CURRENT TRENDS IN INSECTICIDE RESISTANCE IN VECTORS OF DISEASES

by

S. L. Perty and S. K. Ranganathan,

Defence Research Laboratory (Stores), Kanpur

ABSTRACT

The paper discusses in brief the various aspects of insecticide-resistance developed by insects and reviews briefly the current trends in insecticide-resistance in vectors of diseases.

Introduction

Insecticides play a role of unprecedented importance in improving public health by reducing the prevalence of insect-borne diseases such as malaria, filaria, yellow fever, typhoid, plague, typhus, dengue and many more which have been the scourge of mankind. The role centres round the interruption of transmission and spectacular success has been recorded in many parts of the world. Residual insecticides such as DDT, BHC, dieldrine and chlordane and more recently organic phosphorus compounds have been widely used in vector control programme during the last few years. Over this period evidence has been accumulating that nearly forty species of insects of public health importance have developed resistance to these insecticides. This number is continuously on the increase and public health authorities are therefore faced with the problem of counteracting this resistance so that the insects are still controlled by insecticides. The World Health Organization (WHO) has taken keen interest in this serious problem of insecticide-resistance and during the last few years has promoted research in the field in different countries with the cooperation of the Governments of the countries concerned. The seminars on the susceptibility of insects to insecticides, organised by WHO in Feb-March 1958 in New Delhi, and in Panama in June 1950 have served to focus attention on specific problems created by the insecticide-resistance. The reports of these two seminars (WHO)[1,2] as also the proceedings of the symposium on resistance of insects to insecticides (ICMR)[3] and the reviews published by Metcalf[4], Hoskins and Gordon[5], Kearns[6], Busvine[7], Hoffman[8], Crow[9], Milani[10], and Brown[11] contain a wealth of information on the subject.

Types of insecticide-resistance

Resistance of insects to insecticides can be defined (WHO[41]) as the development of anability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of insects in a normal population of the same species. There are, in general, two main types of insecticide-resistance. These are 'physiological resistance' and 'vigour tolerance'. The physiological type of insecticide-resistance is specific to a particular type of poison and generally results in very high degree of resistance to the insecticide. The serious threat to the control of insects of public health importance is believed to be, by and

large, due to this type of resistance. In vigour tolerance, there is general lowering of susceptibility to one or more types of insecticides depending on 'non-specific' defence machanism due to improved condition, body weight, etc. The tolerance should not be confused with variation in susceptibility due to temperature and humidity (Koshi and Ranganathan)[13,14]. The improved vigour may be due to environmental effects alone such as improved nutrition and is therefore reversible. Besides physiological resistance and vigour tolerance, mosquitoes may exhibit what is known as 'behaviouristic resistance', that is, develop ability to avoid a dose which would prove lethal. Again insecticidal deposits may have an irritant effect on some species of insects and this may keep them away from the treated surface.

The physiological resistance of insects to insecticides is by far the most important and from practical standpoint is essentially of three types (Brown[12]). The first is the 'DDT-resistance' which extends to methoxychlor and DDD. The second is the 'dieldrin or BHC-resistance' which extends to aldrin, endrin, chlordane, heptachlor and toxaphene. The third is the 'organophosphorus' resistance which is of recent origin. One encouraging feature is that the vectors show *generally* resistance to only one of these three groups of insecticides, sometimes to two, but not to the three groups simultaneously.

Insecticide-resistant strains of insects

Standard test methods for detecting and measuring physiological resistance in adult and larval mosquitoes and body lice have been developed by WHO. Techniques for other insects of medical importance are also being developed. Wright[15] and Busvine[16] have described the principles on which existing test methods are based. Several species of insects including houseflies, mosquitoes, human lice and bed bugs have developed resistance to one or more insecticides (Brown[12]) and it is in only three genera of medically important insects namely *Simulium Phlebotomus* and *Glossina*, resistance has not yet been reported (Brown[11]). In the present contributuion, information on the occurrence of insecticide-resistant strains of insects in different parts of the world is mainly drawn from the reviews by Busvine[7] and by Brown[12].

DDT-resistance—The resistance developed by the housefly *Musca domestica* to DDT has been spectacular and widespread. In 1945 there was phenomenal success with DDT for the control of the housefly and within five years this insect became so resistant to DDT that the insecticide had little value in many parts of the world. Resistance was first reported from Sweden in 1946 and it appeared in Italy and Denmark in the following year and by 1948 in all parts of Europe, United States and Canada. In *M. domestica vicina* it appeared in the Eastern Mediterranean region in 1949 and subsequently in tropical South America, Japan, Taiwan and East Africa. Resistance has not been reported in *M. vicina* or *M. nebulo* in India.

In the mosquito, *Anopheles sundaicus*, DDT-resistance was discovered in 1954 in Java and in 1957 in Akyab. In *A. stephensi*, it was first encountered in 1955 in Eastern Arabia and in 1957 in Persian Gulf, Iraq and Iran. In *A. subpictus*, it was reported from Delhi in 1955 although the susceptibility

to γ—BHC or dieldrin was normal. In *A. sacharovi* it has been reported from Greece. Lebanon and South Iran. In *A. maculipennis*, a fairly high tolerance has been shown in Greece, Central Iran and Italy. In the larvae of the salt-marsh species of mosquitoes, *Aedes taeniorhynchus* and *A. sollicitans* in Florida and irrigation-water larvae of *A. nigromaculis* and *A. dorsalis* in California high DDT-resistance was reported in 1948. In 1954 the larvae of the yellow fever mosquito, *Aedes aegypti*, in Trinidad were reported strongly resistant to DDT. DDT-resistance in the larvae and adults of the filaria mosquito, *Culex fatigans*, was first reported from La Reunion in 1951 and from Delhi in 1952 and in *C. tarsalis*, vector of encephalitis, it was first noted in California in 1948 and in Oregon in 1956. In *C. pipiens* it was encountered as early as in 1947 in Italy.

DDT-resistance in the bed bug, *Cimex lectularius*, was first observed in 1947 at Pearl Harbour Hawaii and subsequently in Korea, U.S.A. French Guiana, Israel, Lebanon and Iran. In *C. hemipterus* it appeared in Southern Taiwan and subsequenltly in Hong Kong, Singapore, Bengal, Bombay State and Somalia. The body louse, *Pediculus humanus corporis*, first showed resistance to DDT in South Korea and Japan in 1950-51 and subsequently in Hong Kong, Iran, Turkey, Syria, Jordan, Egypt, Libya, Sudan, Ethiopia, French, West Africa, South Africa, Chile and Peru. The human flea, *Pulex irritans* showed evidence of DDT-resistance in 1951-52 in Ecuador, Brazil, Peru, Greece and the Palestine region. Dog-flea, *Ctenocephalides canis*, and cat-flea, *C. felis* have shown DDT-resistance in Florida. DDT-resistance in the blue-tick, *Boophilus microplus*, appeared in 1954-55 in Australia and in cattle-tick, *B. decoloratus* in South Africa in 1956. In 1955, the American dog-tick, *Dermacentor variabilis*, has shown resistance in New Jersey.

BHC or dieldrin-resistance—BHC-resistance in the housefly, *M. domestica*, in California and in *M. vicina*, in Egypt, first appeared in 1949. Subsequently, it developed in other parts of the United States, Europe and Eastern Mediterranean region where it became added to the DDT-resistance already present but BHC-resistance alone has been reported from Hong Kong, Sudan and parts of Uruguay. Dieldrin-resistance in *M. domestica*, was first reported from Georgia and Arizona in 1950 and subsequently in England, Liberia, Japan and East Africa.

In the mosquito, *Anopheles gambiae*, dieldrin resistance appeared in Northern Nigeria in 1955 and was present in Upper Volta and in Liberia in 1957. In *A. quadrimaculatus*, it was discovered in larvae in Mississippi in 1955. In Greece, *A. sacharovi* has shown considerable tolerance to dieldrin. Strong dieldrin-resistance of adults of the mosquito, *Culex fatigans*, has been encountered in 1957 in Malaya and Liberia and in *C. tarsalis*, in California in 1951. Developed BHC-resistance in larvae of *C. fatigans* has been recorded in Georgetown, Kumbakonam (in Madras State) and French Guiana and dieldrin-resistance at Singapore.

Resistance to BHC and dieldrin was superimposed on DDT-resistance in the bed bug, *C. hemipterus*, in 1956 in Bombay and, dieldrin-resistance developed in Kenya and Tanganyika in 1957. BHC-resistance in the body louse,

P. humanus corporis, has appeared in Iran, Sierra , Leone, Japan, Hong Kong and South Africa. Resistance to chlordane and dieldrin has been encountered in the dog-flea, *C. canis*, and the cat-flea, *C. Felis*, BHC-resistance in blue-tick, *B. decoloratus* appeared in South Africa in 1948 and in the Southern cattle-tick-*B. microplus* in 1952. Resistance to toxaphene and dieldrin was recognized in *B. decoloratus* and *B. microplus* in 1952. The brown dog-tick, *Rhizocephalus sanguineus*, was found in 1955 to have developed resistance to chlordane in New Jersey and in 1956 in other parts of USA.

The German roach, *Blattella germanica*, has been reported to have developed considerable resistance to chlordane and dieldrin. Chlordane-resistance was first noted in Texas in 1951, in Southern USA in 1955 and by 1956 it had become prevalent in the Northern States and the Chicago area.

Organophosphorus-resistance—Resistance to organophosphorus compounds involving parathion, diazinon, and Resitox in the housefly, *M. domestica*, was first encountered in 1955 in Denmark and resistance to malathion was reported from Italy and Switzerland in 1956. In the mosquito, *C. tarsalis*, organophosphorus-resistance was first encountered in 1956 in California.

Behaviouristic-resistance—A striking example of behaviouristic resistance is that of *Anopheles albimanus*, to DDT reported in Panama in 1952. Such resistance has also been reported in *A. gambiae*, in Nigeria and Brazil, in *A. quadrimaculatus* in USA and in *A. superpictus*, in Turkey. *Aedes sollicitans* and *A. taeniorhynchus* in USA have also exhibited behaviouristic resistance.

Mechanism of insecticide-resistance

Earlier workers attempted to establish morphological or biological differences between susceptible and resistant strains. Length of life cycle, biotic potential, preferential resting habits, darker pigmentation, thicker cuticle and smaller diameter pulvilli have all been reported as related to resistance in insects but none seem to have universal applicability. The reduced rate of insecticide penetration has also been shown not to be the cause of resistance since similar resistance is evident when the insecticide is applied by injection. Of late, the rapid rise of resistant strains of insects from 2 in 1946 to 40 in 1958 has necessitated attention on several important fundamental aspects of the problem such as genetics, physiology and biochemistry of resistance. From the results of systematic studies undertaken by various workers it is possible to throw some light on the mechanism of insecticide-resistance and also to forecast measures to counteract resistance.

Genetics of insecticide-resistance—Studies on the inheritance of resistant factors have been carried out on a number of species of medical importance and the genetic concepts of insecticide resistance have been critically reviewd by Davidson [17], Crow[9], Milani [10] and Busvine [18]. It is now well accepted that inheritance of resistance is not sex-linked and is not due to induced mutation in insects caused by the insecticide. There is also no post-adaptation or habituation to the insecticide by the insects and the resistance is not due to the

acquired ability of the insects to adapt themselves to the changed environment. Recent researches (Milani [10] and Crow [9]) have shown that resistance is a pre-adaptive phenomenon and that the appearance of resistant strains of insects can be explained on the basis that few resistant individuals already existed in the population and by the use of insecticides gradual selection has been made in favour of such individuals. Thus the development of resistance is due to selection by insecticides of a few resistant individuals already present in the population. The selected individuals possess an innate ability to withstand the insecticide and this ability is acquired by breeding from those individuals which survive exposure to the insecticide (Hoskins and Gordon)[5]. Thus insecticides act as selective agents (Metcalf [4, 19] and Crow [9]) and not as mutagenic agents.

Spiller [20] has suggested that there are 'specific' genes for resistance. Until recently (WHO[21]) it was believed that DDT-resistance in *Musca domestica* was due to the accumulated action of a number of genes. It is now well established (Milani[10]) that the resistance is due primarily to a single gene in a given strain. The gene in the housefly is partially dominant for kill and is consistently on the recessive side for knockdown. DDT-resistance in *Anophelessundaicus* and *Aedes aegypti* is also due to a single factor that is recessive (Brown [12]). Dieldrin-resistance in *Anopheles gambiae*, has also been shown to be due to a single factor that is neither dominant nor recessive. Three possible genotypes-resistant homozygotes, intermediate heterozygotes and susceptible homozygotes—could be identified in a population, the first genotype being eight hundred times as resistant as the last. BHC-resistance in the housefly has been shown to have a separate genetic factor or rather origin from that of DDT-resistance and to-be due probably to more than one gene. Multiple genes are probably also involved in the BHC-resistance of *Anopheles sacharovi*.

Physiological and biochemical mechanisms of insecticide-resistance—Current knowledge of the physiological and biochemical aspects of insecticide resistance has been reviewed by Metcalf [19], Winteringham and Barnes [22], Hoskins and Gordon[5], Crow[9], Winteringham [23, 24], Brown [12] and Babers[25]. The main cause of DDT-resistance in the housefly is an increased rate of detoxification of the insecticide. DDT-resistant flies have been shown to metabolize or dehydrochlorinate this insecticide to its ethylene analogue, DDE, in proportion to the intensity of their resistance, (Sternburg, Kearns and Bruce[26], Perry and Hoskins [27], and Babers and Pratt[28]) whereas susceptible strains are either very slow or incapable of converting DDT to the non-toxic analogue. A part of topically applied DDT in a normal strain of the housefly is metabolized to DDE (Damodar, Dixit, Perti, Ranganathan and Srivastava,[29] and unpublished work in this laboratory). Sternburg, Vinson and Kearns[30, 31], and Kearns[6] have shown that the dehydrochlorination of DDT to DDE is due to an enzyme, DDT-dehydrochlorinase. This enzyme could be isolated and when activated by glutathione can produce DDE *in vitro*, in DDT-resistant but not in susceptible strains of flies. Miyake, Kearns and Lipke [32] consider that the internal tissues in houseflies have sufficient dehydrochlorinating ability to protect the vital site from DDT. The fact that DDT-dehydrochlorinase enzyme could be found only in the resistant strains of flies shows that dehydrochlorination is the chief mechanism in the resistance of flies to DDT. Thus the modern toxicological theory of insecticide-resistance almost exclusively relates the mechanism of insecticide-resistance to specific interference with biochemical systems, largely

enzymatic in nature. According to this theory detoxification systems have been developed by the insects at the same time that resistance builds up so that the insect can withstand doses of insecticides which otherwise would be fatal. Kerr, Venables, Roulston and Schnitzerling [33] suggest that the parent colony of the housefly contains a small proportion of DDT resistant individuals possessing the enzyme DDT-dehydrochlorinase and this proportion is increased either by selection with DDT or by slow pre-adult development.

Brown and Perry [34] have shown that resistant mosquitoes also dehydrochlorinate DDT to DDE. DDT-resistance in *Aedes aegypti* has been found associated with DDE production *in vivo* but not *in vitro*. The same occurs in an induced DDT-resistant strain of *Blatella*. Resistant *Pediculus* contain a DDT-dehydrochlorinating enzyme. The DDT-resistant *Aedes taeniorhynchus* larvae can produce DDE *in vivo* and DDT-resistant *Anopheles sundaicus* could produce DDE *in vitro* (Brown, [12]).

Oppenoorth[35] has shown that resistance to BHC in the housefly is due to an increased ability to detoxify the insecticide., Sternburg and Kearns [31] could not detect DDT-dehydrochlorinase enzyme in a BHC-resistant strain of houseflies. It has, however, been found that by some other mechanism BHC-resistant flies can detoxify or dehydrochlorinate BHC to *pentachlorocyclohexene* and subsequently to several water-soluble derivatives Brown [12]– Dieldrin-resistant strains of the housefly have been reported to excrete a sulphur analogue of dieldrin about twice as rapidly as a normal strain (Brown [12]) and this may be a factor in dieldrin-resistance.

Oppenoorth [36] has reported that a parathion-resistant strain of housfly detoxifies paraoxon (a toxin produced by the insect by oxidising the insecticide) more rapidly than a normal strain. Injected paraoxon was also removed more rapidly in the resistant strains. Lord and Solly [37], however, stated that a normal strain destroyed paraoxon as fast as a strain with twice the tolerance to organophosphorus.

It is believed (Bradbury, Campbell and O' Carroll[38]), that DDT-resistance is also achieved in part by changes in the quantity and nature of fatty tissues of the insect so as to direct the insecticide preferentially to fatty sites in the insect body where it cannot exert its toxic action. Brown[12] has stated that DDT-resistant strains of the housefly contain not only more total lipoid but also more tarsal lipoid than normal and this fat dissolves more DDT and contributes towards defence mechanism. A diazinon-resistant strain of the housefly has been shown to have twice the lipoid content of a normal strain.

Counteraction of insecticide-resistance

The results of field observations and laboratory investigations have shown that, wherever possible, control measures should be intensified to achieve eradication of the disease before the appearance of resistant strains of vector species. However, the development of resistance of insects to insecticides at an alarming rate necessitates synthesis of new residual insecticides which are more toxic to resistant strains of insects than to normal ones. This development appears

to be still confined to laboratory experimentation but there can be no doubt that such 'negatively correlated' insecticides (Mitlin, Babers and Barthel [39]) hold the key to most promising counter measures against insecticide-resistance in future. One such compound is cetyl bromoacetate. Ascher[40] had first tested this chemical on houseflies by a tarsal-contact method and found that the rate of knockdown was greater in strains selected for resistance to DDT and other insecticides than in susceptible strains. Other promising 'negatively correlated' compounds are *diisopropyl* tetrachloroethyl phosphate, and phenyl thiourea or phenyl thiocarbamide (Brown [12]). The development of synergists for insecticides against which resistance has developed is another landmark on the road to counteracting resistance. It has been found that the pyrethrin synergists, piperonyl cyclonene and piperonyl butoxide (WHO [21]) are effective in increasing the activity of DDT against resistant flies. The miticide DMC has also been found to be an effective synergist for DDT (Brown[12]). This increase in effectiveness has been shown to be due to the inhibition of the process of enzymatic detoxification.

The future

It appears appropriate to conclude this article with the observations made by the WHO Expert Committee on isecticides (WHO[41]) on the need for more intensified research on the subject– "In order to keep physiological understanding in step with resistance developments at the rate at which these are now occurring, it is estimated that at least some four or five times the present efforts would be required but lack of fundamental research on these problems is a hindrance to attainment of this objective at the presnt time". It will be appreciated that such future research is closely integrated and is always aimful.

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