Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1967, 37, 287-292

Insecticide-Resistance in *Culex pipiens fatigans**

J. R. BUSVINE [1]

The three main subspecies of the Culex pipiens *complex*—pipiens, fatigans *and* molestus—*have similar "normal" levels of susceptibility to insecticides.* C. p. fatigans *larvae resemble those of anopheline mosquitos in sensitivity to dieldrin, but are more resistant to DDT; the adults are unusually resistant to chlorinated insecticides, but do not differ greatly from other species of mosquito in susceptibility to organophosphorus compounds.*

There is evidence of conversion of DDT to DDE in C. p. fatigans *and other mosquitos, but this may well not be the only cause of resistance. Dieldrin-resistance in* C. p. fatigans *involves cross-resistance to HCH, but at a considerably lower level; the mechanism of resistance is still obscure. Resistance to organophosphorus insecticides has been observed; the cross-resistance to diazinon indicates that the mechanism is not solely one of carboxyesterase detoxication.*

Mechanisms of inheritance of resistance have been investigated. The most recent results indicate the monofactorial inheritance of resistance by a single pair of nearly dominant genes on chromosome 2 for DDT-resistance and by a single pair of genes on chromosome 3 with intermediate dominance for HCH dieldrin-resistance.

NORMAL SUSCEPTIBILITY LEVELS

Many workers have reported the results of measurements of susceptibility or resistance to insecticides of field and laboratory strains of members of the *Culex pipiens* complex. Some 50 references have been consulted for the present survey, mainly those cited in issues of the *WHO Information Circular on Insecticide Resistance*. "Normal" susceptibility levels were assessed from the lowest values reported on the basis of adequate experimental data. After careful comparison of results for *pipiens*, *fatigans* and *molestus*, it was decided that all three forms have similar "normal" levels. This is supported by recent work in which field strains of *C. p. fatigans* inbred in the laboratory were found to have the same susceptibility as a *C. p. molestus* colony. LC_{50} values for various insecticides are given in Tables 1 and 2.

LC_{50} values, especially those obtained on field strains, are of limited value unless the homogeneity of the strains can be guaranteed. Some authorities prefer to use discriminating dosages (i.e., those giving a complete kill). The data in Table 3, obtained by Davidson (1964), give a clear comparison of the susceptibility characteristics of normal *C. p. fatigans* and anopheline mosquitos. The Elliott larvicide test was used and concentrations of insecticide required to kill all fourth-stage larvae 5 hours after a 1-h exposure were obtained.

These results suggest that *C. p. fatigans* larvae behave similarly to those of anopheline mosquitos in tolerance of dieldrin but are more sensitive to DDT. Adult *C. p. fatigans*, however, show exceptional tolerance of chlorinated insecticides. Most anopheline mosquitos are killed by 4% DDT or 0.4% dieldrin after a 1-h exposure in the standard WHO test (WHO Expert Committee on Insecticides, 1963). In contrast, to give a complete kill of *C. p. fatigans* requires 4 h with 4% DDT or 1 h with 4% dieldrin. It may be noted that, whereas there is a sharp division between normal and dieldrin-resistant *C. p. fatigans*, it is less easy to fix the discriminating dosages for DDT-resistance. Thus, the presence of dieldrin-resistance seems to increase tolerance to DDT, so that exposure to 4% DDT must be increased to 6 h, or even 8 h, to give a complete kill. (Truly DDT-resistant strains show negligible mortality after such exposures, however.)

In contrast to their reaction towards chlorinated insecticides, normal adult *C. p. fatigans*, do not

* Revised version of paper presented at Seminar on the Ecology, Biology and Control of the *Culex pipiens* Complex, Geneva, 1965; this Seminar was supported by the World Health Organization and by a United States Public Health Service research grant (EF. 00194.04) to the World Health Organization.

[1] Professor in Entomology as Applied to Hygiene, London School of Hygiene and Tropical Medicine, London, England.

TABLE 1

LC50 VALUES FOR DIFFERENT INSECTICIDES ON *C. P. FATIGANS* AND *C. P. PIPIENS* LARVAE [a]

Insecticide	LC50 (ppm)	Locality
	C. p. fatigans	
DDT	0.01	Fiji [b]
Dieldrin	0.004	
HCH	0.008	
Malathion	0.02	Upper Volta [c]
Diazinon	0.03	
Fenthion	0.002	
	C. p. pipiens	
Parathion	0.0045	USA [d]
Azinphos (Guthion)	0.007	USA [e]
Coumaphos (Co-Ral)	0.02	
Dichlorvos (DDVP)	0.005	
Dicapthon	0.018	
Fenchlorphos (Ronnel)	0.010	
Trichlorphon (Dipterex)	0.088	

[a] 24-hour larvicide tests of normal larvae by WHO method.
[b] Burnett & Ash (1961).
[c] Hamon (1960).
[d] Mulla, Axelrod & Isaak (1961).
[e] Sutherland & Darsie (1960).

differ in susceptibility to organophosphorus compounds from other species of mosquito. (Table 4).

EXTENT OF RESISTANCE

Some impression of the growth and extent of resistance will be gained from Table 5, which was compiled from information in reviews (Wood, 1959; Brown, 1961), brought up to date by that in issues of the *WHO Information Circular on Insecticide Resistance*.

TOXICOLOGY OF RESISTANCE

DDT-resistance

Since the normal susceptibility of *C. p. fatigans* adults is low, whereas that of larvae is high, it is evident that incipient resistance (e.g., resistance in a proportion of the population) will be much more evident in adults than in larvae. Although this may be important in practice, from the theoretical standpoint it appears that there is no evidence for independent resistance in the two stages.

TABLE 2

LC50 VALUES FOR DIFFERENT INSECTICIDES ON *C. P. FATIGANS* ADULTS [a]

Insecticide	LC50 (%)	Locality
DDT	3.7	Tanzania [b]
Dieldrin	0.46	Malaya [c]
Malathion	0.3-1.0	Tanzania [b]
	1.06	Upper Volta [d]
Fenthion	0.21-0.37	Upper Volta [d]

[a] 1-hour adulticide tests of normal adults by WHO method.
[b] Smith & Bransby-Williams (1962).
[c] Wharton (1955).
[d] Hamon & Sales (1963).

TABLE 3

SUSCEPTIBILITY TO INSECTICIDES OF *C. P. FATIGANS* AND ANOPHELINE MOSQUITOS

Species	Insecticide conc. (ppm) required to kill all fourth-stage larvae in Elliott test
	DDT
C. p. fatigans	5
Anopheles albimanus	20
A. sundaicus	50
A. stephensi	100
	Dieldrin
C. p. fatigans	1
A. albimanus	2
A. quadrimaculatus	0.2

TABLE 4

LC50 AND LC100 VALUES FOR MALATHION AND FENTHION ON DIFFERENT SPECIES OF MOSQUITO [a]

Species	Malathion		Fenthion	
	LC50%	LC100%	LC50%	LC100%
C. p. fatigans	1.06	3.2	0.21-0.37	0.8-1.6
Aedes aegypti	0.8-1.1	3.2	0.26	1.6
Anopheles gambiae	1.1	3.2	0.55	1.6
A. funestus	0.81	3.2	0.26	1.6

[a] Hamon & Sales (1963).

TABLE 5
GROWTH AND EXTENT OF INSECTICIDE RESISTANCE OF *CULEX PIPIENS* COMPLEX

Culex fatigans

DDT	HCH/dieldrin
1952 India	1951 California
1953 Réunion (Indian Ocean)	1953 India; Malaya
1956 Venezuela; Taiwan	1956 Singapore
1957 Okinawa	1957 West Africa
1958 Malaya; Panama	1958 Panama; Tanzania
1959 Hawaii; Congo; Brazil	1959 Brazil; Congo
1960 Upper Volta; Cameroon; Ecuador	1960 Cameroon
1961 Tanzania; Fiji; China	1961 Fiji; China; Trinidad
1962 Madagascar	1962 Madagascar
Organophosphorus:	1959 Cameroon 1965 Okinawa

Culex pipiens and *molestus*

DDT	HCH/dieldrin
1947 Italy	1950 Italy
1953 Ohio	1955 Israel
1954 Greece	1959 Japan
1955 Massachusetts	
1958 Israel	
1959 Japan; California	
1961 Illinois	
1962 Turkey	
1964 Bulgaria	
1965 Albania; Korea	

Bami et al. (1957) obtained evidence that both normal (S) and resistant (R) *C. p. fatigans* convert DDT to DDE. A dose of 1 μg per mosquito was applied and the quantities of DDE detected 24 hours later (by the Schechter-Haller method) were 0.36 μg and 0.57 μg in S and R strains, respectively. The dose used was 5 times the LD_{50} for the S mosquitos and only 1/5th of the LD_{50} for the R strain; thus, the extra conversion could have been a result of prolonged survival rather than a cause of resistance.

Hoskins, Miskus & Eldefrawi [1] examined the DDT-metabolites from larvae of S and R strains of *C. p. fatigans*, using radioactive chromatography. They found considerably more DDE in the R strain, 24-72 hours after treatment, and also indications of more polar metabolites. Once again, however, the difference could have been due to longer survival of the R larvae.

Perry (1960) investigated the uptake of DDT and the production of DDE in mosquitos in Greece and Italy. In tests on *C. p. molestus* (using the Schechter-Haller method), he obtained evidence of conversion to DDE, ranging from 46% of a 0.1-μg dose to 18% of a 0.37-μg dose. He was cautious about the relevance of this metabolism to resistance.

A little further light on the subject was thrown by the experiments of Kalra & Joshi (1962). They compared the susceptibility of various mosquito larvae to *pp'*-DDT and *op'*-DDT. Their normal strains were more susceptible to *pp'*-DDT, in conformity with data obtained on other insects. A resistant *Anopheles subpictus* colony, however, showed the opposite effect (i.e., *op'*-DDT was more toxic). They ascribe this to the probable specificity of a DDT-detoxifying enzyme. Both their laboratory strains and resistant field populations of *C. p. fatigans* showed greater susceptibility to *op'*-DDT and they suggest that this may be an anomaly of the species. But since the LC_{50} of their laboratory colony was 0.56 ppm, it seems likely that this was not a truly susceptible strain. The inference is that resistance in *C. p. fatigans*, too, may be due to a detoxifying enzyme specific for *pp'*-DDT. Furthermore, the failure to cope with *op'*-DDT is reminiscent of that of the DDT-dehydrochlorinase of resistant houseflies (Sternburg, Kearns & Moorefield, 1954).

Kimura, Duffy & Brown (1965) established the existence of a dehydrochlorinating system in larvae of both normal and resistant *C. p. fatigans*, as well as in resistant *C. tarsalis* larvae. The enzyme in the resistant *C. fatigans* was extremely efficient and could convert 99% of a 1-ppm dose of DDT to DDE. This compares with a 50%-72% conversion by resistant *Aedes aegypti* and one of 34%-45% by resistant *C. tarsalis*. To this extent, conversion efficiency paralleled levels of resistance; on the other hand, normal *C. p. fatigans* could convert twice as much DDT as resistant *C. tarsalis*, although the latter were over 20 times as resistant as the former.

HCH/dieldrin-resistance

The mechanism of resistance to HCH and the chlorinated cyclodiene insecticides is still obscure.

[1] Hoskins, W. M., Miskus, R. & Eldefrawi, M. E. (1958): *The biochemistry of DDT-resistance in insects.* In: *Seminar on the susceptibility of insects to insecticides. Report. Panama, 26-28 June 1958*, Pan American Sanitary Bureau, p. 239 (unpublished).

In *C. p. fatigans*, dieldrin-resistance always involves cross-resistance to γ-HCH, but at a considerably lower level. Thus, in Tanganyika, Tanzania, the LC_{50} values for adult *C. p. fatigans* were 0.4% dieldrin for 24 h and 0.16% γ-HCH for 1 h in one locality and 0.31% dieldrin for 24 h and 0.06% γ-HCH for 1 h in another.

Oonnithan & Miskus (1964) studied the metabolism of ^{14}C-labelled dieldrin in resistant *C. p. fatigans* adults. They found that 85% of a 0.04-μg dose was absorbed into the mosquito in two days. Radiometric measurements of paper chromatograms were made at intervals of 2, 6 and 9 days after treatment and showed a gradual decline in the amount of dieldrin, accompanied by a rise in a more polar metabolite (which had the same R_f value as aldrin glycol). Approximate percentages (read from their graph) were:

Days after treatment	*Dieldrin (%)*	*Metabolite (%)*
2	55	18
6	45	20
9	25	35

The authors note that 24%-43% of the applied dose was lost.

Interesting though these results may be, they do little to explain dieldrin-resistance because (*a*) there are no comparative data for susceptible mosquitos, (*b*) the metabolism of the dieldrin is slow compared to its toxic action, and (*c*) other work (on houseflies) has shown that strains with high levels of dieldrin-resistance do not differ in dieldrin metabolism from susceptible strains.

Organophosphorus-resistance

The first recorded values of organophosphorus-resistance were obtained from Cameroon (Mouchet et al., 1960), where LC_{50} values for malathion and diazinon against larvae were found to be 1.8 ppm and 1.65 ppm, respectively; these values compare with 0.023 ppm and 0.03 ppm for susceptible strains. Cross-resistance to diazinon suggests that the mechanism involved is not a carboxyesterase detoxication.

More recent records have been obtained from two localities in Okinawa, where LC_{50} values for malathion against larvae were reported as 1.6 ppm or more (source given as "AEHA" in *WHO Information Circular on Insecticide Resistance*, No. 54, p. 28).

Carbamate-resistance

In selecting laboratory colonies of *C. p. fatigans*, Georghiou (1965) failed to increase resistance to *m*-isopropylphenylmethyl carbamate more than 2- to 3- fold even after 50 generations. However, on selecting a strain from southern California that was known to be resistant to chlorinated insecticides, he increased the resistance to *o*-isopropoxyphenylmethyl carbamate 11-fold in 17 generations. Moderate (× 2 to × 8) cross-resistance was shown to other carbamates and also to some organophosphorus insecticides. A slight indication of specificity was shown in rather higher levels of resistance to ortho-substituted phenylmethyl carbamates.

Investigations of the detoxication powers of normal and resistant strains were made by subjecting larvae to ^{14}C-labelled *o*-isopropoxyphenylmethyl carbamate. Chromatograms of extracted larvae showed the presence of 12% metabolites from the susceptible strain and 30% from the resistant strain, a two-and-a-half-fold increase.

GENETICS OF RESISTANCE

DDT-resistance

The first investigation of the mode of inheritance of DDT-resistance was made by Pal & Singh (1958). Susceptibility levels of adults were determined by topical application tests and of larvae by 24-h exposures to DDT suspensions. Pal & Singh concluded that resistance was due to a single pair of nearly recessive genes and their results suggested some (maternal) cytoplasmic effect. However, examination of their data reveals considerable overlap between regression lines of the resistant and susceptible strains and there is some doubt whether the resistant colony was homogeneous.

The problem was further investigated by Rozeboom & Hobbs (1960), using strains from the Philippines and Texas. Inheritance patterns were judged by larval mortalities in the two colonies and in hybrids exposed to a single concentration of DDT (0.1 ppm). The results were consistent with monofactorial inheritance of a resistant gene with partial dominance; however, the authors conselled caution since they could not guarantee their resistant strain to be completely homozygous.

A further investigation was made by Davidson (1964), who selected and inbred his colonies to ensure their homogeneity. This enabled him to use discriminating dosages, both for larvae (using the Elliott test) and adults (using the Busvine-Nash method). His results indicate monofactorial inheritance of resistance by a dominant gene.

More recently, the matter has been further investigated in detail by Brown & Tadano (1965), using a strain from Rangoon, selected to a high level of DDT-resistance in the laboratory. By means of serial dosage tests on the larvae they obtained strong evidence that a single pair of nearly dominant genes governed resistance. Using genetically marked susceptible strains for backcrossing, they located the resistance genes on chromosome 2.

HCH/dieldrin-resistance

Pennell & Hoskins (1964) isolated resistant and susceptible strains of *C. p. fatigans* (= *C. p. quinquefasciatus*) from a heterogeneous colony, using discriminating concentrations on the larvae. By crossbreeding, hybrids of intermediate susceptibility were obtained. Repeated crossbreeding, with elimination of susceptibles, gave results indicating monofactorial inheritance. The ratio of the LC_{50} values for larvae of susceptible, hybrid and resistant types was 1 : 19 : 196.

The problem was investigated independently by Davidson (1964), who found it possible to use discriminating dosages with adults as well as with larvae. His results agree with those discussed above, indicating monofactorial inheritance with intermediate dominance.

These conclusions have been further confirmed by larval tests made by Brown & Tadano (1965), although the colony they used was not highly resistant (it was selected from an LC_{50} of 0.008 ppm to one of 0.023 ppm in 10 generations). By means of backcrossing with susceptible marked colonies they obtained evidence that the pair of genes governing resistance was located on chromosome 3.

RÉSUMÉ

Il résulte de la compilation des résultats de nombreux travaux que le taux « normal » de sensibilité aux insecticides est semblable chez les trois principales sous-espèces du complexe *Culex pipiens*: *pipiens*, *fatigans* et *molestus*. Les larves de *C. p. fatigans* ont la même tolérance à la dieldrine que les larves d'anophèles mais sont plus sensibles au DDT; les adultes montrent cependant une résistance exceptionnelle aux insecticides chlorés. Il faut remarquer qu'une distinction nette peut être faite entre *C. p. fatigans* normaux et résistants à la dieldrine, mais il est plus difficile de déterminer les degrés de résistance au DDT. Une résistance à la dieldrine semble augmenter la tolérance au DDT. Contrairement à son comportement devant les insecticides chlorés, *C. p. fatigans* adulte présente la même sensibilité que les autres espèces de moustiques envers les composés organophosphorés.

On a pu démontrer que *C. p. fatigans*, normal ou résistant, transforme le DDT en DDE, comme d'ailleurs d'autres espèces de moustiques. Une enzyme particulièrement active a pu même être mise en évidence chez *C. pipiens fatigans*. Le mécanisme de la résistance à l'HCH et à la dieldrine est encore obscur. Chez *C. p. fatigans*, la résistance à la dieldrine implique toujours une résistance croisée au gamma-HCH, mais d'un degré beaucoup plus faible. Une résistance aux insecticides organophosphorés a également été observée; en raison de l'existence d'une résistance croisée au diazinon, il semble qu'elle ne soit pas due à l'action d'une carboxyestérase. L'exaltation de la résistance aux carbamates est difficile à obtenir. Une légère résistance croisée entre divers carbamates et certains insecticides organophosphorés a pu être observée.

De nombreux travaux, utilisant des méthodes différentes, ont été consacrés aux problèmes posés par la transmission héréditaire de la résistance. Les résultats obtenus indiquent que la résistance au DDT se transmet à l'intervention d'une seule paire de gènes presque dominants, situés sur le chromosome 2. Quant à la paire de gènes responsables de la résistance au HCH et à la dieldrine, elle se trouve sur le chromosome 3.

REFERENCES

Bami, H. L., Sharma, M. T. D. & Kalra, R. L. (1957) *Bull. nat. Soc. India Malar.*, **5**, 236

Brown, A. W. A. (1961) *Bull. ent. Soc. Amer.*, **7**, 6

Brown, A. W. A. & Tadano, T. (1965) *WHO Information Circular on Insecticide Resistance*, No. 52, item 1

Burnett, G. F. & Ash, L. H. (1961) *Bull. Wld Hlth Org.*, **24**, 547

Davidson, G. (1964) *Ann. trop. Med. Parasit.*, **58**, 180

Georghiou, G. P. (1965) *Nature (Lond.)*, **207**, 883

Hamon, J. (1960) *WHO Information Circular on Insecticide Resistance*, No. 25, item 7

Hamon, J. & Sales, S. (1963) *Méd. trop.*, **23**, 621

Kalra, R. L. & Joshi, G. C. (1962) *Indian J. Malar.*, **16**, 327

Kimura, T., Duffy, J. R. & Brown, A. W. A. (1965) *Bull. Wld Hlth Org.*, **32**, 557
Mouchet, J. et al. (1960) *Méd. trop.*, **20**, 447
Mulla, M. S., Axelrod, H. & Isaak, L. W. (1961) *Mosquito News*, **21**, 216
Oonnithan, E. S. & Miskus, R. (1964) *J. econ. Ent.*, **57**, 425
Pal, R. & Singh, N. N. (1958) *Indian J. Malar.*. **12**, 499
Pennell, J. T. & Hoskins, W. M. (1964) *Bull. Wld Hlth Org.*, **31**, 669
Perry, A. S. (1960) *Bull. Wld Hlth Org.*, **22**, 743
Rozeboom, L. E. & Hobbs, J. (1960) *Bull. Wld Hlth Org.*, **22**, 587
Smith, A. & Bransby-Williams, W. R. (1962) *Bull. Wld Hlth Org.*, **27**, 603
Sternburg, J., Kearns, C. W. & Moorefield, H. (1954) *Agr. Food Chem.*, **2**, 1125
Sutherland, D. J. & Darsie (1960)In: *Proceedings of the 47th Meeting of the New Jersey Mosquito Extermination Association*, p. 139
Wharton, R. H. (1955) *Bull. ent. Res.*, **46**, 301
WHO Expert Committee on Insecticides (1963) *Wld Hlth Org. techn. Rep. Ser.*, **265**
Wood, R. J. (1959) *Ann. appl. Biol.*, **47**, 620