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INSECTICIDE RESISTANCE

AND VECTOR CONTROL

Seventeenth Report
of the WHO Expert Committee
on Insecticides

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INSECTICIDE RESISTANCE

AND VECTOR CONTROL

Seventeenth Report
of the WHO Expert Committee on Insecticides

The WHO Expert Committee on Insecticides met in Geneva from 19 to 25 November 1968. Dr P. Dorolle, Deputy Director-General, opened the meeting on behalf of the Director-General.

1. PRESENT STATUS OF RESISTANCE

1.1 Resistance of arthropods to insecticides

In 1962, the WHO Expert Committee on Insecticides reported that there was clear evidence of developed resistance in 81 arthropod species of medical or veterinary importance, and indications of resistance in about 10 more. In 1968, the number of species which had developed resistance was 102, with isolated indications in 4 more. Many of them have developed 2 or even 3 types of resistance, the comparison over the 6-year period being as follows :

	DDT resistance	*Dieldrin resistance*	*Organophosphorus resistance*
1962	47	65	8
1968	56	84	17

Among the anopheline mosquitos, 38 species have developed resistance to 1 or more insecticide, 36 having developed resistance to dieldrin, 15 to DDT (Table 1), and increased tolerance to malathion developed in 1 species. Among the culicine mosquitos 19 have developed resistance, 16 to DDT, 12 to dieldrin, and 9 to organophosphorus compounds (Table 2), with 4 additional isolated cases which will be mentioned later.

In the housefly, resistance to malathion or to diazinon is now becoming quite widespread in Western Europe, North America and Japan, and diazinon resistance has appeared in Australia (Table 3). Resistance to ronnel is now common in California, with cross-tolerance to fenthion ; cross-resistance to dimethoate has been low, but control failures with

TABLE 1.

RESISTANCE OF ANOPHELINE MOSQUITOS TO DDT AND DIELDRIN a

Insecticide	Mosquito	Year	Area
DDT	A. sacharovi	1951	Greece ; Iran ; Turkey
	A. sundaicus	1954	Java ; Sumatra
	A. stephensi	1955	Saudi Arabia ; Iraq ; Iran ; S. India
	A. subpictus	1956	N. India ; W. Pakistan ; Nepal ; Java
	A. albimanus	1958	El Salvador ; Nicaragua ; Guatemala ; Honduras ; Mexico ; Cuba
	A. pharoensis	1959	Egypt ; Sudan
	A. quadrimaculatus	1959	Georgia, USA ; Maryland, USA ; Mexico
	A. annularis	1959	India
	A. culicifacies	1960	W. & S. India ; W. Pakistan ; Nepal
	A. albitarsis	1961	Colombia
	A. nuneztovari	1961	Venezuela
	A. aconitus	1962	Java
	A. fluviatilis	1963	W. India
	A. hyrcanus sinensis	1964	Ryukyus
	A. gambiae	1967	Upper Volta ; Senegal
Dieldrin	A. sacharovi	1952	Greece
	A. quadrimaculatus	1953	Mississippi, USA ; Georgia, USA ; Mexico
	A. gambiae	1955	Nigeria ; Liberia ; Ivory Coast ; Dahomey ; Upper Volta ; Cameroon ; Sierra Leone ; Togo ; Ghana ; Mali ; Congo (Brazzaville) ; Sudan ; Mauritania ; Madagascar
	A. subpictus	1957	Java ; Ceylon ; N. India ; W. Pakistan
	A. coustani and A. pulcherrimus	1957	Saudi Arabia
	A. albimanus	1958	El Salvador ; Guatemala ; Nicaragua ; Honduras ; Jamaica ; Ecuador ; Mexico ; British Honduras ; Cuba ; Dominican Republic ; Haiti ; Colombia
	A. pseudopunctipennis	1958	Mexico ; Nicaragua ; Peru ;, Venezuela ; Ecuador
	A. aquasalis	1958	Trinidad ; Venezuela ; Brazil
	A. culicifacies	1958	W. India ; Nepal
	A. vagus	1958	Java, Philippines
	A. barbirostris and A. annularis	1958	Java
	A. sergenti	1958	Jordan
	A. fluviatilis	1958	Saudi Arabia
	A. splendidus	1958	N. India
	A. stephensi	1959	Iran ; Iraq
	A. minimus flavirostris	1959	Philippines ; Java
	A. pharoensis	1959	Egypt ; Sudan ; Israel
	A. albitarsis	1959	Colombia ; Venezuela
	A. labranchiae	1959	Morocco ; Algeria
	A. strodei	1959	Venezuela
	A. triannulatus	1959	Venezuela ; Colombia
	A. sundaicus	1960	Java ; Sumatra ; Sabah
	A. aconitus	1960	Java ; India
	A. neomaculipalpus	1960	Trinidad ; Colombia
	A. crucians	1960	S. Carolina, USA ; Dominican Republic
	A. filipinae	1960	Philippines
	A. maculipennis	1961	Romania
	A. rangeli	1961	Venezuela
	A. maculipennis messeae	1961	Romania
	A. labranchiae atroparvus	1961	Romania ; Bulgaria
	A. philippinensis	1962	Sabah
	A. funestus	1962	Nigeria ; Ghana ; Kenya
	A. nili	1966	Ghana
	A. rufipes	1968	Mali

a This table covers only the first reported instance of resistance in any area.

TABLE 2. RESISTANCE OF CULICINE MOSQUITOS TO THREE INSECTICIDE GROUPS a

Mosquito	DDT group — Year	DDT group — Area	HCH-dieldrin group — Year	HCH-dieldrin group — Area	Organophosphorus group b — Year	Organophosphorus group b — Area
Culex fatigans (quinquefasciatus)	1952 1953 1956 1957 1958 1959 1961 1964 1966	India Reunion Venezuela ; Taiwan Puerto Rico W. Africa ; S. Australia ; Panama Tanganyika ; Hawaii ; Congo Madagascar ; Cuba ; Georgia, USA China (mainland) Ryukyus ; Queensland, Australia	1951 1953 1954 1956 1957 1958 1959 1960 1961 1964 1966	California, USA Malaya ; India E. Asia S. America W. Africa Panama Zanzibar ; Congo Texas, USA Mali ; Madagascar ; Brazil Tanganyika ; China (mainland) Togo ; Ivory Coast ; Queensland, Australia	1959 1960 1963 1967	Cameroon (O) California, USA (M) Sierra Leone (O) Ryukyus (M)
C. pipiens	1947 1955 1959 1961 1965	Italy Massachusetts, USA ; New Jersey, USA Israel ; Japan ; California, USA New York, USA ; Maryland, USA ; Illinois, USA ; Utah, USA France ; Korea ; Turkey	1950 1955 1959 1965 1966	Italy Israel France ; Japan Korea Morocco		
C. tarsalis	1951 1956 1961	California, USA Oregon, USA Washington, USA ; Utah, USA	1951 1961	California, USA Oregon, USA	1956 1961	California, USA (M) Oregon, USA (M)
C. coronator	1958	Panama				
C. tritaeniorhynchus	1958	Ryukyus	1959 1965	Dahomey ; Ryukyus Korea	1967	Ryukyus (M)
C. peus	1961	Oregon, USA			1967	California, USA (M)
Aedes aegypti	1954 1955 1956 1957 1958 1959	Trinidad ; Dominican Republic Venezuela Haiti Antigua, Colombia S. Viet-Nam Puerto Rico ; Jamaica ; Guadeloupe ; French Guinea	1959 1962 1963 1964 1965 1966	Puerto Rico Jamaica ; Haiti ; Curaçao Virgin Islands Surinam ; Guyana ; Cambodia ; S. Viet-Nam Texas, USA Cameroon ; Tahiti ; Thailand ; Congo ; Senegal ; Ivory Coast	1965 1966	Jamaica ; Venezuela (M) Thailand ; S. Viet-Nam (M) ; Congo (Brazzaville)

TABLE 2. RESISTANCE OF CULICINE MOSQUITOS TO THREE INSECTICIDE GROUPS a (concluded)

Mosquito	DDT group		HCH-dieldrin group		Organophosphorus group b	
	Year	Area	Year	Area	Year	Area
Aedes aegypti (cont.)	1961 1963 1965 1966 1968	Florida, USA; Guyana; Thailand; E. India; Japan; Texas, USA; W. India; St Vincent; Liberia; Ivory Coast; Dahomey; Cameroon	1968	Liberia; Togo; Nigeria; Upper Volta		
A. sollicitans	1947 1951	Florida, USA Delaware, USA	1951 1958	Florida, USA Delaware, USA		
A. taeniorhynchus	1949 1959	Florida, USA Georgia, USA	1951 1959	Florida, USA Georgia, USA		
A. nigromaculis	1949	California, USA	1951	California, USA	1958 1960	California, USA (P) California, USA (M)
A. melanimon c	1951	California, USA	1951	California, USA	1962	California, USA (P)
A. dorsalis					1966	New Mexico, USA (O)
A. cantator	1960	New Brunswick, Canada	1960	New Brunswick, Canada		
A. cantans	1958	Germany				
A. detritus	1959	France				
A. albopictus	1964	S. Viet-Nam, S. India				
A. vittatus	1964	W. India				
Psorophora confinnis			1954	Mississippi, USA		
P. discolor			1954	Mississippi, USA		

a This table covers only the first reported instance of resistance in any area.

b M = malathion resistance; P = parathion resistance; O = general organophosphorus resistance.

c Formerly called A. dorsalis.

TABLE 3. RESISTANCE TO THREE INSECTICIDE GROUPS BY NOXIOUS DIPTERA a

Species	DDT-group		HCH-dieldrin group		Organophosphorus group	
	Year	Area	Year	Area	Year	Area
Musca domestica	1946 1947 1948 1949	Sweden; Denmark USA; Mediterranean New Zealand; S. America W. Europe; Canada	1949 1950 1951 1952	California, USA; Sardinia; Egypt USA; Scandinavia S. America Africa	1955 1956 1957 1958	Denmark; Florida, USA Switzerland; Italy; Georgia, USA New Jersey, USA California, USA; Arizona, USA; Louisiana, USA
	1950 1953 1956 1958 1960	USSR; Africa Japan China Czechoslovakia; Poland India	1953 1954 1957 1961 1962	USSR Japan India Caribbean Romania	1960 1961 1962 1967	Japan Germany; France Australia USSR
Stomoxys calcitrans	1948 1958 1966	Sweden Norway Germany; Italy	1958 1965 1966	Norway Florida, USA Germany		
Phaenicia cuprina			1957	Australia	1966	Australia
Phaenicia sericata			1959	New Zealand; S. Africa		
Chrysomyia putoria			1949 1960 1961	Congo (Kinshasa) Madagascar Zanzibar	1954	Congo (Kinshasa)
Protophormia terraenovae	1964	European Russia				
Haematobia irritans			1959	Texas, USA	1962	Louisiana, USA
Fannia canicularis	1953 1962 1967	Spain Japan England; California, USA	1967	California, USA		

TABLE 3. RESISTANCE TO THREE INSECTICIDE GROUPS BY NOXIOUS DIPTERA a **(concluded)**

Species	DDT group		HCH-dieldrin group		Organophosphorus group	
	Year	Area	Year	Area	Year	Area
F. fennoralis	1967	California, USA	1967	California, USA		
Simulium aokii	1963	Japan				
S. ornatum	1966	Japan				
S. venustum	1967	Quebec, Canada				
Chironomus zealandicus			1966	New Zealand		
Glyptotendipes paripes			1953	Florida, USA	1955	Florida, USA
Chaoborus astictopus	1961	California, USA				
Psychoda alternata	1949	Illinois, USA	1953	England		
Leptoconops kerteszii	1961	California, USA				
Culicoides furens			1958 1959	Florida, USA Panama		
Hippelates collusor			1957	California, USA		
Leptocera hirtula	1955	Malaya	1955	Malaya		
Drosophila virilis b	1952	Japan				

a This table covers only the first reported instance of resistance in any area.
b Also D. melanogaster, both field strains.

dimethoate started to appear in Denmark and New Jersey in 1966. Resistance to trichlorfon in baits and sprays has been encountered first in Florida, then in Denmark, and very recently in the USSR. The use of dichlorvos vapour can induce resistance, but very slowly. Resistance to coumithioate has developed in Italy ; fenitrothion and Gardona [1] have not yet been used long enough for their liability to resistance to be determined.

The stable fly, *Stomoxys*, has developed DDT resistance in Western Europe and dieldrin resistance in Florida. The latrine fly, *Fannia cani-cularis*, has become resistant to DDT in England and Japan, and in addition is resistant to dieldrin in California where *F. femoralis* has also developed resistance to both insecticides. The blowfly, *Protophormia terraenovae*, has developed DDT resistance in European Russia. The horn fly, *Haematobia*, first developed resistance to toxaphene in Texas, and then to ronnel in Louisiana. The sheep blowfly, *Phaenicia cuprina*, is gradually developing resistance to diazinon in New South Wales, Australia. Whether the same change has occurred in the dieldrin-resistant species *P. sericata* in South Africa (wrongly listed as *P. cuprina* in the Committee's thirteenth report) has yet to be established. Increased tolerance to DDT and dieldrin in *Leucophyra leucostoma* in California is not yet high enough to be listed. Resistance has not yet been recorded in *Phlebotomus*, or in *Glossina*.

One of the most serious developments has been the recent appearance of populations of *Simulium* blackflies resistant to the larvicides which have been used against them, DDT resistance and some HCH tolerance has been reported in a population of *S. aokii* near Tokyo, and DDT resistance coupled with tolerance to fenthion in *S. ornatum* at Chino, Japan. A third species, *S. venustum*, has developed a population 10 times as resistant to DDT as normal in an area of Quebec Province, Canada, which had been treated with this insecticide for the preceding 10 years. A recent control failure with DDT against *S. damnosum* on the lower Volta River in Ghana may have been caused by an increased tolerance to DDT in this important vector of onchocerciasis. Among the midges, the only addition since 1962 is *Chironomus zealandicus*, which has developed resistance to HCH and cyclodiene insecticides in New Zealand.

Among body lice, new records of DDT-resistant populations have come from Hungary and Romania, and strong HCH resistance has recently been found in Turkey (Table 4). There have been a number of new reports of resistance among lice of veterinary importance and these are listed in Table 4.

New records of chlordane resistance in the German cockroach, *Blattella*, have come from Germany, Denmark, Hawaii, New South Wales (Australia) and New Guinea. Diazinon resistance has been found in Texas as well as in Kentucky and Indiana, and malathion resistance in Louisiana, Texas

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[1] Dimethyl 2,4,5-trichloro-alpha-(chloromethylene)benzyl phosphate.

TABLE 4. RESISTANCE OF CERTAIN ARTHROPODS OF PUBLIC HEALTH AND VETERINARY IMPORTANCE [a]

Species	DDT group		HCH-dieldrin group		Organophosphorus group	
	Year	Area	Year	Area	Year	Area
Pediculus corporis	1951 1952 1955 1956 1958 1959 1961 1964	Korea ; Japan Egypt ; E. Mediterranean (UNRWA camps) Iran ; Turkey ; Ethiopia ; W. Africa ; Peru ; Chile France Yugoslavia ; Libya ; Afghanistan ; India Mexico ; Uganda Sudan Romania	1955 1956 1957 1958 1959 1961	France ; Japan W. Africa ; S. Africa Iran India ; Korea Tanganyika Sudan		
Linognathus vituli	1957 1966	Virginia, USA Alberta, Canada				
L. africanus and *L. stenopsis*			1967	S. Africa		
Haematopinus eurysternus	1964	Alberta, Canada	1964	Alberta, Canada	1966	Alberta, Canada
Boophilus limbata and *B. caprae*			1957	Texas, USA		
Blatta orientalis	1964	Czechoslovakia	1958 1964	Germany Czechoslovakia		
Blattella germanica	1958 1959 1961	France ; Germany ; Cuba ; Bahamas ; Puerto Rico Trinidad ; Poland England	1951 1955 1956 1958 1959 1961 1963	Texas, USA S. E. USA N. E. USA California, USA ; Panama ; Cuba ; Puerto Rico Canada ; Trinidad ; Japan ; Poland England ; Germany Denmark ; Hawaii ; Australia ; New Guinea	1960 1964 1966	Kentucky, USA ; Indiana, USA Texas, USA Louisiana, USA

	Year	Locality	1968	Florida, USA	1968	Florida, USA
Periplaneta brunnea					1968	Florida, USA
Cimex lectularius	1947 1952 1953 1955 1956 1957 1959 1960 1961 1962 1967	Hawaii Ohio, USA; Illinois, USA; Indiana, USA; Utah, USA; Congo; Israel; Korea; Greece Japan; Italy Iran; Colorado, USA; Pennsylvania, USA; Texas, USA French Guiana Trinidad; Turkey Hungary; Poland; Rhodesia Borneo; Indonesia; Colombia S. India S. Africa Egypt	1954 1956 1960 1961 1962 1965 1967	Italy Israel Indonesia; Zambia; Rhodesia; Borneo S. India S. Africa N. India Egypt	1964	Israel
C. hemipterus	1952 1954 1955 1957 1960 1961	Taiwan Hong Kong; Singapore W. India Somalia; Kenya Malaya; Gambia; Thailand Madagascar; S. India	1956 1957 1959 1960 1961	W. India Tanzania; Kenya; Upper Volta Dahomeh; Zanzibar Malaya; Gambia Madagascar; S. India		
Pulex irritans	1949 1950 1951 1952 1965 1967	Peru Ecuador Greece Brazil; Palestine Turkey Egypt	1959 1965 1967	Tanzania Turkey Egypt		
Ctenocephalides canis and/or *C. felis*	1952 1953 1956 1958	Georgia, USA French & British Guiana; Colombia Scattered areas of USA Hawaii	1956 1957 1958	USA Hong Kong Hawaii; Japan		
Xenopsylla cheopis	1959 1960 1961 1964 1966 1967	W. India S. India N. India S. Viet-Nam Thailand Egypt	1962 1966	W. India; S.E. India Thailand		
X. astia	1961 1962	N. India S. India	1962	S. India		

TABLE 4. RESISTANCE OF CERTAIN ARTHROPODS OF PUBLIC HEALTH AND VETERINARY IMPORTANCE ᵃ (concluded)

Species	DDT group		HCH-dieldrin group		Organophosphorus group	
	Year	Area	Year	Area	Year	Area
Boophilus decoloratus	1956	Cape Province, S. Africa	1948 1952 1956	Cape Province, S. Africa Transvaal N. Rhodesia		
B. microplus	1954 1956	Queensland, Australia Brazil	1950 1952 1960	Queensland, Australia Brazil N. India ; Guadeloupe	1964	Queensland, Australia
Amblyomma americanum			1954 1963	Oklahoma, USA Madagascar		
Rhipicephalus sanguineus			1954 1958 1961	New Jersey, USA Panama ; Texas, USA Puerto Rico		
R. evertsi			1960	S. Africa		
R. appendiculatus			1965	S. Africa		
Dermacentor variabilis	1959	Massachusetts, USA	1959	Massachusetts, USA		

ᵃ This table covers only the first reported instance of resistance in any area.

and Colorado. The cross-resistance to fenthion ranged up to 5-fold in Texas and 11-fold in Louisiana, and in the latter State cross-resistances ranged up to 14-fold to the carbamate insecticide OMS-33.[1] The oriental cockroach, *Blatta*, is reported to have developed resistance to DDT and dieldrin in Czechoslovakia.

Populations resistant to DDT or the cyclodiene insecticides or both are now frequent among bedbugs, *Cimex hemipterus* and *C. lectularius*, in most regions of the world. Moreover, a most serious development has appeared in Israel, where *C. lectularius* has become resistant to malathion and fenthion. By contrast, reduviid bugs such as *Rhodnius* and *Triatoma* have not provided any proven instance of resistance to HCH or the cyclodiene compounds despite their long history of use against these vectors of Chagas' disease.

When DDT resistance was discovered in the oriental rat fleas, *Xenopsylla cheopis* and *X. astia*, in Maharashtra, Mysore and Uttar Pradesh, in India in 1960, it was accompanied by increased tolerance to HCH and dieldrin. The situation was the same in flea populations in Andhra Pradesh, India, where plague broke out in 1964. Nevertheless, these Indian populations are still reported to be amenable to control with HCH formulations. More recently, DDT resistance has been found in *X. cheopis* in Thailand and Vietnam; at certain places in Thailand dieldrin resistance was also found. In areas of Vietnam open to the hazard of plague, diazinon has proved effective against flea populations resistant to organochlorines. Application of the WHO standard test has discovered strong DDT resistance and some dieldrin resistance in *Pulex irritans* at several places in Turkey.

The brown ear tick, *Rhipicephalus appendiculatus*, has now joined *R. evertsi* and *Boophilus decoloratus* in showing resistance to cattle-dips containing toxaphene or gamma-HCH in the East London area of South Africa. The Australian cattle tick, *B. microplus*, was found to have developed resistance to the organophosphorus compound dioxathion at Rockhampton, Queensland in 1964. Cross-resistance was shown to carbophenothion but not to most of the other organophosphorus compounds, although elsewhere in Queensland a more universal type of organophosphorus resistance is developing.

Among culicine mosquitos, the number of species which have developed organophosphorus resistance has increased from 5 to 9, consequent on the increasing use of organophosphorus compounds as larcivides. *Culex pipiens fatigans* has developed malathion resistance at Douala, Cameroon; Freetown, Sierra Leone; and Okinawa among the Ryukyu islands. *Culex*

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[1] This compound has also been known as Bayer 39007, Baygon, IMPC, UNDEN and propoxur; the name " arprocarb" has been used but this name has also been applied to another carbamate.

tritaeniorhynchus has also developed malathion resistance on Okinawa. In California, malathion resistance has developed in *C. peus* but has largely disappeared in *C. tarsalis*.

Whereas resistance to organochlorines in *C. fatigans* is a general occurrence, new records of such resistance in *C. pipiens* have come from France, Morocco, Turkey, and Korea. Isolated cases of DDT resistance have been found in *C. salinarius* in New Jersey, but not elsewhere in eastern North America, and in *C. erythrothorax* in California ; dieldrin resistance was evident in a larval sample of *C. restuans* in New York.

In *Aedes aegypti*, DDT resistance has now been recorded from India, Thailand, Japan, the Americas and West Africa. Dieldrin resistance accompanies DDT resistance throughout the Caribbean area, and has developed in parts of West Africa (e.g., Upper Volta) in the absence of DDT resistance ; it is also present in the large cities in Cambodia and Vietnam, and on the island of Tahiti. Populations of *Ae. aegypti* with increased tolerance to malathion have been found in Jamaica, Venezuela, Congo (Brazzaville), Thailand, and Vietnam.

It has become evident that the salt-marsh mosquito, *Ae. taeniorhynchus*, has developed malathion tolerance in several parts of Florida where organophosphorus compounds have been consistently applied as adulticides. The irrigation-water mosquito *Ae. nigromaculis* in the south-central part of the San Joaquin valley of California has developed, in succession, resistance to parathion, methyl parathion, fenthion, and even to Dursban,[1] although remaining controllable by OMS-33.[2] Organophosphorus resistance is also intensifying in *Ae. melanimon*, but more slowly. Fenthion resistance has recently been encountered in *Ae. dorsalis* in New Mexico, USA.

Ae. albopictus has developed DDT resistance in Saigon, Vietnam and Bangalore, India, while *Ae. vittatus* has been found to be DDT-resistant at Baroda, India. An isolated case of increased DDT tolerance was found in *Ae. vexans* in, British Columbia, but elsewhere this species has remained susceptible. *Ae. atropalpus* was found to be DDT-tolerant in every sample tested in North America except one, and *Ae. fijiensis* proved to be DDT-resistant in every sample tested in Fiji ; whether these were developed or pre-existing tolerances is not clear.

In the anopheline vectors of malaria, DDT resistance has now developed in *Anopheles gambiae*, appearing first in the A form in Upper Volta and then in the B form in Senegal. Records of dieldrin resistance from outside West Africa have now come from the Sudan, Kenya, Rhodesia and Madagascar ; both A and B forms have been involved.

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[1] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate.

[2] This compound has also been known as Bayer 39007, Baygon, IMPC, UNDEN and propoxur ; the name " arprocarb " has been used but this name has also been applied to another carbamate.

During the past 6 years, *An. funestus* has developed dieldrin resistance in Nigeria, Ghana, Upper Volta and Mali in West Africa. Tolerance to malathion has been reported in *An. albimanus* on the Pacific slopes of Guatemala and Nicaragua. Otherwise the new records of resistance since 1962 have not been numerous and have been confined to dieldrin resistance in *An. philippinensis* in Sabah, *An. nili* in Ghana, and *An. rufipes* in Mali, and DDT resistance in *A. fluviatilis* in Maharashtra State, India, and *An. hyrcanus sinensis* in Okinawa. In addition, the development of dieldrin resistance in the *maculipennis* group in general, mentioned in the 1962 report, has produced specific examples in *An. maculipennis* (spp. *messeae* and *maculipennis*) in Romania, and in *An. labranchiae atroparvus* in Romania and Bulgaria.

In anophelines of the *maculipennis* group, DDT has remained effective for interrupting malaria transmission despite a certain development of DDT resistance (e.g., *An. sacharovi*); it has been concluded that this effect is due to their pronounced irritability, which reduces not only the inter-domiciliary contact between mosquito and man but also the selection pressure for DDT resistance. Populations and laboratory strains of anophelines (and culicines) which have developed DDT resistance are found to have become less DDT-irritable. Changes in behavioural response to DDT have been reported in anophelines without any change in susceptibility level. A population of *An. pseudopunctipennis* in Mexico, appeared to have developed an increased ability to escape from sprayed houses alive, and this was probably the characteristic which was developed by the Chagres River population of *An. albimanus* in Panama. The avoidance of DDT deposits and enhanced escape from treated houses observed in *An. sundaicus* at Tjilatjap (Java, Indonesia) was on the other hand found to be a pre-existing characteristic of Javan populations. The procedures devised to test DDT irritability of anophelines, have yielded useful information, despite the high variance, but require reinforcement by additional tests, particularly to measure flight and escape activity.

The WHO standard test for physiological resistance in adult mosquitos has given an extensive and precise understanding of the resistance situation in many of the anopheline species during the past 12 years but there still remain a number of important species on which insufficient data are available. The standard test for mosquito larvae has achieved similar success for *Aedes aegypti*, *Culex fatigans*, and other culicines, while providing confirmatory evidence for resistance in anophelines.

1.2 Resistance of insects to chemosterilants and bacterial toxins

In anticipation of their possible use against insects of public health importance, chemical sterilants and bacterial toxins have been investigated in the laboratory for their ability to induce resistance.

Selection of 2 strains of *Aedes aegypti*, one by apholate, the other by metepa, resulted in a 20-fold and a 3-fold increase in tolerance, respectively, to the selecting agents and was associated in the metepa-selected strain, with an increased detoxification of the chemosterilant. In a third experiment, selection by hempa induced at first an increased tolerance; but in the sixth generation, the sterility had become higher than that observed originally, owing to the acquisition of inheritable recessive genetic defects.

Similar selection experiments with metepa and apholate on *Musca domestica* suggest that the balance between physiological resistance and inheritable defects is struck at an earlier stage in the selection regime in this species.

Selection of a house-fly strain with spores of *Bacillus thuringiensis* at a level well above 50 % mortality induced an 8-fold to 14-fold resistance after 27–50 generations, which was fairly stable when selection was discontinued. A similar selection at a lower level failed to develop this resistance.

1.3 Resistance of rodents to rodenticides

Resistance of the brown rat, *Rattus norvegicus*, to warfarin was discovered in the vicinity of Glasgow, Scotland, in 1958. Resistant individuals are now common in a 10 km × 20 km area north and east of Glasgow, with occasional resistant rats being found as far east as Edinburgh, a distance of about 70 km. In 1960, warfarin resistance was found near Welshpool, Wales, and by 1965 the area affected was 50 km in diameter. A resistant population was also discovered at Minehead, Somerset. In 1962, warfarin resistance was found in the eastern Vejle district of Denmark, and by 1967 was present over a 10 km × 20 km area in this coastal peninsula of Jutland. In 1966, difficulty of control by warfarin was reported from Sleen, Drenthe Province, Netherlands, and the resistance in that area was confirmed by laboratory test.

In the Jutland population, at least 55% of the rats were warfarin resistant, there being a definite phenotypic discontinuity between them and susceptible rats. The resistance extends to other anticoagulants such as coumachlor (another coumarin derivative) and diphacinone (an indandione derivative). Crosses and back-crosses of the Scottish strain with susceptible rats have indicated the resistance to be due to a single semi-dominant autosomal gene. Similar work with the Danish resistant rats, however, indicated that more than one gene was involved. The Danish resistant rats have a lower reproductive rate, and in a colony reared in the absence of warfarin susceptible rats steadily decreased.

Reports of warfarin resistance in populations of the house mouse, *Mus musculus*, in England commenced in 1959. Mice which survived control failures in Harrogate, Yorkshire, were tested and found to be

resistant to warfarin. Other resistant populations have now been found in at least 7 English counties, including the cities of Huddersfield, Leeds, Norwich and London. The resistant mice are cross-resistant to the anti-coagulant rodenticides pindone and chlorophacinone. The resistance is inheritable in the laboratory, and seems to be recessive ; work to date can go no further than to indicate that it is either polygenic or due to a gene under the influence of modifiers.

Control failures with warfarin developed in the canefield rat, *Holochilus sciureus*, in British Guiana in 1962 ; control failures have also been reported with warfarin and coumachlor in the paddyfield mole-rat, *Gunomys gracilis*, in north-western Ceylon. That these failures were due to developed resistance has not yet been conclusively proved.

Resistance to endrin, used as a rodenticide in apple orchards, has been developed by the pine mouse, *Pitymys pinetorum*, in the Shenandoah valley of Virginia, USA. Laboratory experiments are under way to investigate the physiology and genetics of this resistance.

1.4 Resistance of non-target organisms to insecticides

In the cotton-growing areas of Mississippi, one of the regions of the world most heavily treated with insecticides, resistance of non-target organisms to insecticides has been confirmed by appropriate tests. Strong resistance to DDT and aldrin was found in the cricket-frogs *Acris crepitans* and *A. gryllus*. The mosquito-fish, *Gambusia affinis*, in this area showed a 4-fold tolerance of DDT and as much as 120-fold resistance to dieldrin and other cyclodiene derivatives. Populations of the green sunfish, *Lepomis cyanellus*, the blue-gill sunfish, *L. macrochirus*, and the golden shiner, *Notemigonus crysoleucas*, were found to be some 50 times more resistant than normal to cyclodiene insecticides, but were still susceptible to DDT although it had been widely used in the area.

Among the mammals tested, populations of the cotton rat, *Sigmodon hispidulus*, were found to be no more DDT-tolerant than those from untreated areas. However, in the laboratory it has been found possible to develop a slightly DDT-resistant strain of white mice, *Mus musculus* ; this strain showed cross-tolerance to dieldrin and gamma-HCH, and was characterized by containing 15–30% more total lipoid than the unselected colony.

Among invertebrates, the aquatic nymphs of the mayflies, *Heptagenia hebe* and *Stenonema fuscum*, have developed a 15-fold DDT resistance in areas of New Brunswick, Canada, repeatedly sprayed with DDT for spruce-budworm control. The paddyfield crab, *Paratelphusa ceylonensis*, has developed endrin resistance in northwestern Ceylon, the parasitic copepod, *Argulus*, has developed HCH resistance in Israel, and the freshwater shrimp, *Palaemonetes kadakiensis*, has developed a slight tolerance to organochlorine insecticides in Mississippi. DDT resistance is also known

to have developed in colonies of the honey bee, *Apis mellifera*, from California, Louisiana and England. Among beneficial insects, 2 species of parasitic wasps, *Macrocentrus ancylivorus* and *Bracon mellitor*, were purposely selected in the laboratory for DDT resistance so that they could be safely introduced into areas being treated with DDT for the control of agricultural pests. The mite predator, *Typhlodromus occidentalis*, has developed parathion resistance in the apple orchards of the Okanagan valley in British Columbia, Canada.

2. IMPLICATIONS OF RESISTANCE IN VECTOR CONTROL

2.1 A global survey of the impact of resistance

In an attempt to gather the required information on the impact of insecticide resistance on vector and disease control, a questionnaire was prepared to inquire about the following matters :

(*a*) Control schemes in a given country—insecticides used and the scale of operations ;

(*b*) Insecticide resistance developed—insecticide group ; resistance suspected or confirmed ; effects of resistance on control (moderate or severe) ; which insecticide(s) had been abandoned ; possibility of increase of disease due to resistance.

This enquiry was sent to 114 public health authorities in different parts of the world and 70 replies were received.[1]

The committee reviewed the information collected and concluded that DDT, HCH and dieldrin have been the principal insecticides used in vector control programmes and that an increasing number of species has now developed resistance to one or more of these toxicants in different geographical areas. The effect of such resistance on the control of arthropods of medical importance is outlined in the following discussion.

2.1.1 *Anopheline mosquitos*

Resistance has occurred in the principal malaria vectors in many places during malaria eradication programmes. The effects have ranged from being an inconvenience to an apparently insuperable obstacle. Thus, in temperate regions, such as Europe, where malaria does not reach hyperendemic levels, resistance has not prevented the achievement of eradication. In these regions dieldrin resistance was generally more intense and DDT was usually used to complete the campaigns, as dieldrin was abandoned.

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[1] See Busvine, J. R. & Pal, R. (1969) *Bull. Wld Hlth Org.*, **40**, 731.

In warmer climates, where malaria is more fully established, there are, even now, areas where resistance challenges the outcome of the campaigns (e.g., the areas around the Persian Gulf, Mexico and several countries in Central America). Again, resistance to dieldrin usually leads to this insecticide being abandoned while a simultaneous resistance to DDT (which is less intense) renders the final stages excessively difficult.

In the African continent, the difficulties of control and eradication are very considerable and could be attacked only by a highly effective insecticide. DDT is not completely satisfactory and the widespread dieldrin resistance in West Africa renders both this compound and HCH useless.

2.1.2 *Culicine mosquitos*

(a) *Aedes aegypti.* A campaign to eradicate this urban vector from the Americas was initiated about 15 years ago. At first, there was good progress and a number of countries claimed to be free : later, trouble due to DDT resistance and double resistance was encountered in the Caribbean islands. In recent years there has been reinvasion of several mainland countries : double resistance is appearing in these countries and there is a serious chance that it will jeopardize the whole undertaking.

(b) *Culex fatigans.* This species has developed double resistance to DDT and dieldrin in most places. The loss is serious although the insect can still be controlled by organophosphorus larvicides.

(c) *Nuisance mosquitos.* Coastal breeding culicines in South-east Asia and the USA and flood water culicines in California provided the first examples of insecticides resistance in mosquitos. Control is being achieved by the use of organophosphorus insecticides.

2.1.3 *Lice, vectors of typhus and relapsing fever*

A world survey of *Pediculus humanus* in 1965 showed that DDT resistance is prevalent in many places. Usually this develops as a result of intermittent de-lousing campaigns conducted by various health authorities. So far, resistance to DDT has not interfered with typhus control except perhaps in a limited area in South Africa. Should an outbreak occur in a DDT-resistant area, it should be possible to employ an alternative insecticide such as malathion.

2.1.4 *Flea vectors of plague*

Among the most important chemical weapons against plague are insecticides to kill the fleas and modern rodenticides to reduce the number of rats. Such measures have been generally successful and plague has disappeared from most of India, for example, with only small pockets remaining in

Madras and Mysore. Although epidemics are now exceedingly rare, outbreaks have occurred in rural and urban parts of Vietnam, and control efforts are handicapped by resistance to DDT in the vectors. In India, it is said that the use of insecticides in the malaria campaign has induced flea resistance, and this may hamper the elimination of remaining foci.

2.1.5 *Flies and fly-borne diseases*

Two groups of diseases are associated with flies : (*a*) enteric diseases (dysenteries) spread by *Musca domestica* (in Africa probably also by *Chrysomya putoria*) ; and (*b*) ophthalmic diseases, the most serious being trachoma, spread by *Musca domestica* and *M. sorbens*. In both cases, there are other means of transmission of the disease, so that the part played by flies is less easy to assess than in infections that are exclusively insect-borne. Nevertheless, the association with flies is firmly established, since, in the early days before resistance was widespread, the use of DDT house-spraying was shown to reduce enteric infections. Resistance of houseflies is now so widespread and has extended to so many types of insecticides, that it is not possible to rely on chemical control alone in any region where conditions promote extensive breeding.

2.1.6 *Vectors of onchocerciasis*

The use of DDT as a larvicide against *Simulium* seems a promising strategy, particularly in relation to the control of onchocerciasis, since applications to rivers can be effective for miles downstream. Treatments must be repeated at intervals, otherwise reinfestation occurs from adults that are not affected by the treatment. Sometimes it has been possible to achieve eradication by successive treatments over an isolated area. Further successes of this kind might be possible given suitable conditions. Quite recently, however, resistance has been confirmed in certain pest populations of *Simulium* in Japan and Canada, so that the development of resistance in vector species elsewhere cannot be excluded ; vigorous extension of control measures is likely to accentuate this trouble, but organophosphorus insecticides are a possible alternative.

2.1.7 *Bedbug infestations*

Insecticide resistance of the bedbugs, *C. lectularius* and *C. hemipterus*, is now exceedingly common in warm and tropical countries. The impact of resistance to organochlorine compounds goes far beyond losing a simple method of controlling a nuisance. By causing suspicion and antagonism in householders when spraying fails to kill bugs and houseflies, the success of antimalarial campaigns can be jeopardized.

2.1.8 *Cockroach infestations*

Cockroaches are given separate consideration from insects that are merely nuisances, in view of their possible implication in disease transmission. Since *Blattella germanica*, the German cockroach, is not very susceptible to DDT and since dieldrin resistance is widespread, increasing reliance will have to be placed on organophosphorus and carbamate insecticides.

2.1.9 *Other vectors and diseases*

So far, there have been no proven reports of resistance among tsetse flies, sandflies, reduviid bugs or tick or mite vectors of human disease. This is not entirely a matter for complacency, because so far use of pesticides against all these vectors has been mainly restricted to moderate or large-scale field trials. It seems possible that extension of usage to a national scale might well provoke resistance.

2.1.10 *Conclusions*

The problems arising out of resistance to the organochlorine insecticides have been apparent for some time and it has been equally evident that alternative insecticides are required. Research in the past 5 years (much of it sponsored by WHO) has shown the value of various organophosphorus and carbamate insecticides. It is to be hoped that these will adequately meet the requirements for most of the vectors mentioned, but attention must be focused on all facets of their use. Some of the new compounds are rather specific in their action and all are more expensive than the organochlorine insecticides. The emergence of resistance to the new compounds is always a threat, and research on the development of alternative insecticides must continue together with the exploration of new methods of control.

2.2 Indications of resistance that warrant a change in operational insecticide

In view of the possibility of the development of resistance, it is highly desirable, when planning a control programme, to decide upon a suitable alternative insecticide that may be readily procured for use, should the need arise.

A watch for incipient resistance should be maintained as follows:

Following the establishment of a base-line for dosage–mortality, resistance tests should be simplified to the use of single-dose tests to detect the first signs of resistance, as discussed in section 5. These tests should be used over as wide a geographical area as possible and should also be done (in the same way) with the alternative insecticides, that may be required.

When evidence of resistance occurs, the following steps should be taken :

(*a*) Assess the effect of the resistance on the density of the vectors in the field, making sure that any increase in numbers is not due to other factors (e.g., inadequate treatment, optimum conditions for arthropod propagation).

(*b*) Re-treat a selected part of the area thoroughly and measure the effect on arthropod densities.

(*c*) If the experimental re-treatment fails to control the vector, even at increased doses, apply and evaluate an alternative pesticide in a similar way before adopting it for the entire control operation.

The knowledge available on resistance indicates that where dieldrin resistance occurs, it rapidly develops to the point where control cannot be achieved by increased dosage. Resistance to DDT and organophosphorus compounds appears to be less absolute than dieldrin resistance and in some instances has not markedly altered the efficacy of control measures, at increased dosages. The type of resistance involved should be taken into consideration.

3. RESEARCH ON INSECTICIDE RESISTANCE

3.1 Present status of research

In the 6 years since the thirteenth meeting of the WHO Expert Committee on Insecticides, research on the subject of resistance has made most progress in the field of genetics. The importance of genetics in the study of resistance was emphasized by the WHO Scientific Group on Genetics in 1964, because the development of resistance in the field is essentially a problem of population genetics. In addition to this, the identification of resistance mechanisms with particular genes, assists understanding of the complexity of different resistance problems and the probable success of countermeasures.

Resistance has been found to be due to single principal genes in nearly all cases, and genes have been discovered that give resistance to dieldrin in 17 species, to DDT in 12 species, to organophosphorus compounds in 4 species, and to carbamates in the housefly. Usually DDT resistance is recessive, organophosphorus resistance dominant, and dieldrin resistance intermediate.

The resistance genes have been located on certain chromosomes by discovering their linkage relationships with certain mutant markers. Many of these marker strains have been made available through WHO. Genes have been associated with specific chromosomes in the cases of DDT resistance in *Blattella*, dieldrin resistance in *An. quadrimaculatus*, and both

DDT resistance and dieldrin resistance in *Aedes aegypti* and *Culex fatigans*. In the housefly 3 genes have been discovered which confer DDT resistance, the principal one (*Deh*) on chromosome II, a second (*kdr*) on chromosome III, and a third on chromosome V. The *Deh* gene determines the resistance mechanism of detoxication by the DDT-dehydrochlorinase enzyme, the *kdr* gene is concerned with either reduced cuticular penetration or decreased nerve sensitivity or both, and the third gene with microsomal oxidation of DDT. These findings have an important bearing on measures taken to counter DDT resistance such as the use of enzyme-inhibiting synergists and analogues that do not undergo dehydrochlorination.

Organophosphorus resistance in the housefly has been found to be due to the gene *a*, located on chromosome II close to *Deh*. This gene determines the conversion of the aliesterase enzyme to a detoxifying esterase which in parathion resistance is a phosphatase, and in malathion resistance is a carboxyesterase. Carbamate resistance in the housefly is also conferred by a gene on chromosome II, which determines the microsomal oxidation of carbamates. In *Culex fatigans*, however, carbamate resistance has proved to be polyfactorial. Malathion resistance, which in *C. tarsalis* is due to increased carboxyesterase activity, is in *C. pipiens* associated with a gene in linkage group 2; fenthion resistance in *C. fatigans* due to increased hydrolysis of fenoxon, is due to a gene showing similar linkage relationships.

The mechanism of dieldrin resistance still remains unknown; it is not due to detoxication or to increased lipoid content, but may be connected with the ultrastructural components of the nerve ganglia. Flies resistant to gamma-HCH carry this factor, and in addition at least one other factor concerned with reduced absorption or increased detoxication, or both.

3.2 Research needs to counter resistance

Basic research on resistance seems to have become centred in a limited number of laboratories and investigators of the biochemistry and genetics of resistance now have a considerable background in this field. It is now necessary, however, to extend such studies from the organochlorine insecticides to the organophosphorus and carbamate compounds which are becoming available in considerable variety. Some questions need to be answered as soon as possible, such as the relation between organophosphorus detoxication, which seems to be hydrolytic, and carbamate detoxication, which seems to be oxidative, while both are associated with a chromosome-II gene in the housefly. The solution of this basic problem may throw light on the all important points of the cross-resistance between organophosphorus compounds and carbamates.

More studies are needed on the detoxifying ability of the cell microsome fraction. The esterases should be analysed by electrophoresis on zymo-

grams and special attention should be paid to the bands which can detoxify organophosphorus compounds.

Whenever new types of organophosphorus resistance or carbamate resistance are encountered in the field, consideration should be given to their basic study in the laboratory or if necessary under field conditions. Malathion-resistant bedbugs have lately been taken under study, and organophosphorus-resistant *Aedes nigromaculis* are being colonized for biochemical investigation. Where malathion-tolerant *Ae. aegypti* and *An. albimanus* are found in the field, it would be advantageous to determine their resistance mechanism in the laboratory.

Laboratory selection experiments can give some indication, though by no means infallible, of the future of an insecticide in the field. Although laboratory strains usually suffer from a contraction of the gene pool, laboratory selection can occasionally reveal potentialities that field observations cannot detect, e.g., DDT resistance in *Ae. aegypti* in West Africa. Certainly, when resistance is developed in the laboratory, it can be expected in the field. Two examples of forewarnings that laboratory selection has given are (*a*) that body lice can be induced to develop a 300-fold resistance to carbaryl but only a 2-fold tolerance to malathion, and (*b*) that *Culex fatigans* has achieved a 10-fold resistance to fenthion in the laboratory but scarcely changes its susceptibility when selected with Dursban.[1] *Ae. aegypti* has responded less to organophosphorus selection than *Culex fatigans*. In view of the possibility of the development of resistance in the field to Dursban [1] by *Culex fatigans*, and to Abate [2] by *Ae. aegypti*, frequent susceptibility tests should be performed, in this case to obtain a full dosage–mortality regression line. Valid base-line figures for all the new organophosphorus and carbamate compounds become a real necessity as soon as they come into general use for control.

At the same time, cross-resistance figures are urgently needed. A compilation of the results already obtained in houseflies, *C. fatigans* and other species exposed to single known insecticide regimes, would be helpful, for they might confirm whether insects develop resistance and cross resistance to certain organophosphorus compounds (e.g., dimethoate, Dursban [1], etc.) less readily than to others (e.g., carbaryl, chlorthion, etc.). Such findings would provide indispensable information for two of the principal ways of combating resistance, which are (*a*) the introduction of alternative insecticides, and (*b*) the discovery of new insecticides, if possible in new chemical groups. In the latter case, the work of the WHO Collaborative Programme for the Evaluation of New Insecticides has proved of the utmost value. A third countermeasure for resistance is the use of synergists and mixtures; for the understanding of synergists, meta-

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[1] *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate.

[2] *O,O,O′,O′*-tetramethyl *O,O′*-thiodipara-phenylene phosphorothioate.

bolic studies are essential, while for the interpretation of the results with mixtures careful genetic study is necessary. General metabolic studies of vector insects are also advisable to allow for the appraisal of the potentialities of new chemical control agents such as insect hormones. In addition, field research to discover the particular sites at which insecticides are most effective in the field is always necessary with a view to restricting the use of insecticides. Field studies are, of course, also essential to validate the use of control measures other than insecticides ; such measures will, in turn, also reduce the selection pressure that leads to insecticide resistance.

4. PROCEDURES USED FOR THE DETERMINATION OF SUSCEPTIBILITY OR PHYSIOLOGICAL RESISTANCE OF INSECTS TO INSECTICIDES

It is almost 12 years since WHO became actively concerned in organizing measures to counter resistance. One early and important action was the development of standard test methods for detecting and measuring resistance. While there is no doubt that the resistance tests have been of paramount value for defining the changing status of resistance, the time has come to give a careful scrutiny to these methods in order to see whether they are adequately filling present needs and to what extent they will be needed in the future.

In the early years of resistance tests, it was necessary to provide only 2 insecticides (DDT and dieldrin) to detect incipient resistance to the 2 main groups of chlorinated hydrocarbon insecticides. In recent years, however, considerable numbers of organophosphorus insecticides and carbamates have been introduced, and sporadic resistance to these new substances has appeared. Unfortunately it is not possible to choose a single phosphorus compound or carbamate that will, unequivocally, detect resistance to other members of the group concerned. The alternative prospect of providing in the WHO test kits a full range of concentrations of all the new insecticides, would appear to present several difficulties (e.g., increased cost, aggravated by relatively rapid deterioration of the papers on storage). To meet this problem, the Committee adopted certain modifications of existing test methods, as described below.

4.1 Adult mosquitos

4.1.1 *Organochlorine insecticides*

The Committee noted with satisfaction the large body of information that has accumulated on susceptibility to organochlorine insecticides and

resistance levels for most of the major vectors. It is considered that no change in the method of test is required. It is suggested merely that it would be helpful to include with the instructions some data obtained for the more important species for guidance of the field worker (see Annex 1A).

4.1.2 *Organophosphorus and carbamate insecticides*

In view of the impossibility of supplying a full range of concentrations of impregnated papers for the types mentioned, it is recommended that only 2 concentration levels of each be provided. To make up for the reduction in the range of concentrations supplied, tests will be done using different exposure times, in addition to the standard 1-hour period. It is recognized that this procedure will alter the basis of detection and measurement of resistance, since mortalities will be related to exposure times (rather than to concentrations) and from the regression lines the LT_{50} and LT_{95} values, etc. (i.e., exposures causing 50% or 95% mortalities) can be calculated. It was felt that this was justified on two grounds : (*a*) that investigations have shown, at least with organochlorine compounds, that there is a close relationship between exposure time and the dose of insecticide picked up : a close watch should be kept, however, that in newer compounds the close relationship between concentration and time does in fact obtain, particularly with carbamates ; (*b*) that change in the method of detecting resistance is not so serious with these newer compounds, since there is not a large body of comparative data already available.

It is, accordingly, necessary to draft amended instructions for tests with organophosphorus and carbamate insecticides (see Annex 1B).

4.2 Larval mosquitos

4.2.1 *Organochlorine insecticides*

The Committee noted that this test is the most extensively used and felt that no change in the kit or the method is required. For convenience of the field worker, however, some base-line data for certain mosquito species will be added to the instruction sheets (see Annex 2A).

4.2.2 *Organophosphorus and carbamate insecticides*

The Committee reviewed various ways of meeting the demands for solutions of a variety of new insecticides and decided that the best way would be to supply sets of concentrations only of requested compounds. In other respects, the composition of the kit and the procedure remains unchanged. In addition, there would be base-line data provided for some of the more widely used of the new insecticides (see Annex 2B).

4.3 Larvae of biting midges

The Committee noted that field applications against biting midges (e.g., *Culicoides* sp., *Leptoconops* sp.) had been extensively used in some places and that resistance had been reported. It was pointed out that a tentative test method can be conveniently developed based on the larval mosquito test. Accordingly, the Committee decided that instructions should be drawn up to describe the small modifications necessary (see Annex 3).

4.4 Human lice

4.4.1 *Body lice*

The test kit described in the current instructions includes packets of powders containing malathion. It has, however, been found that malathion dusts undergo rapid deterioration on storage, especially at the very low concentrations proposed for use in the test. Accordingly, the feasibility of using impregnated papers was investigated and results placed before the Committee. The results showed that malathion-impregnated papers can be employed satisfactorily.

It would seem a logical step to abandon dusts completely and change to a test based on papers for DDT (and other insecticides, as required). There are 2 objections to this, so far as DDT is concerned : (*a*) extensive data have been collected with the dust method and these have been published by Wright & Brown [1] and Wright & Pal [2]; and (*b*) work by Busvine [3] suggests very strongly that 5% DDT dust makes a very good discriminating dose for detecting resistance.

The instructions for the resistance test for lice have therefore been redrafted to include tests with impregnated papers to detect and measure resistance to malathion, as well as tests with powders to detect resistance to DDT and HCH (see Annex 4).

4.4.2 *Head lice*

It was recognized that head lice present a public health problem in many parts of the world, though not a dangerous one, and it would be desirable to have a test for resistance (especially as unconfirmed reports of resistance have been received). It has been shown that the method used for body lice is not satisfactory for head lice (owing to high control mortality

[1] Wright, J. W. & Brown, A.W.A. (1957) *Bull. Wld Hlth Org.*, **16**, 9.

[2] Wright, J. W. & Pal, R. (1965) *Bull. Wld Hlth Org.*, **33**, 485.

[3] Busvine, J. R. (1967) *Bull. Wld Hlth Org.*, **36**, 431.

of head lice). The Committee recommended that WHO should encourage the development of a tentative test method, by one or more panel members.

4.5 Bedbugs

No change was suggested for this test method (see Annex 5).

4.6 Reduviid bugs

In the thirteenth report of the Committee, these insects were described as " triatomid bugs ", but it has been suggested that " reduviid bugs " is a better description since genera other than *Triatoma* are included.

Only one criticism of the method has been received ; namely, that certain species of *Rhodnius* are able to climb off the treated papers on to the glass walls of the test-tubes used. As a tentative solution, it is proposed to provide glass tubes of the same size as the standard impregnated papers. In addition, the possibility of using the plastic exposure tubes, designed for the adult mosquito test, should be explored. These would be lined completely with impregnated paper.

The test procedure is, at present, unchanged (see Annex 6).

4.7 Fleas

4.7.1 *Adult fleas*

No change is proposed in the test method for adult fleas, which has been fairly widely used and has proved satisfactory (see Annex 7).

4.7.2 *Larval fleas*

The Committee noted that a few investigators have devised a method of testing for resistance in larval fleas, using impregnated papers supplied by WHO. These tests were done with larvae from laboratory colonies.

In view of the difficulty of obtaining flea larvae from natural infestations, and also the fact that insecticides are generally directed against the adult stage, the Committee considers that no useful purpose would be served by developing a standard test for larval fleas.

4.8 Adult sandflies, blackflies, biting midges, eye gnats, etc.

In the thirteenth report of the Committee, separate tests were described for sandflies and adult blackflies. Neither has been very extensively used, and it has been suggested that, in view of the close similarity, they should be combined into a single test, which could also be used for other small diptera. The Committee noted that this test has proved feasible with adult midges

and it seems probable that it could also be used for the eye gnats (*Hippe-lates* spp.). Accordingly instructions for a combined test have been prepared, in which details as to the collection and handling of the different genera have been included (see Annex 8).

4.9 Houseflies, tsetse flies, stable flies, blowflies, etc.

The Committee considered the results provided by collaborating workers who had compared the 2 tentative test methods for houseflies, described in its thirteenth report[1] (i.e. exposure to residues in glass vials and also topical application). In addition, results of tests on a time exposure basis, using treated jars or petri dishes were available. After considerations of these methods and a dip test, the Committee decided to adopt the topical application method as a standard.

It was considered that the housefly susceptibility tests would not be performed individually and sporadically, but in a series of tests to constitute a survey in a given geographical region. Thus they would be performed at a central laboratory, with material brought from various localities and perhaps reared to the first filial generation. It is recommended that WHO should prepare a separate document giving the methods of collection and the general procedure for a housefly susceptibility survey, including a list of the test insecticides available on request.

The Committee noted that the topical application method has given satisfactory results with tsetse flies and stable flies, whereas the method of exposure to impregnated papers (as described in the Committee's thirteenth report) has been found somewhat unsatisfactory. The Committee therefore decided that the test for these 2 insects and similar large diptera (which cannot easily be blown from the treatment tube to the holding tube), could be combined into a single topical application method (see Annex 9).

4.10 Blackfly larvae

The Committee reviewed the merits of the 2 alternative methods recommended in its thirteenth report. It was decided that the second method (the use of shallow trays) should be discarded because of the high mortality in controls. The provisional method now recommended is a modification of the first method suggested in that report. The modified method (see Annex 10) incorporates the following features : exposure of the larvae in 250-ml jars or beakers without a glass plate inserted ; aeration of the water before and after exposure, but not during the exposure ; and manual transfer of the larvae. The Committee noted that this method is not suitable for

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1963, No. 265.

testing larvae of *Simulium neavei*, which live on fresh-water crabs. A test method for this species has been described by Raybould.[1]

4.11 Ticks

Since the thirteenth report of the Committee, the tentative method of test for adult ticks (by topical application) has been found satisfactory with 3 species of hard and 1 species of soft tick. It was therefore decided to adopt it as a provisional test (see Annex 11) though it is pointed out that treatment of very small species (or larvae) might present difficulties. Accordingly it is suggested that further investigation of alternative methods should be pursued. These include dipping and exposure to impregnated papers (the latter method is under consideration by FAO for ticks of veterinary importance).

4.12 Cockroaches

No change is proposed in the method of testing for resistance in cockroaches. It is proposed to supply base-line data for important species together with the test instructions (see Annex 12).

4.13 Persistent fumigants

Considerable development work on this method, using dichlorvos, has been done by collaborating investigators, since the thirteenth report of the Committee. After reviewing the results obtained, the Committee decided that the method should be slightly modified as follows : (*a*) The doses of dichlorvos are applied in a mixture of ethyl methyl ketone and di-octyl phthalate in the proportions of (39 : 1) ; (*b*) time/knock-down curves are obtained at different dosage levels. LT_{50} and LT_{95} values are estimated graphically ; and (*c*) LT_{50} and LT_{95} values are then plotted against doses on log-log paper. From this the dose corresponding to an LT_{50} (or LT_{95}) of 60 min can be calculated. This constitutes the basis for measuring resistance (see Annex 13).

4.14 Rodents

In view of the emergence of resistance of domestic rodents to anti-coagulant rodenticides, WHO requested a collaborating laboratory to describe a test method for detecting and measuring this resistance. A method was drawn up and prototype test kits were sent to a number of laboratories. Results from widely different localities have shown that the test is feasible,

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[1] Raybould, J. (1966) *Bull. Wld Hlth Org.*, **35**, 887.

at least with *Rattus norvegicus*. *R. rattus* and *Mus musculus*. Accordingly, this has been adopted with small modifications as a provisional test method (Annex 14). Base-line data obtained with the method will be included with the instructions.

The Committee was informed of other test methods under investigation which might perhaps be more precise for the determination of physiological resistance. Since insufficient data on these methods are at present available, the Committee recommended that WHO explore these possibilities further.

5. INTERPRETATION OF RESULTS
OBTAINED FROM THE USE OF TEST PROCEDURES
FOR PHYSIOLOGICAL RESISTANCE

· The various test procedures described in the preceding sections have two purposes :

(*a*) to determine the susceptibility of a given species to various insecticides in actual or potential use for control work, and

(*b*) to detect (and measure if possible) any change in susceptibility that may alter the efficacy of control.

Any departure from the base-line characteristics of the species indicates a change in susceptibility. Due regard must be paid to minor variations caused by the non-standard physiological state of the test group or variations in the physical conditions of the test. The most important indication is failure to kill all insects at double the usual 100% mortality dosage (or exposure time) ; this is why such a test is used to monitor incipient resistance. Any survival at this level, in 4 successive tests, is a danger signal indicating the probable existence of resistance in the population and certainly calls for more extensive investigation, using a complete range of concentrations. To evaluate the results of such tests correctly, the explanation in Annex 15 should be consulted.

5.1 Reporting, processing and storage of data

WHO acts as a depository for information on the resistance of insects of public health importance, in order to be fully informed of the status of resistance of different vector species on a global basis. The results of susceptibility tests are received by the Organization from different workers all over the world and are now analysed by computer. The Committee appreciates the value of this programme for the rapid and detailed statistical analysis of data and also for storage of data for subsequent retrieval.

A review of the material received and processed by WHO shows that the data are not entirely representative ; a small proportion of the workers

employing the standard resistance tests use them very extensively; the majority of the available information concerns mosquitos because of the popularity of the larval test. Computer treatment of some existing adult-test data on anopheline mosquitos would be highly desirable. The Committee is of the opinion that the information being accumulated would be even more valuable if there were clearer definitions of the geographical areas, the vector species, and the particular insecticides, for which information was required, in relation to disease prevalence and epidemiological surveillance.

In a number of cases, the data received were inadequate and no interpretation of the results was given. It is therefore essential that the quality of the test data be improved. The Committee considers that the following types of information are needed, and that the requirements should be carefully explained to workers using the test kits :

(a) Base-line data to provide a complete regression line for susceptible populations in respect of new insecticides. The base-line data can be processed automatically by computer.

(b) Routine checks based on tests at a single dose rate (or exposure time) over as wide an area as possible, to provide an over-all surveillance for the emergence of resistance. The single-dose test results can be interpreted in relation to this analysis.

(c) Where there is evidence of decreased susceptibility, tests should be done over the relevant dosage range, to provide an estimate of the proportion of the population that is resistant. Results obtained from mixed field populations, containing susceptible and resistant individuals, would not be suitable for the routine computer processing.

The Committee recognizes that the existence of physiological resistance is difficult to confirm on test results alone. Departure from normality can be established by statistical analysis ; comparisons should always be made with data obtained under similar conditions and thus it is the field worker who is in the best position to make a preliminary diagnosis of resistance.

Tests of the suspect population over the full dosage range may sometimes provide further evidence, since incipient resistance will often result in flexion and flattening of the regression line (as illustrated in Annex 15) whereas changes in the environment will tend merely to displace the normal regression line, without deflecting it.

6. BIOASSAY OF INSECTICIDES

No change was proposed in the 2 test methods for bioassay of insecticides, described in the thirteenth report of this Committee : these were (a) bioassay of insecticidal deposits on wall surfaces (see Annex 16A) and (b) bioassay of persistent fumigants (see Annex 16B).

7. THE EFFECT OF INSECTICIDES ON THE BEHAVIOUR OF INSECTS

The Committee again directs attention to the importance of mosquito behaviour in relation to the insecticidal control of disease transmission, especially when such behaviour may change during control operations. On this subject, the Committee endorses the views expressed in the thirteenth report of the Committee.

At present 2 types of WHO test method are available to study the insecticide response element in this problem, namely (a) the test for physiological resistance and (b) the test for irritability, particularly to DDT, and both have proved their usefulness. It is recommended therefore that the standard irritability test remain unchanged (Annex 17). The combined use of these tests has revealed that DDT-resistant populations or strains of *Anopheles sacharovi*, *An. stephensis* and *An. culicifacies*, as well as those of *Aedes aegypti* and *C. fatigans*, are less irritable to DDT than normal strains, as would be expected on physiological grounds.

The Committee noted the recent report that changes in flight and escape activity have been induced in *Anopheles atroparvus* by laboratory selection ; although the selecting agent was DDT, the changed flight activity was manifested irrespective of the presence of the insecticide.[1] This finding may explain some of the factors underlying behavioural changes observed in the field, e.g., in certain populations of *An. pseudopunctipennis* in Mexico and of *An. albimanus* in Panama, which the two WHO tests had shown did not involve changes in susceptibility or irritability to DDT. The Committee therefore felt that a third type of test should be developed to measure and analyse flight activity and the escape reaction. The laboratory studies in *An. atroparvus* already described would appear promising in the development of such a test.

It remains to be seen what will be the combined effect of changes in susceptibility, irritability and flight activity caused by residual spraying of insecticides in dwellings. The trap-hut has been recognized as the most useful tool for this purpose, and has been used with great success in the ultimate stages of the WHO Collaborative Programme for Evaluation of New Insecticides, under conditions where naturally occurring mosquitos have free access to the hut. The Committee reconsidered the place of a test based on the excito-repellency test box, which had been put forward as a portable substitute for the trap-hut. The Committee reviewed some recent unpublished results obtained with this box, as well as earlier published work, and agreed that further study of this box was needed, especially to define the best dosage level and type of escape orifice. However, it realized that

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[1] Gerold, J. L. & Laarman, J. J. (1967) *Nature (Lond.)*, **215**, 518-520

this test box cannot provide information on such factors as entry and feeding, which are covered with the trap-hut.

8. RECENT DEVELOPMENTS IN VECTOR CONTROL

During the past 5 years there has been an increase in public concern about the use of pesticides for controlling disease vectors and agricultural pests in many parts of the world. Such concern has resulted in a tightening of the regulations governing the use and labelling of pesticides and the allowable tolerances of insecticide residues on foodstuffs. A further result is that greater emphasis has been placed on the development of non-chemical control agents, and more sparing use of insecticides.

The interest in non-insecticidal measures has stimulated increased budgetary support for research on the ecology and behaviour of arthropods, on a search for biological agents inimical to arthropod vectors, on the development of novel methods for arthropod control (e.g., genetic mechanisms, hormones, pheromones) and on the improvement of techniques for assessing population dynamics. These trends are commendable, since this type of basic knowledge relates not only to the biological and genetical control approaches but also to the use of pesticides.

In investigations on chemical control, emphasis remains on the goal of developing effective measures that can be applied without hazard to man and other non-target organisms. However, the Committee noted growing public concern over the contamination of the environment by DDT despite the fact that this compound remains a major and safe weapon for the control or eradication of various vector-borne diseases (e.g., malaria, onchocerciasis, trypanosomiasis). In its report on the Safe Use of Pesticides in Public Health[1] the WHO Expert Committee on Insecticides stated:

In vector control programmes DDT has been in use for over 20 years. Very large quantities continue to be used and no reports of any harmful effects have been recorded among the thousands of people who use it daily in malaria eradication campaigns. Furthermore, DDT remains the insecticide of choice where the vector is susceptible to its action.

The concern that has been expressed in recent years about contamination of the environment by this very stable and persistent insecticide should not, in the opinion of the Committee, be considered sufficient reason for substituting other insecticides for indoor residual spraying against mosquitos. The safety record of DDT remains outstanding.

The Committee recognized the desirability of using compounds less persistent than DDT when long residual action is not essential to protect the population from arthropod-borne diseases. However, the consensus

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 356.

is that DDT will continue for some time to be a major pesticide in the control of arthropod-borne diseases and it was therefore stressed that the pertinent facts of its role in the field of human health be brought to the attention of appropriate authorities in those countries where DDT is manufactured or used. Such action would provide the authorities with the data and background information necessary to reach an unbiased judgment.

Co-operative efforts among organizations and individuals involved in insecticide evaluation, vector control programmes and wild-life preservation have advanced during the past few years. One example resulting from such co-ordination is the replacement of DDT with methoxychlor for blackfly control in the north-eastern part of the USA. When investigations revealed that DDT was affecting fish reproduction in lakes, other pesticides were evaluated for this purpose, and methoxychlor was shown to be safe for fish propagation and at the same time effective against *Simulium* larvae. The substitution resulted in an increased cost to the programme but the control effort was not adversely affected. The Committee noted, however, that the cost factor could well be a critical one in the use of alternative pesticides in developing countries.

Among the various advances in control technology, the recent use of ultra-low-volume (ULV) aerial sprays against adult mosquitos represents an outstanding development in the methods of combating outbreaks of mosquito-borne diseases. This technique allows the dispersal of certain technical grade insecticides at the extremely low rates of 56 ml – 450 ml per hectare. In an encephalitis outbreak in the USA in 1966, ULV treatment with malathion resulted in almost complete elimination of the infected *Culex* population. In 1967 the same method was used to treat approximately 12 000 km² to prevent mosquito multiplication following a hurricane disaster. The Committee noted the recent studies by WHO to evaluate the feasibility of this technique against *Ae. aegypti* and *Culex fatigans* in Thailand and *Ae. simpsoni* in Ethiopia.

8.1 Chemical control

The Committee believes that control of arthropod vectors and rodent reservoirs of disease must rely for the most part on chemical pesticides for many years to come since it is not realistic to expect that alternative methods will become available in the near future. The tremendous reductions in human mortality and morbidity consequent upon the use of organic insecticides must be recognized. In many parts of the world the public has come to expect that pesticide campaigns would continue to give freedom from arthropod pests and arthropod-borne disease.

Sanitation services have often lagged behind the rapid, unplanned urbanization that has occurred, especially in many of the developing countries; this has resulted in serious increases in the populations of

such vectors as *Culex pipiens fatigans* and *Aedes aegypti*, with consequent increases in the prevalence of the diseases that these species transmit. Until environmental sanitation can be improved sufficiently to control these vectors, programmes based on chemical pesticides will be necessary.

Another factor in the continuing use of pesticides in public health programmes has been the deferment of some insect or disease eradication programmes necessitating the establishment of long-term control programmes, based on pesticides, to keep vector populations at low levels and disease transmission at a minimum. When epidemic outbreaks of arthropod-borne disease occur, insecticides are the method of choice to achieve immediate interruption of transmission, for instance by ULV application of insecticides against mosquitos or the application of dusts against the vectors of plague and epidemic typhus.

As the use of pesticides remains an integral and often dominant part of vector control methods the Committee emphasizes that consideration should be given to ways of making this use more selective and of minimizing its effects on non-target organisms. With greater knowledge of the biology and ecology of vectors, more judicious use can be made of existing pesticides, by applying them at a critical point in the life cycle of the species. In many cases it may only be necessary to reduce the density or longevity of the vector population to a point at which disease transmission no longer occurs, with a concomitant saving of pesticide and money.

The Committee noted the extensive research being carried out in many countries in an effort to develop new, effective and safe pesticides for vector control: particular note was taken of the WHO Programme for the Evaluation and Testing of New Insecticides, now in its eighth year of operation; more than 1400 insecticides have been examined to determine their possible use against arthropods of public health importance. This international collaborative research programme involving 9 co-operating laboratories and a number of WHO field-research units was initiated with the object of taking the fullest possible advantage of the great resources and research potential of the chemical industry in the development of new vector control measures, especially new pesticides.

8.2 Biological control

The Committee reviewed many of the advances that have been made during the past 5 years in regard to biological control agents. The review was based on contributions and suggestions received from a number of workers active in this field.

8.2.1 *Predators*

The mosquito fish, *Gambusia affinis*, is still the most effective agent for the control of mosquito larvae where it can be used. Both this species and

the guppy, *Lebistes reticulatus*, have received much attention but maximum use is not being made of them. *Gambusia* has been effectively used in Iran during recent years, and probably there are many situations elsewhere throughout the world where it could help in the control of mosquito larvae. The existence of insecticide-resistant strains of *Gambusia* could perhaps be exploited (see section 1.4). Note was taken of the investigations being made at present in Rangoon on the value of *Poecilia*.

Several species of " annual " fish (cyprinodontids) have been suggested as promising candidates for mosquito control in alternately flooded and dry habitats in tropical and mild temperate climates. However, it was urged that the introduction of any kind of fish should not be contemplated without thorough prior ecological studies of the habitats. Moreover, where introductions have been made, it is essential that the effects on the flora and fauna be carefully assessed.

Invertebrate predators may help to reduce vector populations and when insecticides are applied every effort should be made to conserve them.

Much interest was expressed in the possible introduction of Japanese weasels into a Pacific island for the control of rats. It is emphasized that a full study of the island and its fauna should be undertaken before weasels are introduced and that an assessment of possible harmful effects of the weasels should also be made.

8.2.2 *Pathogens and parasites*

The Committee examined the characteristics that would be desirable in pathogens and parasites considered for the biological control of vectors and concluded that they should show as many as possible of the following :

(*a*) Interference with some part of the host's life cycle, such as (*i*) stopping egg production through destruction of reproductive tissues, (*ii*) depriving the adult of all fat or protein, thereby inhibiting the production of eggs, (*iii*) causing the death of all immature stages, (*iv*) metabolizing so much of the immature host's fat that the larva enters the pupal stage with insufficient reserves and therefore dies as a pupa or as a newly emerged adult, and (*v*) shortening the life-span so as to preclude the full development of the vector and the transmission of the pathogens and parasites infective to man.

(*b*) A high degree of infectivity for the host, including an ability to penetrate the host's natural protective barriers.

(*c*) The ability to reproduce within the host, either sexually or asexually.

(*d*) The production of large numbers of resistant spores, or a similar dormant stage, adapted to withstand environmental stress.

(*e*) The ability to colonize alternative hosts when numbers of the vector are substantially reduced.

Among the various agents considered, *Coelomomyces* fungi seemed to offer the most promise. Recent work in the USA on infections of *An. quadrimaculatus* by *C. punctatus* has shown that high infection rates under field conditions can be achieved, and progress is being made there and elsewhere with research on *C. indicus* and *C. anophelesicus* which are parasitic on several important anopheline vectors. Progress has also been made in the study of *Coelomycidium simulii* in relation to blackflies.

In view of the promise shown by work on this group of pathogens, the Committee considers that WHO should encourage further field studies, and in due course field trials, when the remaining technical and scientific problems have been resolved.

The other potential agents that were discussed included fungi other than *Coelomomyces*, and extracts of entomotoxigenic strains of algae, bacilli, protozoa and mermithid nematodes. The general opinion of the Committee was that none of these agents had been sufficiently well studied for a valid assessment to be given on their possible uses, and that active support of research on their further development should continue.

8.2.3 *General considerations*

The Committee strongly supported WHO's programme for the collection of pathogens and parasites of arthropods of public health importance. This programme has been designed specifically to find agents with the characteristics listed above that might be used for biological control.

It was emphasized that all proposals for experiments or field trials involving biological control agents, should take account of the possible effects of the introduced agents on non-target organisms, including man. The possible hazards associated with the manipulation of biological agents were outlined in a previous report,[1] and those recommendations were endorsed.

Finally the Committee agreed that the use of biological control methods should not be viewed as an alternative to other control methods but rather as an additional approach. In the planning of integrated control programmes full consideration should be given to the possible role of biological control agents.

8.3 Genetic control

Genetic control has been defined as " the use of any condition or treatment that can reduce the reproductive potential of noxious forms by altering or replacing the hereditary material ".[2]

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 356.
[2] *Wld Hlth Org. techn. Rep. Ser.*, 1964, No. 268.

The aspect of genetic control that has received most attention involves the release of males sterilized by gamma-irradiation or chemosterilants. In another approach, insects have been induced to feed on baits or to enter traps treated with chemosterilants. The basic principle is that the sterilized males seek out and mate with the wild females in the natural populations, thus nullifying their reproductive potential.

In addition to the traditional sterile-male technique, it may be possible to use other genetic mechanisms that result in either complete or high levels of male sterility or in distorted sex ratios for controlling natural populations of arthropods. Since both radiation and chemosterilants may reduce mating competitiveness in sterilized individuals, techniques using naturally occurring sterility mechanisms, such as cytoplasmic incompatibility and hybrid sterility may be promising.

In one recent experiment, for example, virtual elimination of *Culex fatigans* was obtained through releases of incompatible males in Okpo, an isolated village near Rangoon, Burma. The field trial was undertaken under WHO sponsorship and complete control was brought about in approximately 3 months or 5–6 generations. This successful field experiment indicates that this species of mosquito might be amenable to genetic control measures.

Another example concerns the members of the *Anopheles gambiae* complex, which consists of at least 5 sibling species. Crosses between any 2 of these 5 species result in hybrid male sterility though the degree of this differs with different crosses. Furthermore, crosses involving species A and B males with females of *A. melas*, *A. merus*, or species C, usually produce predominantly male offspring (sometimes 100 %); and because of their hybrid nature, the sterile males show increased competitive mating ability. Thus, this method may provide several important advantages over mutagen-induced sterility. Laboratory experiments with these hybrid males have proved very encouraging. WHO is currently undertaking a field experiment in the village of Pala near Bobo-Dioulasso, Upper Volta.

Release of males with translocations offers a method of introducing a sterility mechanism into the population. In *Aedes aegypti*, for example, a radiation-induced translocation, which is transmitted from males to their male progeny and causes sterility, represents a possible control method, particularly if heterosis is added to the males by appropriate outbreeding before release.

Males in certain strains of *Ae. aegypti* bearing a sex-distorting allele could be released and thus introduce a mechanism for higher production of males and lower production of females in the population. The potential of this method has been demonstrated in *Ae. aegypti* in population cage experiments in which varying proportions of " male-producing males ", " normal males " and so-called " sensitive females " were used.

Experiments are now in progress on the control of *Glossina morsitans* on an island in Lake Kariba by the release of chemosterilized males, and the control of *Ornithodoros tholozani* in caves in Israel by the release of males sterilized by radiation. An extensive study on the feasibility of village or larger scale trials for the control of *Culex fatigans* or *Ae. aegypti* by the release of chemosterilized, radiosterilized or incompatible males is planned for the near future under the auspices of WHO.

Although recognizing the difficulties inherent in the development of the technique, the Committee strongly supports the continuation of investigations into the genetic control of species of public health importance. In addition to the possibility of developing an improved control method, such studies will yield data on population dynamics of the vector that will be of great value to control methods using biological agents or pesticides.

9. ECOLOGY IN VECTOR CONTROL

The Committee stressed the importance of ecological studies in all programmes of vector control and reviewed the parameters that require measurement. It agreed that different techniques are required for the study of vectors with different life-cycles and that in many cases satisfactory techniques are not available. Strong support is therefore expressed for all experimental studies designed to meet this need.

A number of topics that are common to all vector studies and that require attention were discussed. These are considered under 7 headings.

9.1 Distribution

Mapping of the distribution of a vector should be a primary consideration. This implies the recognition and definition of different biotopes within the range of the species, and may require the utilization of special survey maps and charts, such as those prepared from topographical, hydrological, geobotanical and geological surveys. Such detailed distribution maps are required before the start of control operations, and they are essential for an understanding of the factors influencing the abundance of vector species. Besides providing one of the base-lines for a control programme, they may be used to pinpoint the areas where control proves difficult and allow correlations to be made between such areas and environmental or climatological features.

Note was taken of the progress being made in the USSR on the preparation of such distribution maps and also of the WHO programme to map the distribution of major vectors on a world-wide basis, using modern data-processing techniques.

9.2 Life-table analysis

Carefully constructed life-tables are required for all vector species. In the case of mosquitos, a description of life-tables and their analysis was given by a WHO Scientific Group.[1]

These tables would allow an estimate to be made of the duration of each stage in the life-cycle, would provide quantitative data on the probability of survival of an individual through each stage, and would be helpful in making a decision on the stage of the life-cycle at which control measures might be applied most effectively and economically. In addition, an analysis of the factors causing natural mortality might suggest new methods of control.

New and better methods for the estimation of numbers of vectors are required for an understanding of population dynamics. At present one may attempt to count the total population of vectors by isolating the area and collecting every single individual, as in the case of mites, ticks, fleas and reduviid bugs. (This technique has also been applied to rodents in the USSR.) An alternative method is the mark-release-recapture technique which is the most suitable method of providing estimates of absolute population size of some arthropods. Where these techniques cannot be applied, methods of providing data on relative changes in populations are available. Biting rates, resting collections, and artificial traps all contribute valuable information. Although such data on relative numbers are never as informative as absolute population size estimates, they may be sufficient for most purposes.

Mortality rates may be directly or indirectly related to population size and the mark-release-recapture technique can provide estimates of this parameter. However, there may be more convenient and more efficient techniques. In the case of mosquitos an estimate of daily mortality rate can be obtained from the parous rate provided there are data on the duration of the first and subsequent gonotrophic cycles. Some species of mosquito and tsetse fly can be individually age-graded with respect to the number of gonotrophic cycles through which they have passed, and invaluable information on the age structure of a population can be derived in this way.

Equally important is the measurement of natality in a population. The best data will be collected by direct observation in the field. When this is not possible, fecundity data may be obtained by an experimental assessment in the laboratory based on field-collected material.

All the data gathered for life-table analysis provide essential information required for evaluation of control measures. Since the impact of control measures on different stages of the life-cycle may vary, measurement of as many parameters as possible is recommended.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 368.

9.3 Population fluctuations

Vector populations commonly show seasonal or cyclical fluctuations and the recognition and measurement of these may suggest the time when control measures can be applied most effectively. Particular attention should be given to those populations that are regularly reduced to a very low level as a result of climatic changes, and control measures should be planned to take maximum advantage of the natural decline.

9.4 Dispersal

Although terms have been devised for different degrees of dispersal and correlations between the physiology of arthropods and their movement can be made, the definition of movement in quantitative terms is far from easy. Ideally, it would be desirable to define the probability of an individual moving, or being carried, different distances after different intervals of time, but this is probably not possible for most vectors. Many experiments have been made on flight range by marking insects, releasing them together from a central point, and recapturing them at distances from the release point. In other experiments it has been possible to make collections in the resting place of a species and individuals have been marked and returned with less disturbance than occurs in mass release. Even more useful is the technique in which dyes or radioisotopes are introduced into the blood of host animals at a particular site and then the arthropods that have ingested the marker are identified in subsequent collections. Besides providing information on dispersal, the latter technique can be modified for studies of host preference of vectors and their frequency of feeding.

Some information on rates of dispersal might be provided by the introduction of marker genes into natural populations. If the latter could be linked with some factor conferring a selective advantage, so much the better. Such a study might contribute towards an understanding of the rate of spread of genes conferring insecticide resistance.

9.5 Behaviour

The different stages in the life-cycle of a vector often have different habits. A study of these habits is therefore necessary in order to judge the stage at which control measures could be directed most profitably. The control operations may well affect the vector's feeding and resting places and perhaps also the host preference. If some individuals of the vector population behave differently from the rest and escape the lethal effect of the pesticide, then the continued use of the pesticide will favour this section of the population.

To detect and measure such possible changes requires quantitative data obtained before control measures are started. The effect on disease

transmission will be determined in part by the changes, if any, in the choice of host.

9.6 Population surviving control pressure

Small vector populations that survive control measures in isolated foci deserve special attention. It has been observed that such populations may differ significantly in their dispersal and other behaviour patterns from the pre-control population; individuals from these populations often move much greater distances than normal. Of particular importance is the rate of reproduction of residual populations. This rate is generally higher than normal, and this may result in rapid reinvasion of the vector-free areas.

The detection of these changes requires a continuing study of all facets of the ecology of such populations. This point is emphasized since any change in the structure or behaviour of populations might require a corresponding change in the vector control techniques or in the strategy of control operations.

9.7 Mathematical models of populations

The mathematical study of theoretical and natural populations is of considerable interest. A theoretical model can be devised to describe population dynamics in which the parameters are given values that would maintain the population in a stable condition. The model can then be used to estimate the results of altering one or other of the parameters, and population fluctuations can be simulated. The value of these techniques has been more fully outlined elsewhere.[1]

It was recognized that adequate data from natural vector populations were not always available for the full exploitation of computer simulation models. This emphasizes the need for additional ecological studies in all fields of vector control.

10. CHEMICAL METHODS
FOR THE CONTROL OF VECTORS AND PESTS
OF PUBLIC HEALTH IMPORTANCE

In the light of recent developments the Committee reviewed Annex 17 of the thirteenth report of the WHO Expert Committee on Insecticides[2] in which

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 398.

[2] The original version and the first and second revisions appeared, respectively, in the eighth, tenth and thirteenth reports of WHO Expert Committee on Insecticides (*Wld Hlth Org. techn. Rep. Ser.*, 1958, No. 153 ; 1960, No. 191 ; 1963, No. 265).

techniques for use against arthropod vectors and rodents are described. This review was based on information that had been considered by 49 scientists from different parts of the world.

The review resulted in the recommendations being revised and the new recommendations are given in Annex 18 to the present report under the new title " Chemical methods for the control of vectors and pests of public health importance ". The revised recommendations describe the various commercially marketed pesticides and methods of control that have proved effective under field conditions in one or more geographical area, The decision to apply any compound or to use a specific method rests with the agency or individual concerned.

An introductory section discusses the over-all role of pesticides in vector control, the factors influencing their efficacy, insecticide resistance, and the need for efficient technical and managerial supervision. Later sections concern such matters as repellants, pesticide formulation, application rates, conversion factors, and the safe use of pesticides. The arthropods and rodents are considered on the basis of the major groups (i.e., mosquitos, flies, etc.) with each of these categories subdivided into species or genera. Thus mosquitos are considered under 5 categories, i.e., *Anopheles*, *Aedes aegypti* and other domestic *Aedes*, salt-marsh *Aedes* and other flood-water *Aedes*, *Culex* and *Mansonia*. Under each category the information is presented on the bases of area treated, insecticide, application procedure, treatment cycle and precautions.

In view of rapid developments in the field of vector control, it is recommended that further revisions of the document be made every 2–3 years. The new arrangement will facilitate such revisions and will ensure that public health workers are kept abreast of the most recent advances in vector-borne disease control.

Annex 1A

INSTRUCTIONS FOR DETERMINING
THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOS
TO ORGANOCHLORINE INSECTICIDES

1. Introduction

Purpose and limitations of the test

This method measures the levels of susceptibility of a population of adult mosquitos to a given organochlorine insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. In many cases, the first indication of incipient resistance may be an increase in the number of mosquitos in sprayed houses. Nevertheless, it is desirable to detect resistance as early as possible, before it is widely established in the insect population. For this purpose, it is necessary (*a*) to establish the susceptibility levels of normal populations of mosquitos of the species concerned, and (*b*) to make subsequent routine checks of the susceptibility levels at periodic intervals. The test is specifically devised to detect physiological resistance. However, the effect of resistance on the transmission of disease and the advisability of continuing to use the same insecticide in a given area cannot be determined by resistance tests alone ; general epidemiological appraisal and entomological assessment by other means are necessary. The resistance test is not designed to evaluate the effectiveness of insecticides in the field ; for this purpose other techniques must be used.

Establishing the base-line

Batches of mosquitos are exposed to different concentrations of insecticide and the mortality at each level is determined. It is suggested that a preliminary test be made at each concentration in the complete range provided, using the standard exposure of 1 hour. This will indicate the general level of susceptibility ; further tests should then be made on this basis. A series of 4 concentrations should be chosen, some of which will give partial mortalities (i.e., at least one of them should give 100% mortality and one should give less than 50% mortality). Tests at these concentrations should be repeated 4 times with samples from the same population of mosquitos. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable. Even if a true base-line (i.e., for an untreated population) cannot be established owing to previous applications of insecticides for

any purpose, the susceptibility level of the vector species should be determined immediately.

Subsequent routine checks

A concentration double the lowest concentration that has consistently given 100% mortality in the 4 successive tests is chosen for the routine checks. These should be made periodically with 5 replicates ; when mosquitos are scarce, a minimum of 2 replicates is permissible. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on 3 successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include 4 tests at each of the concentrations used in establishing the original base-line. To validate the results, the investigator must make every effort to collect sufficient specimens to provide at least 15 mosquitos per tube. It should be emphasized that the confirmation of resistance in its early stages is difficult when the genes responsible are recessive (as has been noted in certain cases of DDT resistance). In such cases it may be helpful to keep survivors from the exposure at the check concentration and rear their progeny. If the progeny give a distinctly lower level of susceptibility than the parents, this is a strong indication of resistance (however, this does not always occur with a recessive type of resistance).

Condition of mosquitos

Females should be used exclusively. It is recommended that the mosquitos selected for test be females that have recently fed and show the presence of a blood meal. If mosquitos are scarce, it is permissible to use a mixture of fed and unfed females, provided the proportion of each is recorded. Mosquitos may be collected from sprayed and unsprayed premises in the zone, but their source should be reported on the form provided. In instances where it is not possible to collect a sufficient number of adult mosquitos for testing, these specimens may sometimes be provided by collecting the immature stages and rearing the adult therefrom. In some circumstances females without a blood meal may be used exclusively, e.g., those recently emerged from a collection of larvae.

Conditions of test

The experiments should be carried out indoors, if possible, in buildings free from insecticidal contamination and extremes of temperature, humidity, illumination, and wind. Where possible, subsequent comparison tests should be made under similar conditions of temperature and humidity. Transport of insects to a base laboratory often results in mortality from causes other than the insecticide ; this will be evident as high mortality in the controls.

2. Composition of test kit

(a) 20 plastic tubes, 125 mm in length and 44 mm in diameter ; 8 of these (with red dot) are used for exposing the mosquitos to the insecticide ; 2 (with green dot) are used for the control exposure without insecticide ; 10 (with green dot) are used as holding-tubes for pre-test sorting and post-exposure observation. Each tube is fitted at one end with a 16-mesh screen. In order to identify the concentrations used with them, the red exposure tubes should be numbered 1 to 8, the green control exposure tubes 9 and 10, and the holding-tubes 1a to 10a.

(b) 10 slide-units, each with a screw-cap on either side and provided with a 20-mm filling hole.

(c) 5 packages of papers impregnated with DDT (p,p'-isomer) in mineral oil (Risella 17, Shell) at concentrations of 0.25%, 0.5%, 1.0%, 2.0%, and 4.0%, respectively, and 1 package treated with oil only ; 7 packages of papers impregnated with dieldrin (purified HEOD) in mineral oil at concentrations of 0.05%, 0.1 %, 0.2%, 0.4%, 0.8%, 1.6%, and 4.0%, and 1 package treated with oil only. (Each package contains 8 papers.)

(d) Sheets of clean paper (12×15 cm) for lining the holding-tubes.

(e) 20 spring-wire clips to hold the papers in position against the walls of the tubes. The 12 silver clips should be used only for the holding-tubes and the control exposure tubes ; the 8 copper clips should be used only for the insecticide exposure tubes.

(f) 2 glass aspirator tubes, 12 mm in internal diameter, together with 60 cm of tubing.

(g) 1 roll of self-adhesive plastic tape.

(h) Instruction sheets, a set of report forms, and 3 sheets of log-probability paper for plotting regression lines.

3. Procedure

(a) First, a preliminary test is performed with the complete range of concentrations. Into each of the holding-tubes (6 for DDT, 7 for dieldrin), insert a piece of clean white paper rolled into a cylinder to line the wall and fasten it in position with a spring-wire clip (silver). Attach the slides to the tubes.

(b) Collect approximately 200 female mosquitos with the aspirator provided (Fig. 1, A). Damage resulting from careless handling of mosquitos during collecting may produce misleadingly high mortalities. Mosquitos should be collected in lots of not more than 10 (Fig. 1, B) and gently transferred to the holding-tubes through the filling-hole in each slide (Fig. 1, C) to give 15 to 25 per tube. Any departure from these figures may impair the reliability of the results.

FIG. 1.   METHOD FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOS TO ORGANOCHLORINE INSECTICIDES
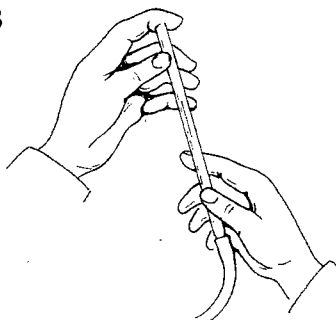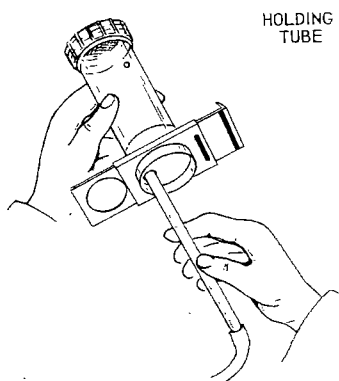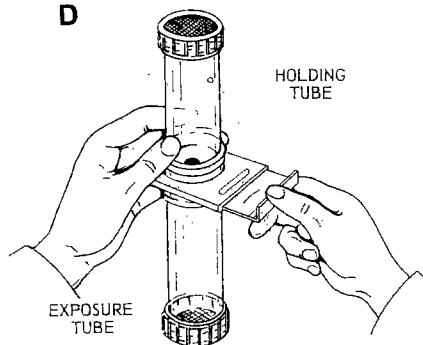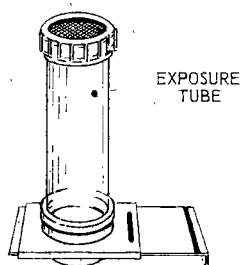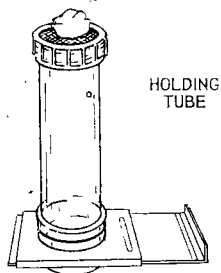
(c) A pre-test holding period may be necessary to guard against including damaged specimens in the test. For this purpose, the holding-tubes are set upright, screen end up, for 1 hour or more. At the end of this time, the damaged insects are removed.

(d) Into each of the exposure tubes (6 for DDT, 7 for dieldrin) introduce a sheet of impregnated paper, rolled into a cylinder to line the wall, and fastened into position with an appropriate spring-wire clip. One paper impregnated with each of the concentrations of insecticide provided should be used, and one control paper impregnated with oil alone.

(e) Introduce the mosquitos into the exposure tube by attaching it to the vacant screw-top in the slide (Fig. 1, D). The slide should be pulled out to a point beyond the filling-hole so that no part of it occludes the tube openings ; the mosquitos are then blown gently down into the exposure tube. (If necessary, the small safety knob on the slide may be filed down to facilitate this operation.) Close the slide. Detach the holding-tube and set it aside.

(f) Leave the exposure tubes standing upright with screen end up for 1 hour (Fig. 1, E) under conditions of moderate, diffuse illumination.

(g) At the end of the exposure period, transfer the mosquitos to the holding-tubes by reversing procedure (e). When some mosquitos have been knocked down in the course of an exposure, the exposure tubes should be held horizontally and tapped to dislodge the insects from the slide before the latter is withdrawn. Attach the holding-tube, open the slide, and gently blow the mosquitos into the holding-tube ; close the slide and remove the exposure tube. Then set the holding-tube so that it stands on the slide and place a pad of wet cotton-wool on the screen (Fig. 1, F). Cardboard cartons or cups or other suitable containers may be used instead of the holding-tubes, provided that they are used consistently.

(h) Keep the holding-tubes for 24 hours in a secluded, shaded place, where the temperature does not exceed 30°C. Wherever feasible, the maximum and minimum temperature of the room during the 24 hours should be recorded. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. If conditions are very hot and dry, a moist chamber may be prepared by suspending damp towelling in a container.

(i) Mortality counts are made after 24 hours. Remove the dead mosquitos by gently detaching the slide and cautiously moving the tube aside. Affected specimens that are unable to walk are to be counted as dead. As an aid to counting the living specimens, they may be stunned by a sharp jerk of the tube or stupefied by chloroform or ether. (The anaesthetics should not be allowed to come into direct contact with the plastic tube and

screw cap, which are soluble in these compounds.) The results should be recorded on the forms provided. Copies of completed forms should be distributed in accordance with the instructions on page 54.

(*j*) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of 4 concentrations. Four replicates, including the preliminary test where appropriate, should be performed at each of these chosen concentrations. The 20 tubes provided in the kit are sufficient for 1 series of 2 replicates at each of the 4 chosen concentrations, together with 2 controls.

(*k*) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(*l*) When the test has been repeated 4 times with the same population of mosquitos, adequate data should be available for constructing a base-line of susceptibility as described below (section 5).

4. General remarks

(*a*) Each impregnated paper may be used up to 20 times, and up to 3 weeks after removal from the package, provided all possible precautions are taken against evaporation of the oil. To this end, the papers should be left in the tubes, with the open end well wrapped, and placed in the kit box, which in turn should be kept in a cool place. No paper should be used more than 3 weeks after removal from the package.

(*b*) After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as too low a temperature may cause crystallization in the higher insecticidal concentrations. Prolonged storage at high temperatures should be avoided. Papers should not be used after the expiry date shown on the box. Chlorinated hydrocarbon papers have a useful life of 5 years from the date of impregnation, provided the packages are kept sealed at all times.

(*c*) If the species of mosquito concerned is exceptionally insensitive, the exposure period may be increased to 2, 4, or 8 hours. In all cases, the 1-hour exposure period should be used first and the result recorded. To decide on a suitable exposure period, conduct tests with the highest concentration available until complete mortality is obtained. The complete range of concentration is then employed to give a concentration mortality regression line.

(*d*) The accompanying table gives specimen results obtained with this method for susceptible strains of a number of mosquito species. These results indicate the approximate levels of susceptibility expected.

SPECIMEN RESULTS OBTAINED WITH WHO ADULT TEST METHOD

Insecticide	Species	LC_{50} (%)	LC_{95-100} (%)	Ref.
DDT	Anopheles gambiae	0.95	4	1
	An. atroparvus	1.0	>3	2
	An. maculatus	0.4	1.8	2
	An. quadrimaculatus	0.65	4	3
	An. albimanus	0.5	2	4
	An. stephensi	0.5	2	5
	An. fluviatilis	0.3	0.64	6
	Aedes aegypti	1.1	4.0	7
	Ae. caspius	0.8	2.9	8
	Ae. albopictus	1.3	3	2
	Culex molestus	1.6	3	2
	C. fatigans	1.9	—	9
		—	4 (4 h)	10
	C. gelidus	0.3	1.8	2
	Culiseta fumipennis	0.58	1.5	11
	Mansonia longipalpis	0.8	1.8	2
dieldrin	Anopheles gambiae	0.05	0.2	12
	An. atroparvus	0.14	0.3	2
	An. albimanus	<0.1	0.2	4
	An. quadrimaculatus	0.14	0.4	3
	Aedes aegypti	0.13	0.4	2
	Ae. albopictus	0.23	0.6	2
	Culex fatigans	0.46	1.8	2
		0.4	4.0	10
	Mansonia longipalpis	0.14	0.18	2

[1] Hamon, J. & Mouchet, J. (1961) Med. trop. (Madr.), **21**, 565.
[2] Wharton, H. (1955) Bull. ent. Res., **46**, 301.
[3] Davidson, G. (1963a) Bull. Wld Hlth Org., **29**, 177.
[4] Davidson, G. (1963b) Bull. Wld Hlth Org., **28**, 25.
[5] Davidson, G. & Jackson, E. (1961) Bull. Wld Hlth Org., **25**, 209.
[6] Eshgy, N. (1967) Unpublished report to WHO.
[7] Busvine, J. R. & Harrison, M. (1953) Bull. ent. Res., **44**, 729.
[8] Phillips, R. J. (1964a) Unpublished report to WHO.
[9] Smith, A. (1958) Indian J. Malar., **12**, 341.
[10] Davidson, G. (1964) Ann. trop. Med. Parasit., **58**, 180.
[11] Phillips, R. J. (1964b) Unpublished report to WHO.
[12] Davidson, G. (1956) Nature (Lond.), **178**, 863.

5. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-

probability paper provided. The regression line may be fitted by eye, and the LC_{50} or LC_{95} [1] read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC_{50}, the worker is referred to those described by Swaroop [2] and Litchfield & Wilcoxon.[3]

(b) If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

$$\frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

(c) The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per tube (25) decreases the effect of individual differences in response. Regression lines based on similar numbers of specimens are more reliable.

6. Interpretation of results

See Annex 15: *Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides.*

7. Distribution of reports

It is of considerable importance to WHO to receive copies of results obtained from the use of this test kit. It is therefore requested that copies of all reports be sent to the following addresses:

For anopheline species:

1. World Health Organization, Division of Malaria Eradication, 1211 Geneva, Switzerland;

2. The appropriate WHO Regional Office; [4] and

3. Project Headquarters.

The 4th copy should be retained by the investigator.

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[1] The LC_{50} and LC_{95} represent, respectively, the insecticide concentrations at which 50% and 95% of the specimens are killed. LC_{50} values for various anophelines will be found in mimeographed documents WHO/Mal/224; WHO/Insecticides/95-WHO/Mal/242; and WHO/Insecticides/100.

[2] Swaroop, S. (1966) *Statistical methods in malaria eradication*, Geneva (*World Health Organization : Monograph Series*, No. 51).

[3] Litchfield, J. T., jr & Wilcoxon, F. (1949) *J. Pharmacol. exp. Ther.*, **96**, 99.

[4] See footnote on next page.

For non-anopheline species :

1. World Health Organization, Vector Biology and Control, 1211
   Geneva, Switzerland ; and

2. The appropriate WHO Regional Office.[1]

The 3rd and 4th copies should be retained by the Investigator.

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[1] Addresses of WHO Regional Offices are as follows :

World Health Organization, Regional Office for Africa, P.O. Box No. 6, Brazzaville, People's Republic of the Congo.

World Health Organization, Regional Office for the Eastern Mediterranean, P.O. Box 1517, Alexandria, United Arab Republic.

World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India.

World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 525, 23rd Street, N. W., Washington, D. C., 20037, USA.

World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark.

World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, Manila, Philippines.

Specimen Report Form

WHO TEST FOR INSECTICIDE RESISTANCE IN ADULT MOSQUITOS

Date: ....

Insecticide : DDT/dieldrin/other (specify) [1] .... Province : .... Species : .... Locality : ....

Country : ....

History of insecticide use (Including agriculture) : ....

Condition of mosquitos : blood-fed/gravid/unfed/sugar-fed/males [1] Where collected : shelters sprayed/unsprayed/outdoors/biting/bred out [1]

Type of test on population : first time/routine check/complete retest [1] Exposure period (minutes) : ....

Tests	Preliminary				Replicate 1				Replicate 2				Replicate 3				Totals (for comparable tests only)			
Date of test																				
Temperature during exposure period (°C)																				
Humidity during exposure period (%)																				
Temperature during 24-h holding period (°C)	Max. Min.				Max. Min.				Max. Min.				Max. Min.							
Insecticide concentration (%)	Dead	Total	Mort. (%) corr.[2]		Dead	Total	Mort. (%) corr.[2]		Dead	Total	Mort. (%) corr.[2]		Dead	Total	Mort. (%) corr.[2]		Dead	Total	Mort. (%) corr.[2]	
....																				
....																				
....																				
Control (oil alone)																				

[1] Cross out what does not apply. [2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks : ....

Interpretation of results : ....

Investigator : Name and postal address : .... Signature : ....

One copy of the completed form should be sent to : (for anopheline species) : World Health Organization, Division of Malaria Eradication, 1211 Geneva, Switzerland ; or to (for non-anopheline species) : World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland. A second copy should be sent to the appropriate WHO Regional Office.

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOS TO ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

1. Introduction

It will be found that this method for detecting and measuring resistance is somewhat different from the method used previously, in that susceptibility levels are now assessed by mortalities produced by different exposure times to a standard concentration (rather than different concentrations at a standard 1-hour exposure).

Purpose and limitations of the test

This method measures the levels of susceptibility of a population of adult mosquitos to a given organophosphorus or carbamate insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. In many cases, the first indication of incipient resistance may be an increase in the number of mosquitos in sprayed houses. Nevertheless, it is desirable to detect resistance as early as possible, before it is widely established in the insect population. For this purpose, it is necessary (a) to establish the susceptibility levels of normal populations of mosquitos of the species concerned, and (b) to make subsequent routine checks of the susceptibility levels at periodic intervals. The test is specifically devised to detect physiological resistance. However, the effect of resistance on the transmission of disease and the advisability of continuing to use the same insecticide in a given area cannot be determined by resistance tests alone ; general epidemiological appraisal and entomological assessment by other means are necessary. The resistance test is not designed to evaluate the effectiveness of insecticides in the field ; for this purpose other techniques must be used.

Establishing the base-line

Batches of mosquitos are exposed to standard impregnated papers for different exposure periods and are subsequently held for 24 hours before mortality determination. Susceptibility levels are assessed from the relations between exposure and mortality. Preliminary tests are carried out with a

wide range of exposure periods, at logarithmic intervals, as follows : ½, 1, 2, and 4 hours. A suitable range of 4 exposure times is chosen, such that the longest exposure gives 100% mortality and the shortest gives less than 50% mortality. Tests at these exposure times should be repeated 4 times with samples from the same population of mosquitos. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable. Even if a true base-line (i.e., for an untreated population) cannot be established owing to previous applications of insecticides for any purpose, the susceptibility level of the vector species should be determined immediately. The exposure time for the control must be the longest exposure time used with insecticide-impregnated paper.

Subsequent routine checks

An exposure time double the shortest exposure that has consistently given 100% mortality in the 4 successive tests is chosen for the routine checks. These should be made periodically with 5 replicates ; when mosquitos are scarce, a minimum of 2 replicates is permissible. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on 3 successive occasions) constitutes a signal calling for further investigations, which should include 4 tests at each of the exposure times used in establishing the original base-line. To validate the results, the investigator must make every effort to collect sufficient specimens for providing at least 15 mosquitos per tube.

Condition of mosquitos

Females should be used exclusively. It is recommended that the mosquitos selected for testing be females that have recently fed and show the presence of a blood meal. If mosquitos are scarce, it is permissible to use a mixture of fed and unfed females, provided the proportion of each is recorded. Mosquitos may be collected from sprayed and unsprayed premises in the zone, but their source should be reported on the form provided. When it is not possible to collect a sufficient number of adult mosquitos for testing, specimens may sometimes be provided by collecting the immature stages and rearing the adults therefrom. In some circumstances females without a blood meal may be used exclusively—e.g., those recently emerged from a collection of larvae.

Conditions of test

The experiments should be done indoors, if possible, in buildings free from insecticidal contamination and extremes of temperature, humidity,

illumination, and wind. Where possible, subsequent comparison tests should be made under similar conditions of temperature and humidity. Transport of insects to a base laboratory often results in mortality from causes other than the insecticide ; this will be evident as high mortality in the controls.

2. Composition of test kit

(a) 20 plastic tubes, 125 mm in length and 44 mm in diameter : 8 of these (with red dot) are used for exposing the mosquitos to the insecticide ; 2 (with green dot) are used for the control exposure without insecticide ; 10 (with green dot) are used as holding-tubes for pre-test sorting and post-exposure observation. Each tube is fitted at one end with a 16-mesh screen.

In order to identify the exposure time used with them, the red exposure tubes should be numbered 1 to 8, the green control exposure tubes 9 and 10, and the holding-tubes 1a to 10a.

(b) 10 slide-units, each with a screw-cap on either side and provided with a 20-mm filling hole.

(c) Packages of papers impregnated with insecticides are available on request as follows : malathion, 0.5%, 5% ; fenthion, 0.25%, 2.5% ; fenitrothion, 0.1%, 1.0% ; OMS-33, 0.01%, 0.1%. Also, 2 packages treated with oil only. Of the 2 concentrations available, the lower would normally be suitable for initial tests with susceptible populations.

(d) Sheets of clean paper (12×15 cm) for lining the holding-tubes.

(e) 20 spring-wire clips to hold the papers in position against the walls of the tubes. The 12 silver clips should be used only for the holding-tubes and the control exposure tubes ; the 8 copper clips should be used only for the insecticide exposure tubes.

(f) 2 glass aspirator tubes, 12 mm in internal diameter, together with 60 cm of tubing.

(g) 1 roll of self-adhesive plastic tape.

(h) Instruction sheets, a set of report forms, and 3 sheets of log-probability paper for plotting regression lines.

3. Procedure

(a) First, preliminary tests are undertaken to select suitable exposure periods, using the lower of the 2 concentrations supplied. The exposures should include a period of 1 hour and should form a logarithmic series— e.g., ½, 1, 2, and 4 hours. It may be necessary to extend the series in one

or both directions, in order to include exposures that give (1) 100 % mortality and (2) a mortality less than 50%.

(b) Collect approximately 200 female mosquitos with the aspirator provided (Fig. 1, A). Damage resulting from careless handling of mosquitos during collecting may produce misleadingly high mortalities. Mosquitos should be collected in lots of not more than 10 (Fig. 1, B) and gently transferred to the holding-tubes through the filling-hole in each slide (Fig. 1, C) to give 15–25 per tube. Any departure from these figures may impair the reliability of the results.

(c) A pre-test holding period may be necessary to prevent the inclusion of damaged specimens in the test. For this purpose, the holding-tubes are set upright, screen end up, for 1 hour or more. At the end of this time, the damaged insects are removed.

(d) Into each of the exposure tubes introduce a sheet of impregnated paper, rolled into a cylinder to line the wall, and fastened into position with an appropriate spring-wire clip. Sufficient tubes should be used to cover the desired range of exposure periods.

(e) Introduce the mosquitos into the exposure tube by attaching it to the vacant screw-top in the slide (Fig. 1, D). The slide should be pulled out to a point beyond the filling-hole so that no part of it occludes the tube openings ; the mosquitos are then blown gently down into the exposure tube. (If necessary, the small safety knob on the slide may be filed down to facilitate this operation.) Close the slide. Detach the holding-tube and set it aside.

(f) Leave the exposure tubes standing upright, screen end up, for the exposure periods (Fig. 1, E) under conditions of moderate, diffuse illumination.

(g) At the end of each exposure period, transfer the mosquitos to the holding-tubes by reversing procedure (e). When some mosquitos have been knocked down in the course of an exposure, the exposure tubes should be held horizontally and tapped to dislodge the insects from the slide before the latter is withdrawn. Attach the holding-tube, open the slide, and gently blow the mosquitos into the holding-tube ; close the slide and remove the exposure tube. Then set the holding-tube so that it stands on the slide and place a pad of wet cotton-wool on the screen (Fig. 1, F). Cardboard cartons or cups or other suitable containers may be used instead of the holding-tubes, provided that they are used consistently.

(h) Keep the holding-tubes for 24 hours in a secluded, shaded place, where the temperature does not exceed 30°C. Wherever feasible, the maximum and minimum temperature of the room during the 24 hours should be recorded. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. If conditions are

FIG. 1. METHOD FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOS TO ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

very hot and dry, a moist chamber may be prepared by suspending damp towelling in a container.

(*i*) Mortality counts are made after 24 hours. Remove the dead mosquitos by gently detaching the slide and cautiously moving the tube aside. Affected specimens that are unable to walk are to be counted as dead. As an aid to counting the living specimens, they may be stunned by a sharp jerk of the tube or stupefied by chloroform or ether.[1] The results should be recorded on the forms provided. Copies of completed forms should be distributed in accordance with the instructions on page 63.

(*j*) After the preliminary test has been performed with the complete range of exposure times, tests should be carried out with the chosen series of 4 exposure times. Four replicates, including the preliminary test where appropriate, should be performed at each of these chosen exposure times. The 20 tubes provided in the kit are sufficient for 1 series of 2 replicates at each of the 4 chosen exposure times, together with 2 controls.

(*k*) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(*l*) When the test has been repeated 4 times with the same population of mosquitos, adequate data should be available for constructing a base-line of susceptibility as described below (section 5).

4. General remarks

(*a*) Each impregnated paper may be used up to 20 times, and up to 3 weeks after removal from the package, provided all possible precautions are taken against evaporation of the oil. To this end, the papers should be left in the tubes, with the open end well wrapped, and placed in the kit box, which in turn should be kept in a cool place. No paper should be used more than 3 weeks after removal from the package.

(*b*) After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The package should be kept in a cool place, but not in a refrigerator, as too low a temperature may cause crystallization in the higher insecticidal concentrations. Prolonged storage at high temperatures should be avoided. Papers should not be used after the expiry date shown on the box ; this expiry date is valid only if the packages are kept sealed at all times.

5. Results

(*a*) The user may desire to construct the exposure mortality regression line from the results obtained in the quadruplicate tests at the chosen

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[1] The anaesthetics should not be allowed to come into direct contact with the plastic tube and screw cap, which dissolve in these compounds.

exposure times and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LT_{50} or LT_{95} read from the graph.[1] The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computation, the worker is referred to those described by Swaroop [2] and Litchfield & Wilcoxon.[3]

(*b*) If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula :

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

(*c*) The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per tube (25) decreases the effect of individual differences in response. Regression lines based on similar numbers of specimens are more reliable.

6. Interpretation of results

See Annex 15 : *Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides.*

7. Distribution of reports

It is of considerable importance to WHO to receive copies of results obtained from the use of this test kit. It is therefore requested that copies of all reports be sent to the following addresses :

For anopheline species :

1. World Health Organization, Division of Malaria Eradication, 1211 Geneva, Switzerland ;

2. the appropriate WHO Regional Office ;[4] and

3. Project Headquarters.

The 4th copy should be retained by the investigator.

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[1] The LT_{50} and LT_{95} represent, respectively, the insecticide exposure at which 50% and 95% of the specimens are killed.

[2] Swaroop, S. (1966) *Statistical methods in malaria eradication*, Geneva (*World Health Organization : Monograph series*, No. 51).

[3] Litchfield, J. T., jr & Wilcoxon, F. (1949) *J. Pharmacol. exp. Ther.*, **96**, 99.

[4] See footnote on next page.

For non-anopheline species :

1. World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland ; and

2. the appropriate WHO Regional Office.[1]

The 3rd and 4th copies should be retained by the investigator.

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[1] Addresses of WHO Regional Offices are as follows :

World Health Organization, Regional Office for Africa, P.O. Box No. 6, Brazzaville, People's Republic of the Congo.

World Health Organization, Regional Office for the Eastern Mediterranean, P.O. Box 1517, Alexandria, United Arab Republic.

World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India.

World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 525, 23rd Street, N. W., Washington, D. C., 20037, USA.

World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark.

World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, Manila, Philippines.

Specimen Report Form

WHO TEST FOR INSECTICIDE RESISTANCE IN ADULT MOSQUITOS

Date: ....

Insecticide : malathion/other (specify) [1] .... Species : ....

Country : .... Province : .... Locality : ....

History of insecticide treatment (including agriculture) : ....

Condition of mosquitos : blood-fed/gravid/unfed/sugar-fed/males [1]   Where collected : shelters sprayed/unsprayed/outdoors/biting/bred out [1]

Type of test on population : first time/routine check/complete retest [1]   Exposure period (minutes) : ....

Tests	Preliminary			Replicate 1			Replicate 2			Replicate 3			Totals (for comparable tests only)		
	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]
Date of test															
Temperature during exposure period (°C)															
Humidity during exposure period (%)															
Temperature during 24-h holding period (°C)	Max. Min.			Max. Min.			Max. Min.			Max. Min.					
Insecticide concentr. (%)	Ex- posure (h)														
Control (oil alone)															

[1] Cross out what does not apply.   [2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks : ....

Interpretation of results : ....

Investigator : Name and postal address : ....   Signature : ....

One copy of the completed form should be sent to (for anopheline species) : World Health Organization, Division of Malaria Eradication, 1211 Geneva, Switzerland ; or to (for non-anopheline species) : World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland. A second copy should be sent to the appropriate WHO Regional Office.

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF MOSQUITO LARVAE TO ORGANOCHLORINE INSECTICIDES

1. Introduction

In order to detect the emergence of an insecticide-resistant strain of a mosquito, it is necessary to establish a base-line for the species, either before the wide use of insecticides or with specimens' from an untreated area. Where regular larvicide operations are undertaken to control mosquitos, the normal susceptibility levels of the larvae should be determined as early as possible. To this end, several tests (a minimum of 8) should be performed at various localities and seasons, to assess normal biological variation. Tests should then be continued at regular intervals to determine any significant reduction in susceptibility.

The history of the use of insecticides in the area, for both mosquito control and major agricultural uses, should be noted.

It is stressed that this test is not designed to indicate the relative effectiveness of the insecticides in the field.

2. Composition of test kit

(a) Solutions in ethanol at 5 different concentrations of each of the following insecticides: DDT (p,p'-isomer), gamma-HCH or lindane (pure gamma-isomer), and dieldrin (HEOD). The concentrations indicated on the labels (0.004, 0.02, 0.10, 0.50, and 2.50 ppm) are those obtained when 1 ml of solution is added to 249 ml of water. The kit contains 50 ml of each concentration and 50 ml of ethanol for the control.[1]

(b) 4 pipettes (1 ml), 1 for each insecticide and 1 for ethanol. Each pipette is equipped with a small rubber suction bulb for drawing up test solutions.

(c) 3 eye-droppers for transfer of the larvae.

(d) The following materials for use in making a strainer: 2 wire loops, 1 piece of nylon netting, and 1 tube of cement. It is suggested that 2 pieces of netting, measuring approximately 5 cm × 5 cm, be cut and cemented to opposite sides of the large end of a wire loop. More cement

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[1]. Solutions of the above insecticides that give a concentration of 12.5 ppm when diluted are available on request.

should then be applied around the outside of the loop to join the 2 pieces of netting. When dry, the netting may be trimmed with scissors. The kit contains sufficient netting for replacement purposes.

The user is expected to provide his own collecting and test vessels.

(e) Instruction sheets, a set of report forms, and 3 sheets of log-probability paper for plotting regression lines.

3. Procedure

(a) For a complete test with one insecticide, sufficient larvae should be collected from the field in order that about 300 individuals of the same species may be selected; they should be in their third or early fourth instar and should be retained in the water in which they were collected until selected for testing. Any larvae showing abnormalities, for example a fuzzy appearance due to the presence of parasites on the body surface, should be discarded. Lots of 20–25 larvae are distributed in each of 12 small beakers, each containing 25 ml of water. Their transfer is effected either by means of the strainer or by means of an eye-dropper; during the process they should be rinsed lightly in clean water.

(b) Into each of 12 glass vessels, approximately 7.5–10 cm in diameter, (jars, bowls, or 500-ml beakers) place 225 ml of water. The vessels should be such that the depth of water is between 2.5 and 7.5 cm. Distilled water, rain-water, or tap-water may be used, or even water obtained from a well or stream, but it should be as free as possible from chlorine or organic contaminants. It should be noted that distilled water obtained commercially may contain traces of poisonous heavy metals. Certain species, such as salt-marsh or tree-hole mosquitos, may suffer on transfer to relatively pure water, an effect that will be reflected in high control mortalities; in this case, water from the breeding site should be used, provided that it is free from insecticides and care is exercised to exclude detritus. The average temperature of the water should be recorded and should be approximately 25°C; it must not be below 20°C or above 30°C.

(c) Prepare the test concentrations by pipetting 1 ml of the appropriate standard insecticide solution under the surface of the water in each of the glass vessels and stirring vigorously for 30 seconds with the pipette. In preparing a series of concentrations, the most dilute should be prepared first. There should be 2 replicates at each concentration, and 2 control replicates. The 2 controls should be prepared by the addition of 1 ml of ethanol to the water in each container.

(d) Within 15–30 minutes of the preparation of the test concentrations, add the mosquito larvae to them by tipping the contents of the small beakers into the vessels.

(*e*) After a period of 24 hours, make mortality counts. In recording the percentage mortalities for each concentration, the moribund and dead larvae in both replicates should be combined. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface (within a reasonable period of time) or of showing the characteristic diving reaction when the water is disturbed; they may also show discolouration, unnatural positions, tremors, incoordination, or rigor.

(*f*) Discard the larvae that have pupated during the test. If more than 10% of the control larvae pupate in the course of the experiment, the test should be discarded. Tests with a control mortality of 20% or more are unsatisfactory and should be repeated.

(*g*) It is of importance to obtain not less than 3 mortality counts between 10% and 90%. With some species it has not been possible to do this using only the standard concentrations in the test kit, and it is then necessary to prepare additional concentrations both above and below 0.004 ppm. Additional intermediate concentrations may be prepared by diluting a portion of a standard solution with pure ethanol (e.g., a concentration of 0.01 ppm may be obtained by diluting the 0.02-ppm standard with an equal quantity of ethanol before taking the 1 ml for addition to the water in the vessel). If higher concentrations are required, they may be obtained from WHO.

(*h*) When 4 replicates have been performed with the same population of mosquito larvae, adequate data should be available for constructing a base-line of susceptibility.[1] The results should be recorded on the forms provided. Completed forms should be distributed in accordance with the instructions on page 70.

4. General remarks

(*a*) The accuracy of the concentrations provided will be affected if the alcohol is allowed to evaporate from the standard solutions. The bottles should therefore be tightly stoppered after use. The contents should no longer be used when they have decreased below 5 ml; fresh standard solutions should then be obtained from WHO.

(*b*) Test vessels should be carefully cleaned after use to remove traces of insecticide. They should be thoroughly rinsed, scrubbed with detergent and water (or cleaned with potassium dichromate and sulfuric acid), and

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[1] When physiological resistance is demonstrated in a population, the effect on the transmission of the disease and on the advisability of continuing to use the same insecticide in the area can be evaluated only by epidemiological appraisal and by entomological assessment by other means.

rinsed again. Pipettes should be thoroughly cleaned with acetone or alcohol.

(c) The accompanying table gives specimen results obtained with this method for susceptible strains of a number of mosquito species. These results indicate the approximate levels of susceptibility expected.

SPECIMEN RESULTS OBTAINED WITH WHO TEST FOR MOSQUITO LARVAE

Insecticide	Species	LC_{50} (ppm)	LC_{95} (ppm)	Ref.
DDT	Anopheles quadrimaculatus	0.0074	0.16	1
	An. albimanus	0.02	0.04	2
	Aedes aegypti	0.0052	0.013	3
	Ae. caspius	0.0007	0.0035	4
	Culex fatigans	0.012	0.036	5
	C. sitiens	0.0027	0.0084	6
	C. gelidus	0.0009	0.0026	6
	C. tritaeniorhynchus	0.013	0.072	7
dieldrin	Anopheles quadrimaculatus	0.0082	0.08	1
	An. albimanus	0.020	0.040	2
	Aedes aegypti	0.0042	0.0082	3
	Culex fatigans	0.004	0.01	5
	C. pipiens	0.017	0.16	3
	C. sitiens	0.0006	0.0010	6
	C. gelidus	0.0006	0.0018	6
HCH	Anopheles quadrimaculatus	0.048	0.08	2
	An. albimanus	0.05	0.08	2
	Aedes aegypti	0.010	0.035	5
	Culex fatigans	0.008	0.032	5
	C. pipiens	0.042	0.128	2
	C. sitiens	0.0032	0.0043	6
	C. gelidus	0.0024	0.0091	6

[1] Klassen, W. et al. (1964) Mosquito News, 24, 192.
[2] Keppler, W. J. et al. (1963) Mosquito News, 25, 415.
[3] Sutherland, D. J. (1964) Proc. New Jers. Mosq. Exterm. Ass., 51, 107.
[4] Abedi, Z. H. (1964) Unpublished report to WHO.
[5] Burnett, G. F. & Ash, L. H. (1961) Bull. Wld Hlth Org., 24, 547.
[6] Kurihara, T. (1965) Unpublished reports to WHO.
[7] Kinzer, H. G. (1963) Unpublished report to WHO.

5. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression

line may be fitted by eye, and the LC_{50} or LC_{95} read from the graph.[1] The regression line should not be extended (extrapolated) beyond the highest mortality obtained.

(b) If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula :

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

(c) The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per test (25) decreases the effect of individual differences in response. Regression lines based on similar numbers of specimens offer greater reliability.

(d) It appears that resistance may be suspected in mosquito larvae if the LC_{50} is 10–15 times the original figure, or when a proportion of the population can no longer be killed by the highest concentration in the kit. From the comparatively few data available at present, the indications are that when an LC_{50} for DDT in excess of 0.1 ppm is found for *Aedes* or *Anopheles* spp., or an LC_{50} above 1 ppm for *Culex* spp., resistance should be suspected.

6. Interpretation of results

See Annex 15 : *Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides.*

7. Distribution of reports

It is of considerable importance to WHO to receive copies of results obtained from the use of this test kit. It is therefore requested that copies of all reports be sent to the following addresses :

For anopheline species :

1. World Health Organization, Division of Malaria Eradication, 1211 Geneva, Switzerland ;

2. the appropriate WHO Regional Office ;[2] and

3. Project Headquarters.

The 4th copy should be retained by the investigator.

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[1] The LC_{50} and LC_{95} represent, respectively, the insecticide concentrations at which 50% and 95% of the specimens are killed.

[2] See footnote on next page.

For non-anopheline species :

1. World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland ; and

2. the appropriate WHO Regional Office.[1]

The 3rd and 4th copies should be retained by the investigator.

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[1] Addresses of WHO Regional Offices are as follows :

World Health Organization, Regional Office for Africa, P.O. Box No. 6, Brazzaville, People's Republic of the Congo.

World Health Organization, Regional Office for the Eastern Mediterranean, P.O. Box 1517, Alexandria, United Arab Republic.

World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India.

World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 525, 23rd Street, N.W., Washington, D.C., 20037, USA.

World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark.

World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, Manila, Philippines.

Specimen Report Form

WHO TEST FOR INSECTICIDE RESISTANCE IN MOSQUITO LARVAE

Date: ....

Insecticide : DDT/dieldrin/HCH/other (specify) [1] .... Species : ....

Country : .... Province : .... Locality : ....

History of insecticide treatment (including agriculture) : ....

Condition of larvae : Instar .... reared/collected/other [1] ....

Results of test (abbreviations : "M" — moribund ; "D" — dead)

Tests	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Totals (for comparable tests only)		
	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]
Date of test															
Temperature during test (°C)															
Insecticide concentration (ppm)															
Control 1															

[1] Cross out what does not apply. [2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks : ....

Investigator : Name and postal address : .... Signature : ....

One copy of the completed form should be sent to (for anopheline species) : World Health Organization, Division of Malaria Eradication, 1211 Geneva, Switzerland ; or to (for non-anopheline species) : World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland. A second copy should be sent to the appropriate WHO Regional Office.

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF MOSQUITO LARVAE TO ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

1. Introduction

In order to detect the emergence of an insecticide-resistant strain of a mosquito, it is necessary to establish a base-line for the species, either before the wide use of insecticides or with specimens from an untreated area. Where regular larvicide operations are undertaken to control mosquitos, the normal susceptibility levels of the larvae should be determined as early as possible. To this end, several tests (a minimum of 8) should be performed at various localities and seasons, to assess normal biological variation. Tests should then be continued at regular intervals to determine any significant reduction in susceptibility.

The history of the use of insecticides in the area, for both mosquito control and major agricultural uses, should be noted.

It is stressed that this test is not designed to indicate the relative effectiveness of the insecticides in the field.

2. Composition of test kit

(*a*) Solutions in ethanol at 4 different concentrations of each of the requested insecticides, covering a suitable range. The concentrations indicated on the labels are those obtained when 1 ml of solution is added to 249 ml of water. Examples of the compounds and concentrations (in ppm) that are available are as follows: Abate (0.025, 0.005, 0.001, 0.0002), bromophos (0.125, 0.025, 0.005, 0.001), Dursban (0.025, 0.005, 0.001, 0.0002), fenitrothion (0.125, 0.025, 0.005, 0.001), fenthion (0.625, 0.125, 0.025, 0.005), malathion (3.125, 0.625, 0.125, 0.025), and parathion (0.125, 0.025, 0.005, 0.001). The kit contains 50 ml of each insecticide solution and 50 ml of ethanol for the control.

(*b*) 4 pipettes (1 ml), 1 for each insecticide and 1 for ethanol. Each pipette is equipped with a small rubber suction bulb for drawing up test solutions.

(*c*) 2 eye-droppers for transfer of the larvae.

(*d*) The following materials for use in making a strainer: 2 wire loops, 1 piece of nylon netting, and 1 tube of cement. It is suggested that 2 pieces of netting, measuring approximately 5 cm × 5 cm, be cut and cemented

to opposite sides of the large end of a wire loop. More cement should then be applied around the outside of the loop to join the 2 pieces of netting. When dry, the netting may be trimmed with scissors. The kit contains sufficient netting for replacement purposes.

The user is expected to provide his own collecting and test vessels.

(*e*) Instruction sheets, a set of report forms, and 3 sheets of log-probability paper for plotting regression lines.

3. Procedure

(*a*) For a complete test with one insecticide, sufficient larvae should be collected from the field in order that about 300 individuals of the same species may be selected; they should be in their third or early fourth instar and should be retained in the water in which they were collected until selected for testing. Any larvae showing abnormalities, for example a fuzzy appearance due to the presence of parasites on the body surface, should be discarded. Lots of 20–25 larvae are distributed in each of 12 small beakers, each containing 25 ml of water. Their transfer is effected either by means of the strainer provided, or by means of an eye-dropper; during the process they should be rinsed lightly in clean water.

(*b*) Into each of 12 glass vessels, approximately 7.5–10 cm in diameter, (jars, bowls, or 500-ml beakers) place 225 ml of water. The vessels should be such that the depth of water is between 2.5 and 7.5 cm. Distilled water, rain-water, or tap-water may be used, or even water obtained from a well or stream, but it should be as free as possible from chlorine or organic contaminants. It should be noted that distilled water obtained commercially may contain traces of poisonous heavy metals. Certain species, such as salt-marsh or tree-hole mosquitos, may suffer on transfer to relatively pure water, an effect that will be reflected in high control mortalities; in this case, water from the breeding site should be used, provided that it is free from insecticides and care is exercised to exclude detritus. The average temperature of the water should be recorded and should be approximately 25°C; it must not be below 20°C or above 30°C.

(*c*) Prepare the test concentrations by pipetting 1 ml of the appropriate standard insecticide solution under the surface of the water in each of the glass vessels and stirring vigorously for 30 seconds with the pipette. In preparing a series of concentrations, the most dilute should be prepared first. There should be 2 replicates at each concentration, and 2 control replicates. The 2 controls should be prepared by the addition of 1 ml of ethanol to the water in each container.

(*d*) Within 15–30 minutes of the preparation of the test concentrations, add the mosquito larvae to them by tipping the contents of the small beakers into the vessels.

(*e*) After a period of 24 hours, make mortality counts. In recording the percentage mortalities for each concentration, the moribund and dead larvae in both replicates should be combined. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface (within a reasonable period of time) or of showing the characteristic diving reaction when the water is disturbed; they may also show discolouration, unnatural positions, tremors, incoordination, or rigor.

(*f*) Discard the larvae that have pupated during the test. If more than 10% of the control larvae pupate in the course of the experiment, the test should be discarded. Tests with a control mortality of 20% or more are unsatisfactory and should be repeated.

(*g*) It is of importance to obtain not less than 3 mortality counts between 10% and 90%. With some species it has not been possible to do this using only the standard concentrations in the test kit, and it is then necessary to prepare additional concentrations both above and below 0.004 ppm. Additional intermediate concentrations may be prepared by diluting a portion of a standard solution with pure ethanol (e.g., a concentration of 0.01 ppm may be obtained by diluting the 0.02-ppm standard with an equal quantity of ethanol before taking the 1 ml for addition to the water in the vessel). If higher concentrations are required, they may be obtained from WHO.

(*h*) When 4 replicates have been performed with the same population of mosquito larvae, adequate data should be available for constructing a base-line of susceptibility.[1] The results should be recorded on the forms provided. Completed forms should be distributed in accordance with the instructions on page 76.

4. General remarks

(*a*) The accuracy of the concentrations provided will be affected if the alcohol is allowed to evaporate from the standard solutions. The bottles should therefore be tightly stoppered after use. The contents should no longer be used when they have decreased below 5 ml; fresh standard solutions should then be obtained from WHO.

(*b*) Test vessels should be carefully cleaned after use to remove traces of insecticide. They should be thoroughly rinsed, scrubbed with detergent and water (or cleaned with potassium dichromate and sulfuric acid), and rinsed again. Pipettes should be thoroughly cleaned with acetone or alcohol.

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[1] When physiological resistance is demonstrated in a population, the effect on the transmission of the disease and on the advisability of continuing to use the same insecticide in the area can be evaluated only by epidemiological appraisal and by entomological assessment by other means.

(c) The accompanying table gives specimen results obtained with this method for susceptible strains of a number of mosquito species. These results indicate the approximate levels of susceptibility expected.

5. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC_{50} or LC_{95} read from the graph.[1] The regression line should not be extended (extrapolated) beyond the highest mortality obtained.

(b) If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula :

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

(c) The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per test (25) decreases the effect of individual differences in response. Regression lines based on similar numbers of specimens offer greater reliability.

6. Interpretation of results

See Annex 15 : *Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides.*

7. Distribution of reports

It is of considerable importance to WHO to receive copies of results obtained from the use of this test kit. It is therefore requested that copies of all reports be sent to the following addresses :

For anopheline species :

1. World Health Organization, Division of Malaria Eradication, 1211 Geneva, Switzerland ;

2. the WHO Regional Office ; [2] and

3. Project Headquarters.

The 4th copy should be retained by the investigator.

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[1] The LC_{50} and LC_{95} represent, respectively, the insecticide concentrations at which 50% and 95% of the specimens are killed.

[2] See footnote on next page.

For non-anopheline species :

1. World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland ; and

2. the appropriate WHO Regional Office.[1]

The 3rd and 4th copies should be retained by the investigator.

SPECIMEN RESULTS OBTAINED WITH THE WHO TEST FOR RESISTANCE IN MOSQUITO LARVAE

Insecticide	Species	LC_{50} (ppm)	LC_{95} (ppm)	Ref.
malathion	*Anopheles quadrimaculatus*	0.19	0.64	1
	An. albimanus	0.26	0.64	2
	Aedes aegypti	0.029	0.26	3
	Ae. caspius	0 023	0.043	4
	Ae. detritus	0.020	0.044	4
	Culex fatigans	0.033	0.050	5
	C. pipiens	0.035	0.073	6
	C. sitiens	0.025	0.099	5
	C. gelidus	0.0022	0.012	5
	C. tritaeniorhynchus	0.024	0.10	7
diazinon	*Aedes aegypti*	0.0033	0.13	3
	Culex fatigans	0.032	0.082	7
	C. sitiens	0.009	0.017	5
	C. gelidus	0.009	0.019	5
	C. tritaeniorhynchus	0.010	0.023	8
fenthion	*Anopheles quadrimaculatus*	0.072	0.16	2
	An. albimanus	0.026	0.08	2
	Aedes aegypti	0.0026	0.0063	9
	Ae. caspius	0.0021	0.0032	4
	Ae. detritus	0.0015	0.0024	4
	Culex fatigans	0.0020	0.0029	10
	C. pipiens	0.0023	0.0048	11
	C. sitiens	0.0019	0.0039	5
	C. gelidus	0.0007	0.0024	5
	C. tritaeniorhynchus	0.0015	0.0028	12
	Armigeres obturbans	0.028	0.064	5

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[1] Addresses of WHO Regional Offices are as follows :

World Health Organization, Regional Office for Africa, P.O. Box No. 6, Brazzaville, People's Republic of the Congo.

World Health Organization, Regional Office for the Eastern Mediterranean, P.O. Box 1517, Alexandria, United Arab Republic.

World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India.

World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 525, 23rd Street, N.W., Washington, D.C., 20037 USA.

World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark.

World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, Manila, Philippines.

Insecticide	Species	LC_{50} (ppm)	LC_{95} (ppm)	Ref.
parathion	*Anopheles quadrimaculatus*	0.0064	0.02	1
	An. albimanus	0.029	0.16	2
	Aedes aegypti	0.010	0.022	13
	Culex fatigans	0.0025	0.0036	10
fenitrothion	*Anopheles quadrimaculatus*	0.020	0.04	2
	An. albimanus	0.032	0.08	2
	Aedes aegypti	0.0081	0.013	14
	Culex pipiens	0.0041	0.01	2
Abate	*Aedes aegypti*	0.00056	0.0021	9
	Culex fatigans	0.00039	0.0007	15
Dursban	*Aedes aegypti*	0.0007	0.0016	16
	Culex fatigans	0.0003	0.0006	15
bromophos	*Aedes aegypti*	0.0018	0.0033	14
	Culex fatigans	0.0027	0.0072	10
OMS-33	*Anopheles quadrimaculatus*	0.52	1.28	1
	An. albimanus	0.67	2.65	2
	Culex pipiens	0.25	0.64	2

[1] Klassen, W. et al. (1964) *Mosquito News*, **24**, 192.
[2] Keppler, W. J. et al. (1965) *Mosquito News*, **25**, 415.
[3] Tu, M. (1966) Unpublished reports to WHO.
[4] Gras, G. et al. (1966) *Bull. Soc. Path. exot.*, **59**, 898.
[5] Kurihara, T. (1965) Unpublished reports to WHO.
[6] Mouchet, J. (1966) Unpublished report to WHO.
[7] Pennington, N. E. (1965) Unpublished reports to WHO.
[8] Pennington, N. E. (1967) Unpublished report to WHO.
[9] Bang, Y. H. (1966) Unpublished reports to WHO.
[10] Hamon, J. & Sales, S. (1967) Unpublished reports to WHO.
[11] Kuhlow, F. (1964) Unpublished report to WHO.
[12] Abedi, Z. H. (1964) Unpublished report to WHO.
[13] Sutherland, D. J. (1964) *Proc. New Jers. Mosq. Exterm. Ass.*, **51**, 107.
[14] Mouchet, J. (1967) Unpublished reports to WHO.
[15] Mouchet, J. (1966) Unpublished reports to WHO.
[16] Hamon, J. (1967) Unpublished report to WHO.

Specimen Report Form

WHO TEST FOR INSECTICIDE RESISTANCE IN MOSQUITO LARVAE

Date: ....

Insecticide (specify): .... Species: ....

Country: .... Province: .... Locality: ....

History of insecticide treatment (including agriculture): ....

Condition of larvae: instar .... reared/collected/other [1]

Results of test (abbreviations: "M" — moribund; "D" — dead)

Tests	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Totals (for comparable tests only)		
	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]
Date of test															
Temperature during test (°C)															
Insecticide concentration (ppm)															
Control 1															

[1] Cross out what does not apply.   [2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks: ....

Investigator: Name and postal address: ....   Signature: ....

One copy of the completed form should be sent to (for anopheline species): World Health Organization, Division of Malaria Eradication, 1211 Geneva, Switzerland; or to (for non-anopheline species): World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland. A second copy should be sent to the appropriate WHO Regional Office.

TENTATIVE INSTRUCTIONS
FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE
OF BITING MIDGE LARVAE TO INSECTICIDES

1. Introduction

In order to detect the emergence of an insecticide-resistant strain of a biting midge, it is necessary to establish a base-line for the species, either before the wide use of insecticides or with specimens from an untreated area. Where regular larvicide operations are undertaken to control biting midges, the normal susceptibility levels of the larvae should be determined as early as possible. To this end, several tests (a minimum of 8) should be performed at various localities and seasons, to assess normal biological variation. Tests should then be continued at regular intervals to determine any significant reduction in susceptibility.

The history of the use of insecticides in the area should be noted.

It is stressed that this test is not designed to indicate the relative effectiveness of the insecticides in the field.

2. Composition of test kit

(a) Solutions in ethanol at 5 different concentrations of each of the following insecticides : DDT (*p,p'*-isomer), gamma-HCH or lindane (pure gamma-isomer), and dieldrin (HEOD). The concentrations indicated on the labels (0.004, 0.02, 0.10, 0.50, and 2.50 ppm) are those obtained when 1 ml of solution is added to 249 ml of water, the kit contains 50 ml of each concentration and 50 ml of ethanol for the control.

(b) 4 pipettes (1 ml), 1 for each insecticide and 1 for ethanol. Each pipette is equipped with a small rubber suction bulb for drawing up test solutions.

(c) 2 eye-droppers and 1 strainer for transfer of the larvae. (The user is expected to provide his own collecting and test vessels.)

(d) Instruction sheets, a set of report forms, and 3 sheets of log-probability paper for plotting regression lines.

3. Procedure

(a) Collect (in tins) samples of mud from the margins of ponds that are known to harbour midge larvae. In the laboratory, place the mud

samples in dishes and soak them with a solution of magnesium sulfate having a specific gravity of 1.11. Pick off the larvae, which float to the surface, with a wire loop and transfer them to watch glasses containing a little water. Place about 20 larvae in each watch glass. (Use 1 glass for each insecticide concentration.)

(b) Into each of several 500-ml beakers, jars, or bowls (one for each concentration) place 249 ml of water. Distilled water, rain-water, or tap-water may be used, or even water obtained from a well or stream, provided it is as free as possible from chlorine and organic contaminants. It should be noted that distilled water obtained commercially may contain traces of poisonous heavy metals. Certain species may suffer on transfer to relatively pure water, an effect that will be reflected in high control mortalities; if this occurs, water from the breeding site should be used, provided it is free from insecticides and care is exercised to exclude detritus. The average temperature of the water should be recorded and should be about 25°C; it must not be below 20°C or above 30°C.

(c) Prepare the test concentrations by pipetting 1 ml of the appropriate standard insecticide solution under the surface of the water in each vessel and stirring vigorously for 30 seconds with the pipette. In preparing a series of concentrations, prepare the most dilute first. Prepare the control by adding 1 ml of ethanol to 249 ml of water in a similar glass vessel.

(d) From each suspension transfer 25 ml into each of two Petri dishes 7.5 cm in diameter (the larvae do not survive in water more than 2.5 cm deep).

(e) Remove larvae from the watch glasses one at a time by means of the wire loop and, after touching them to filter paper to remove water, place them in the test suspensions.

(f) Make mortality counts after 24 hours. In recording the percentage mortalities for each concentration, combine the moribund and dead larvae in both replicates. Dead larvae are those that cannot be induced to move when they are probed with a needle in the cervical region.

(g) Tests with a control mortality of 20% or more are unsatisfactory and should be repeated.

(h) It is of importance to obtain not less than 3 mortality counts between 10% and 90%. With some species it has not been possible to do this using only the standard concentrations in the test kit, and it is then necessary to prepare additional concentrations both above and below 0.004 ppm. Additional intermediate concentrations may be prepared by diluting a portion of a standard solution with pure ethanol (e.g., a concentration of 0.01 ppm may be obtained by diluting the 0.02 ppm-standard with an equal quantity of ethanol before taking the 1 ml for addition to

the water in the vessel). If higher concentrations are required, they may be obtained from WHO.

(*i*) When 4 replicates have been performed with the same population of midge larvae, adequate data should be available for constructing a base-line of susceptibility.[1] The results should be recorded on the forms provided. Completed forms should be distributed in accordance with the instructions on page 83.

4. General remarks

(*a*) The accuracy of the concentrations provided will be affected if the alcohol is allowed to evaporate from the standard solutions. The bottles should therefore be tightly stoppered after use. The content should no longer be used when they have decreased below 5 ml; fresh standard solutions should then be obtained from WHO.

(*b*) Test vessels should be carefully cleaned after use to remove traces of insecticide. They should be thoroughly rinsed, scrubbed with detergent and water (or cleaned with potassium dichromate and sulfuric acid), and rinsed again. Pipettes should be thoroughly cleaned with acetone or alcohol.

5. Results

(*a*) The user may desire to construct the dosage-mortality regression line from the results obtained. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC_{50} or LC_{95} read from the graph.[2] The regression line should not be extended (extrapolated) beyond the highest mortality obtained.

(*b*) If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula :

$$\frac{\%\text{ test mortality} - \%\text{ control mortality}}{100 - \%\text{ control mortality}} \times 100$$

(*c*) The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per test (25) decreases the effect of individual differences in response. Regression lines based on similar numbers of specimens offer greater reliability.

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[1] When physiological resistance is demonstrated in a population, the effect on the transmission of the disease and on the advisability of continuing to use the same insecticide in the area can be evaluated only by epidemiological appraisal and by entomological assessment by other means.

[2] The LC_{50} and LC_{95} represent, respectively, the insecticide concentrations at which 50% and 95% of the specimens are killed.

6. Interpretation of results

See Annex 15 : *Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides.*

7. Distribution of reports

It is of considerable importance to WHO to receive copies of results obtained from the use of this test kit. It is therefore requested that copies of all reports be sent to the following addresses :

1. World Health Organization, Vector Biology and Control,
   1211 Geneva, Switzerland ; and

2. the appropriate WHO Regional Office.[1]

The 3rd and 4th copies should be retained by the investigator.

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[1] Addresses of WHO Regional Offices are as follows :

World Health Organization, Regional Office for Africa, P.O. Box No. 6, Brazzaville, People's Republic of the Congo.

World Health Organization, Regional Office for the Eastern Mediterranean, P.O. Box 1517, Alexandria, United Arab Republic.

World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India.

World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 525, 23rd Street, N.W., Washington, D.C., 20037, USA.

World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark.

World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, Manila, Philippines.

Specimen Report Form

WHO TEST FOR INSECTICIDE RESISTANCE IN BITING MIDGE LARVAE

Date: ....

Insecticide: DDT/dieldrin/HCH/other (specify)[1] .... Species: ....

Country: .... Province: .... Locality: ....

History of insecticide treatment (including agriculture): ....

Condition of larvae: Instar .... reared/collected/other[1] ....

Results of test (abbreviations: "M" — moribund; "D" — dead)

Tests	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Totals (for comparable tests only)		
	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]	M & D	Total	Mort. (%) corr.[2]
Date of test															
Temperature during test (°C)															
Insecticide concentration (ppm)															
Control[1]															

[1] Cross out what does not apply. [2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks: ....

Investigator: Name and postal address: .... Signature: ....

One copy of the completed form should be sent to: World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland. A second copy should be sent to the appropriate WHO Regional Office.

Annex 4

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF BODY LICE TO INSECTICIDES

1. Introduction

This method measures the levels of susceptibility of a population of body lice to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose it is necessary (*a*) to establish the susceptibility levels of normal lice, and (*b*) to make subsequent routine checks of the susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance.

Establishing the base-line [1]

Batches of adult lice are exposed to different concentrations of insecticide and the mortality at each level is determined. It is suggested that a preliminary test be made at each concentration in the complete range provided. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of concentrations should be chosen, some of which give partial mortalities (i.e., at least one of them should give 100% mortality and one less than 50% mortality). Tests at these concentrations should be replicated 4 times with samples from the same population of lice. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable.

Subsequent routine checks [1]

The lowest concentration that has consistently given 100% mortality in the 4 successive tests is chosen for the routine checks. These should be made periodically with 2–5 replicates. The occasional appearance of survivors in such checks may be due to normal variation. However, the

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[1] It is not necessary to establish a base-line for those insect/insecticide combinations that have been fully studied by extensive use of the relevant standard test. For example, extensive data are available for body lice exposed on cloth squares (as described later) to powder preparations of DDT (see Wright, J. W. & Brown, A.W. A (1957) *Bull. Wld Hlth Org.*, **16**, 9 ; Wright, J. W. & Pal, R. (1965) *ibid.*, **33**, 485 ; Busvine, J. R. (1967) *ibid.*, **36**, 431). From these investigations it seems that a suitable concentration of DDT for routine resistance checks is 5%.

Although the investigations of gamma-HCH are less conclusive, it appears that 0.5% is a suitable concentration for checks with this insecticide.

regular occurrence of survivors (e.g., on 3 successive occasions) constitutes a signal calling for further investigations. Such investigations should include 4 tests at each of the concentrations used in establishing the original base-line. In this way, it is possible to detect the presence of physiological resistance and to determine the approximate proportion of resistant lice in the population.

Laboratory tests with 2 colonies of body lice suggest that papers impregnated with 0.4% malathion should give 100% mortality of susceptible lice unless the papers are more than 4 months old.

Condition of lice

Adult lice of either sex can be used provided they are not obviously starved.

Conditions of test

Tests should be carried out in a room free from insecticidal contamination. The lice are exposed in darkness and held at a temperature between 20°C and 30°C and at a relative humidity above 25%.

2. Composition of test kit

(a) 3 packages, each containing 0.5 g of insecticide powder, of each of the following concentrations:

    DDT, technical : 0.04% ; 0.2% ; 1.0% ; 5.0%
    gamma-HCH : 0.02% ; 0.1% ; 0.5% ; 2.5%

(b) 6 packages of papers impregnated with malathion in olive oil, at the following concentrations: 0.1%, 0.2%, 0.4%, 0.8%, 1.6% and 3.2%, and 1 package treated with oil only. These papers should not be used more than 4 months after impregnation; impregnation and expiry dates are marked on the packages.

(c) 50 pieces of cloth, 12.5 cm square.

(d) 1 pair of forceps.

(e) Instruction sheets, a set of report forms, and 3 sheets of log-probability paper for plotting regression lines.

The following items are not included in the kit and must be provided by the investigator:

   14 halves of 9-cm Petri dishes or metal rings from preserving jars (to confine lice on treated cloth);

   14 clean containers (beakers, salve tins, or other smooth-sided containers); and

   1 spatula or table knife (for spreading powders on cloth).

A small amount of acetone or alcohol should be available for cleansing the spatula and forceps after each use.

3. Procedure

Insecticide powders

(*a*) Each package contains 0.5 g of powder, or enough to treat 1 square of cloth. The cloth should be placed on a glass surface (or a piece of white paper), the contents of a package sifted over it, and the powder spread uniformly over one side of the cloth with a spatula or table knife and worked gently into the nap of the fabric. The treated cloth should be carefully placed on a rigid surface (wood or stiff cardboard) and the corners fastened thereto by thumb tacks or narrow strips of tape. Three cloths should be treated as replicates with each concentration of powder, making a total of 39 treated cloths. Three untreated cloths should be similarly mounted for use as controls.

(*b*) Adult lice should be collected in a single container from one individual or, if this is not possible, from a small group of individuals. To assess the resistance level in a locality, additional samples should be taken from a sufficient number of infested individuals. The lice should be divided by random selection into lots of 20 in small containers (beakers, salve tins, or other clean containers). Although it is desirable to use 20 lice in each replicate, if this is not possible the number may be reduced down to an absolute minimum of 10 per replicate.

(*c*) One lot of adult lice should be placed on each treated cloth and confined under half a Petri dish or inside a metal ring. Rubber bands or weights should be employed to hold the dish or metal ring so that the lice cannot escape.

(*d*) Observations of mortality should be made after 24 hours. Only lice capable of co-ordinated movement should be counted as " alive ".

(*e*) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of 4 concentrations giving partial and complete mortality. Three replicates should be performed at each of the chosen concentrations.

(*f*) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(*g*) When the test has been repeated 4 times with the same population of lice, adequate data should be available for constructing a base-line of susceptibility as described in section 4. The results should be recorded on the forms provided.

Impregnated papers

The procedure described above should be followed in all particulars, except that an impregnated paper, placed on a firm impermeable surface, should be used instead of a cloth square treated with powder.

4. Results

(*a*) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC_{50} or LC_{95} read from the graph.[1] The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC_{50} the worker is referred to those described by Swaroop [2] and Litchfield & Wilcoxon.[3]

(*b*) If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula :

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

5. Interpretation of results

See Annex 15 : *Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides.*

6. Distribution of reports

One copy of each completed report form should be sent to the World Health Organisation, Vector Biology and Control, 1211 Geneva, Switzerland, and one copy to the appropriate WHO Regional Office.[4]

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[1] The LC_{50} and LC_{95} represent, respectively, the insecticide concentrations at which 50% and 95% of the specimens are killed.

[2] Swaroop, S. (1966) *Statistical methods in malaria eradication*, Geneva (*World Health Organization : Monograph Series*, No. 51).

[3] Litchfield, J. T., jr & Wilcoxon, F. (1949) *J. Pharmacol. exp. Ther.*, **96**, 99.

[4] Addresses of WHO Regional Offices are as follows :

World Health Organization, Regional Office for Africa, P.O. Box No. 6, Brazzaville, People's Republic of the Congo.

World Health Organization, Regional Office for the Eastern Mediterranean, P.O. Box 1517, Alexandria, United Arab Republic.

World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India.

World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 525, 23rd Street, N.W., Washington, D.C., 20037, USA.

World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark.

World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, Manila, Philippines.

Specimen Report Form

WHO TEST TO DETERMINE THE EFFECTIVENESS OF INSECTICIDE
DUSTING POWDERS OR IMPREGNATED PAPERS AGAINST BODY LICE

Date : _____

Country : _____

Province : _____ Locality : _____

Background information (in narrative form, using additional sheets if need) :

(a) How prevalent are body lice among the population ?
(b) What insecticides or other control measures are in general use ?
(c) For how many years have these insecticides, particularly DDT, been used for control of lice ?
(d) How effective are the various insecticidal treatments against lice at present ?
(e) Is there any evidence that the insecticides in common use are less effective now than in previous years ?

Source of lice : _____

Temperature at which test was performed (°C) : _____

Insecticide & concentration (%) in powder or paper	Condition of lice after 24 hours' exposure					
	First replicate		Second replicate		Third replicate	
	No. tested	No. dead	No. tested	No. dead	No. tested	No. dead
1. Dusts						
DDT :						
0.04						
0.2						
1.0						
5.0						
gamma-HCH :						
0.02						
0.1						
0.5						
2.5						
control						
2. Papers						
malathion :						
0.1						
0.2						
0.4						
0.8						
1.6						
3.2						
control						

Remarks : _____

Investigator : Name and postal address : _____

_____

Signature : _____

One copy of the completed form should be sent to : World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland. A second copy should be sent to the appropriate WHO Regional Office.

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT BEDBUGS TO INSECTICIDES

1. Introduction

This method measures the levels of susceptibility of a population of adult bedbugs to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (*a*) to establish the susceptibility levels of normal bedbugs of the species concerned, and (*b*) to make subsequent routine checks of the susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance. It is stressed that the test is not designed to evaluate the effectiveness of deposits of insecticides in the field, for which other entomological techniques must be used.

Establishing the base-line

Batches of adult bedbugs are exposed to different concentrations of insecticide and the mortality at each level is determined. It is suggested that a preliminary test be made at each concentration in the complete range provided, at the standard exposure of 1 day for organophosphorus compounds, 2 days for dieldrin, and 5 days for DDT. This will indicate the general level of susceptibility ; further tests should then be made on this basis. A series of 4 concentrations should be chosen, some of which give partial mortalities (i.e., at least one of them should give 100% mortality and one less than 50% mortality). Tests at these concentrations should be repeated 4 times with samples from the same population of bedbugs. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable.

Subsequent routine checks

A concentration double the lowest concentration that has consistently given 100% mortality in the 4 successive tests is chosen for the routine checks. These should be made periodically with 2–5 replicates. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on 3 successive occasions) constitutes a signal calling for further investigations. Such investigations should include 4 tests at each of the concentrations used in establishing the original base-line. In this way, it is possible to detect the

presence of physiological resistance and to determine the approximate proportion of resistant bedbugs in the population.

Condition of bedbugs

Adult bedbugs of either sex can be used and their nutritional status should be recorded. Wherever possible fully-fed bedbugs collected in the field should be used. If fully-fed bedbugs are not available (starved specimens are very thin and transparent) the specimens collected may be fed on a suitable host and used on the following day.

Conditions of test

Tests should be carried out in a room free from insecticidal contamination. The bedbugs are exposed and held at a temperature between 20°C and 30°C and at a relative humidity above 25%. Some uniformity in these conditions can be secured by the use of the kit box.

2. Composition of test kit

(*a*) 5 packages each containing 40 papers (5 cm long by 1.5 cm wide, tapered at one end) impregnated with DDT (*p,p'*-isomer) in mineral oil (Risella 17, Shell) at concentrations of 0.25%, 0.5%, 1.0%, 2.0%, and 4.0%, respectively ; 6 packages of papers impregnated with dieldrin (purified HEOD) in mineral oil at concentrations of 0.05%, 0.1%, 0.2%, 0.4%, 0.8%, and 1.6%, and 1 package treated with oil only.[1]

(*b*) 24 glass test-tubes, 17 cm in length and 16 mm in internal diameter.

(*c*) Test-tube rack, plastic covered, holding 24 tubes, together with a black foam plastic mat.

(*d*) 30 pieces of fine-mesh gauze to fit over the tubes.

(*e*) 50 rubber bands.

(*f*) 1 nylon brush, 1 pair of forceps, and 2 fine paint brushes.

(*g*) A holding container.

(*h*) Pill boxes (for feeding bedbugs) with fine-mesh gauze.

(*i*) 1 roll of self-adhesive plastic tape.

(*j*) Instruction sheets, a set of report forms, and 3 sheets of log-probability paper for plotting regression lines.

(*k*) 40 sheets of clean typing paper.

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[1] The following impregnated papers are also available on request : dieldrin (0.025% and 4.0%) and malathion (0.05%, 0.1%, 0.2%, 0.4%, 0.8%, and 1.6%). Since malathion papers lose their efficacy on long storage, their use should be planned well in advance.

3. Procedure

(*a*) Having located infested houses, collect as many adult bedbugs as possible (approximately 100). The area from which collections are made should be restricted as far as possible for tests in a given series. The bed-bugs may be gathered by brushing them from infested sites (bed frames, etc.) into the collecting vessel supplied, taking care not to damage them. The specimens are then brought to the locality of the test. If the bedbugs col-lected are unfed they are introduced into the pill boxes provided, which are then closed with the fine-mesh gauze, secured with rubber bands and applied to suitable surfaces of a convenient host.

(*b*) Set up sufficient test-tubes to include 2 replicates at the range of concentrations required. Into each tube place one of the treated papers. Two papers impregnated with each of the concentrations of insecticide provided should be used and one control paper impregnated with oil alone.

(*c*) Transfer 10 adult bedbugs to each tube and close with the fine-mesh gauze. Set the tubes upright in the holding rack in the box. If necessary, place damp cloths in the box to obviate very low humidity. Close the box for the appropriate exposure period (5 days for DDT, 2 days for dieldrin, or 1 day for organophosphorus compounds).

(*d*) At the end of the exposure time, examine the bedbugs and record the mortality (count bedbugs incapable of any movement, or unable to cling to the test paper, as dead). At the conclusion of the test, discard the test papers, destroy the bedbugs, and cleanse the tubes thoroughly.

(*e*) Following the preliminary test with the complete range of con-centrations, carry out tests with the chosen series of 4 concentrations giving partial and complete mortality. Use 3 replicates at each of the chosen concentrations.

(*f*) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(*g*) When the test has been repeated 4 times with the same population of bedbugs, adequate data should be available for constructing a base-line of susceptibility as described in section 5. The results should be recorded on the forms provided.

4. General remarks

After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as storage at too low a tem-perature may cause crystallization in the higher concentrations. Prolonged storage at high temperatures should be avoided. Papers should not be used after the expiry date shown on the box. Chlorinated hydrocarbon

papers have a useful life of 5 years from the date of impregnation, provided the packages are kept sealed at all times. Additional papers are available on request.

5. Results

(*a*) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC_{50} or LC_{95} read from the graph.[1] The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC_{50}, the worker is referred to those described by Swaroop [2] and Litchfield & Wilcoxon.[3]

(*b*) If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula :

$$\frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

6. Interpretation of results

See Annex 15 : *Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides.*

7. Distribution of reports

One copy of each completed report form should be sent to : World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland, and one copy to the appropriate WHO Regional Office.[4]

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[1] The LC_{50} and LC_{95} represent, respectively, the insecticide concentrations at which 50% and 95% of the specimens are killed.

[2] Swaroop, S. (1966) *Statistical methods in malaria eradication*, Geneva (*World Health Organization : Monograph Series*, No. 51).

[3] Litchfield, J. T., jr & Wilcoxon, F. (1949) *J. Pharmacol. exp. Ther.* **96**, 99.

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World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India.

World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 525, 23rd Street, N.W., Washington, D.C., 20037, USA.

World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark.

World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, Manila, Philippines.

Specimen Report Form

WHO TEST FOR INSECTICIDE RESISTANCE IN ADULT BEDBUGS

Species: .... Date: ....

Country: .... Province: .... Locality: ....

History of insecticide treatment (including agriculture): ....

Condition of bedbugs: blood-fed/unfed [1]

Where collected: human habitation/animal shelter/other [1]: ....

Type of test on population: first time/routine check/complete retest [1]

Exposure period: ....

Tests	Preliminary			Replicate 1			Replicate 2			Replicate 3			Totals (for comparable tests only)		
Date of test															
Temperature range during exposure (°C)	Max. Min.			Max. Min.			Max. Min.			Max. Min.					
Humidity range during exposure (%)	Max. Min.			Max. Min.			Max. Min.			Max. Min.					
Insecticide conc. (%) DDT/dieldrin/other [1]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]	Dead	Total	Mort. (%) corr.[2]
Control (oil alone)															

[1] Cross out what does not apply. [2] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks: ....

Investigator: Name and postal address: .... Signature: ....

One copy of the completed form should be sent to: World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland. A second copy should be sent to the appropriate WHO Regional Office.