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Insecticide resistance in malaria vectors : general review of the neotropical, palearctic and oriental regions *

by

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Reviewing the resistance status of anopheline mosquitos in 1964, DAVIDSON (1965) reported that resistance to chlorinated hydrocarbons had then been found in 19 malaria vectors or suspected vectors. In 11 species double resistance (to DDT and to the dieldrin/HCH group) had been recorded, and DAVIDSON considered that the two resistance factors were on the same chromosome and probably closely linked. He pointed out that DDT, being an insecticide of only moderate efficiency in operational conditions, takes a long time to select out a resistant population. This is one of the considerations making DDT the insecticide of first choice in malaria eradication programmes.

In its eighth summary of resistance reported in malaria vectors (WHO, 1964) the World Health Organization listed 22 species, 21 of which had shown dieldrin-resistance and 9 DDT-resistance. Double resistance (in the same populations) had by then occurred in 6 of the species (*A. quadrimaculatus, A. albimanus, A. albitarsis, A. sacharovi, A. stephensi* and *A. sundaicus*), the countries affected being USA, Mexico, the Central American Republics, Colombia, Greece, Iraq, Iran and Indonesia.

The operational importance of resistance in five major malaria vectors was discussed by HAMON and GARRETT-JONES (1963). The maps they published showed that the know areas of resistance comprised, for each species, only a small part of its geographical range. In some cases, such as that of *A. sacharovi* in Greece and of *A. culicifacies* in Pakistan and India, DDT-resistance did not prevent the continued use of DDT to complete the attack phase and for focal spraying in later stages of malaria eradication. But its rather low efficiency in reducing the vectorial capacity of even many susceptible vector populations may have masked the further loss of efficiency resulting as a vector became resistant — just as these inherent limitations must frequently have masked (or been masked by) operational weaknesses in applying the insecticide. It is thus difficult to assess to what degree there is a causal relationship between DDT-resistance and the non-eradication of malaria in the countries affected.

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In 1965 a further valuable review of the operational importance of resistance was issued by the World Health Organization (UNGUREANU and HAWORTH (Ed.), 1965). The collaborating authors discussed separately the status of *A. albimanus*, *A. pseudopunctipennis*, *A. pharoensis*, *A. sergenti*, *A. stephensi*, *A. sacharovi*, *A. culicifacies*, *A. aconitus* and *A. sundaicus*.

Since the compilation of the reviews referred to, WHO has continued to receive copies of many records of susceptibility tests performed on anopheline mosquitos in the field. From these records periodic summaries of cases of resistance are prepared for internal use, and information is stored in a computer for later retrieval. A majority of the test records on anophelines are of tests on adults at one or two concentrations of insecticide only, since they are carried out as routine checks by operational workers. The construction of a log-probit regression-line to determine the LC_{50} is not possible from these records, nor is it required for practical purposes. As the criterion for determining the presence and degree of resistance, the proportional change in the LC_{50} is here replaced by the proportion of individuals that survive the lowest exposure expected to kill all the susceptible mosquitos. For most anophelines, 1 hour on 4 % DDT or on 0.4 % dieldrin is such an exposure. This is a more sensitive index of incipient resistance, since a substantial proportion of heterozygotes may appear in a population before there is any detectable change in the LC_{50}. Moreover, where heterogeneity produces a regression-line that is even slightly stepped, it is erroneous to assess the LC_{50} from a straight line drawn through the plotted mortalities.

Normally, two replicates, totalling 40 mosquitos tested at the discriminating exposure, are accepted as a minimum sample for a given locality and month, provided that a proper control-test is also run. It is recognized that larger samples are often unobtainable in sprayed areas. But having regard to the survival of occasional specimens owing to exceptional vigour, or perhaps to non-continuous contact during the exposure period, the population is designated "susceptible" whenever the tests show average survivals of 10 % or less. Again, in an epidemiological context, all vector populations with not more than 10 % of resistant individuals are likely to be controlled by the insecticide almost as efficiently as normally-susceptible ones.

Tests showing from 1 % to 10 % of survivors may or may not denote incipient resistance in the vector. But their presence should alert the field entomologist and stimulate him to repeat the tests as soon as possible ; to add tests at double the discriminating exposure ; and to rear and test the progeny of survivors (either as larvae or as adults). Any of these procedures should serve speedily to confirm the presence of resistance and show that it is time to develop contingency plans for a switch to alternative means of vector control in case of need.

Anopheline populations that show from more than ten up to fifty per cent survival at the discriminating exposure are characterized as "intermediate", while those showing still greater survival rates are designated "resistant". It is obvious that "intermediate" refers here, not to the *intensity* of resistance in terms of the dose required to kill the mosquito, but simply to the overall *proportion of mosquitos* exhibiting a physiological response that differs from normal susceptibility. The implication of "intermediate" is that continuing pressure from the same insecticide is likely further to increase that proportion and to decrease the insecticidal control effected.

It is necessary to distinguish tests which are of doubtful validity, most often because of inadequate sampling and replication, or biased sampling. A form of bias that occurs too commonly is the collection of mosquitos for testing from the sprayed houses, where the less-resistant individuals will have been eliminated before the sample is taken. The effect of such bias on the mortality recorded is well demonstrated in the results reported by de ZULUETA *et al* (1968). Separate samples from the same populations of *A. stephensi* in Southern Iraq were collected from DDT-sprayed and from unsprayed shelters, and were tested on 4 % DDT. At Fao in December 1966, the sample from unsprayed shelters showed 19 % mortality, against zero mortality in the one from sprayed shelters. Larger samples, tested at Gezira in May 1967, showed 29.6 % mortality in the 220 mosquitos from unsprayed shelters but only 4 % mortality in the sample of 75 from sprayed shelters. These results are not consistent with homozygosity for DDT-resistance.

The importance of ensuring freedam from sampling bias is clear. Where good numbers of the mosquito cannot be found in unsprayed resting-places it is necessary to collect and test the larvae, or adults bred out in the laboratory.

The WHO records for recent years indicate that there have been few extensions of DDT — or dieldrin-resistance to fresh species of *Anopheles*. This may be due to the fact that most house-haunting species had already come under selection pressure in earlier years, either from insecticides applied in the houses or from those contaminating the breeding-places. In tables 1, 2 and 3 the findings, reported to WHO are summarized for three of the zoogeographical regions (as defined by GORDON and LAVOIPIERRE, 1962). Of the remaining regions, the Ethiopian is not considered in this review, while we have little or no information from the nearctic and the australasian regions.

Table 1 shows that eight *Anopheles* species are involved, and nine countries affected, in the neotropical region. By far the most widespread and serious resistance in the region remains that of *A. albimanus* to both groups of insecticides. This has led to extensive laboratory tests on various strains of *A. albimanus* (see for example GEORGHIOU and GIDDEN, 1965) to assess their potential for selection for resistance to alternative insecticides that may come into operational use against this vector. The other cases are mostly localized and of minimal operational importance.

In the palaearctic region (table 2) 10 species or subspecies are reported resistant, in a total of 12 countries. Malaria was eradicated from Romania (CIUCA *et al*, 1964 ; TEODORESCU and GHEORGIU, 1966) and Portugal before the appearance there of insecticide-resistance in the vectors. But the resistance found in members of the *A. maculipennis* complex is a definite hindrance to the completion of the malaria eradication programmes in Greece and Turkey and probably in Syria. Resistance is also a serious operational problem in other countries of the region, such as UAR (*A. pharoensis*) and particularly so in the Persian Gulf countries, where *A. stephensi* shows double resistance over a large area (CHANG and UNGUREANU, in UNGUREANU and HAWORTH, *ed.*, 1965). There is recent evidence of the further intensification of the DDT-resistance of this vector in Southern Iraq according to de ZULUETA *et al* (1968).

The same species likewise constitutes a serious problem in the oriental region (table 3), where in Southern India DDT-resistance appeared following the treatment of the urban breeding-places of this mosquito. In that region a total of seven anophelines are recorded as resistant, but three of them are non-vectors. The double resistance of two major vectors in Java, Indonesia (*A. sundaicus* and *A. aconitus*) is likely to present difficulties in future operations (SOERONO *et al.*, 1965). But the most widespread technical obstacle in the region may yet arise from the spreading DDT-resistance of *A. culicifacies* in India, Ceylon, Burma, Pakistan and Nepal : hitherto it has been considered that its moderate degree of DDT-resistance would not preclude the continued use of DDT to complete the attack phase of malaria eradication, *A. culicifacies* being a naturally-inefficient vector and producing unstable malaria, on account of its low man-biting habit and limited expectation of life (HAMON and GARRETT-JONES, 1963 ; PAL, in UNGUREANU and HAWORTH, *ed.*, 1965). But it would appear rash to rely entirely on DDT for the further control of *A. culicifacies* in those special conditions which occurring every few years, may give rise to population explosions of this vector and to the catastrophic epidemics known to classical malariology. Since *A. culicifacies* rapidly developed resistance to the dieldrin/HCH group where either of these insecticides was employed against it, we see a pressing need for epidemiological field trials of the performance of newer insecticides in controlling this mosquito in the Indian sub-continent.

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TABLE 1

Summary of cases of anopheline insecticide-resistance reported since 1964 to WHO : neotropical region

Species	Country	Years of test reports indicating resistance to :			
		D.D.T.	Dieldrin	H.C.H.	Other insecticides
A. albimanus	Guatemala	64 - 67	64		malathion : 65
	Mexico	64 - 66			
	Nicaragua	65, 66, 68			malathion : 65
	Cuba	65 - 67			
	El Salvador	64 - 68	64 - 68		
	Costa Rica		65, 66		
	Haiti		65		
	Honduras	66, 68			
A. aquasalis	Brazil	66			
A. galvaoi ...	Brazil	64			
A. pseudo-punctipennis .	Mexico	64 - 67	66, 67		
A. quadrimaculatus	Mexico	64			
A. rondoni ..	Brazil	66			
A. strodei	Brazil	64 - 67			
A. vestitipennis	Mexico	66			

TABLE 2

Summary of cases of anopheline insecticide-resistance reported since 1964 to WHO : palaeartic region

Species	Country	Years of test reports indicating resistance to :			
		D.D.T.	Dieldrin	H.C.H.	Other insecticides
A. fluviatilis .	Saudi Arabia		67		
A. hispaniola .	Tunisia	66			
A. labranchiae atroparvus ..	Romania		64 - 67		
	Portugal	67			
A. maculipennis maculipennis	Romania	64 - 67	64 - 67		
	Turkey		67		
A. maculipennis messeae .	Romania	64 - 67	64 - 67		
	Turkey	67	67		
A. pharoensis	Sudan	65 - 67	65		
	UAR (Egypt)	64 - 67	64 - 67		
A. sacharovi .	Turkey	65 - 67	67		
	Greece	64 - 67	64 - 67		
	Syria	68			
A. sergenti ..	Iraq	68			
	Jordan		64 - 67		
A. sinensis ..	Ryukyu Islands	64 - 68		66	malathion : 66-68 fenthion : 68
A. stephensi .	Iraq	66 - 68	64 - 68		
	Iran	64 - 67	64 - 67		
	Saudi Arabia	67	67		

TABLE 3

Summary of cases of anopheline insecticide-resistance reported since 1964 to WHO : oriental region

Species	Country	Years of test reports indicating resistance to :			
		D.D.T.	Dieldrin	H.C.H.	Other insecticides
A. aconitus ..	Indonesia	64	64		
A. annularis .	E. Pakistan	66			
	India	66			
A. culicifacies	India	64 - 68	64	64 - 67	
	Nepal	64 - 66	64		
	W. Pakistan	66 - 67			
	Burma	67			
	Ceylon	68			
	Thailand	68			
A. stephensi .	India	64 - 68			
	W. Pakistan	64 - 68			
A. subpictus s.l.	Indonesia	64	64		
	Ceylon	66	66		
A. sundaicus .	Indonesia	64	64		
A. vagus s.l. .	Indonesia	64	64		
	Thailand	68			
	Vietnam	64 - 65	64 - 65		