	Health area	One hour exposure												LC50 DDT
			0.25% DDT		0.5% DDT		1.0% DDT		2.0% DDT		4.0% DDT				
			T	M%	T	M%	T	M%	T	M%	T	M%			
1961-1962	1. Ambagaswewa	Hingurakgoda	101	29	102	60	101	88	97	99	102	100	0.40		
	2. Vavunikulam	Vavuniya	103	20	85	67	92	95	79	100	115	100	0.40		
	3. Udugodagama	Gokarella	60	43	53	79	56	93	52	100	65	100	0.32		
	4. Attaragollewa	Hingurakgoda	59	42	57	74	79	81	116	96	125	98	0.43		
	5. Mannar	Mannar	-	-	-	-	19	90	19	79	18	94	-		
1968	6. Dambulla	Dambulla	-	-	18	0	42	5	72	43	131	74	2.39		
	7. Passaradechena	Amparai	-	-	-	-	48	13	90	42	114	87	2.45		
	8. Ambagaswewa	Hingurakgoda	-	-	-	-	-	-	47	43	62	92	2.23		

T = total exposed.

M = mortality.

Investigators: 1961-1962 - Rao & Ariaratnam

1968 - Badawi

TABLE 3. SUSCEPTIBILITY TESTS USING 4.0% DDT IMPREGNATED PAPERS WITH INCREASING EXPOSURE TIME

A. culicifacies - Sri Lanka

Year	Date	Locality	Health area	Exposure time to 4% DDT												Investigator	
				1 hour		2 hours		4 hours		8 hours		16 hours		24 hours			
				T	M%	T	M%	T	M%	T	M%	T	M%	T	M%		
1970	15-19 July	Uduvila	Hambantota	-	-	81	25	-	-	-	-	-	-	-	-	-	RS
1971	15-17 September	Muthiyammegama	Hambantota	75	12	15	24	-	-	-	-	-	-	-	-	-	RS
1972	22-24 June	Panjja Oya	Bibile	18	36	18	45	18	80	20	81	-	-	-	-	-	TS
	15 July	Ulukkatawayayawa	Dambulla	20	21	20	32	20	79	-	-	-	-	-	-	-	MR
	9-12 August	Mangala Oya	Amparai	16	21	17	46	16	50	-	-	-	-	-	-	-	PH
	15-16 August	Mailagama	Hambantota	20	39	-	-	20	71	17	86	-	-	-	-	-	TS
	6-8 September	Kirindigolla	Gokarella	43	30	21	38	20	55	22	73	25	80	25	96	-	PS
	13-16 September	Mailagama	Hambantota	35	29	33	48	17	53	-	-	-	-	-	-	-	PH
	6 November	Gajanayakagama	Tangalle	18	43	16	50	-	-	-	-	-	-	-	-	-	TS
1973	31 May - 2 June	Mailagama	Hambantota	100	37	20	56	20	72	17	79	-	-	-	-	-	TS

T = total exposed.

M% = mortality.

TABLE 4. COMPARISON OF CALCULATED LC₅₀ VALUES

A. culicifacies - Sri Lanka

Health area	Year	Mortality on 4% DDT for one hour	Ct	Indicated LC ₅₀	Apparent increase
Amparai	1968	87%	2.45 x 1	2.45	6.5 times
	1972	21%	4 x 4	16.00	
Dambulla	1968	74%	2.4 x 1	2.40	4 times
	1972	21%	4 x 2.4	9.60	
Gokarella	1961-1962	100%	0.32 x 1	0.32	35 times
	1972	30%	4 x 2.8	11.20	

RESUME

Historique du développement de la résistance au DDT chez Anopheles culicifacies au Sri Lanka de 1961 à 1973.

La CL₅₀ qui était, à l'origine, de 0,3 % à 0,4 % de DDT, dépassait 2,0 % en 1968. Tous les tests de sensibilité faits depuis mars 1969 ont indiqué la présence d'individus résistants.

Les épreuves sont faites actuellement avec la dose discriminatoire (4 % pendant une heure), puis avec la même concentration pendant des périodes d'exposition croissantes. Les valeurs concentration-temps ainsi obtenues permettent de calculer que la CL₅₀ se situait entre 9,6 % et 16 % de DDT en 1972, ce qui indique des augmentations globales de la CL₅₀ de 24 à 40 fois, selon la région.

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