Prevention and management of insecticide resistance in vectors and pests of public health importance

A manual produced by
Insecticide Resistance Action Committee (IRAC)

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IRAC, its aims and organization

Effective insecticide resistance management (IRM) is essential and the Insecticide Resistance Action Committee (IRAC) is dedicated to making this a reality. IRAC was formed in 1984 to provide a coordinated crop protection industry response to prevent or delay the development of resistance in insect and mite pests*. The main aims of IRAC are firstly to facilitate communication and education on insecticide resistance and secondly to promote the development of resistance management strategies in crop protection and vector control so as to maintain efficacy and support sustainable agriculture and improved public health. It is IRAC's view that such activities are the best way to preserve or regain the susceptibility to insecticides that is so vital to effective pest management. In general, it is usually easier to proactively prevent resistance occurring than it is to reactively regain susceptibility.

IRAC is an inter-company organisation that operates as a Specialist Technical Group under the umbrella of CropLife International. IRAC is also recognised by The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations as an advisory body on matters pertaining to resistance to insecticides. The group's activities are coordinated by the IRAC Executive Committee, IRAC International, and Country or Regional Committees with the information disseminated through conferences, meetings, workshops, publications, educational materials and the IRAC website (www.irac-online.org). IRAC International is comprised of key technical personnel from the agrochemical companies affiliated with CropLife through membership in the relevant National Associations (ECPA, CropLife America, etc). Current member companies are BASF, Bayer CropScience, Dow AgroSciences, DuPont, FMC, Sumitomo and Syngenta. The International Committee supports resistance management project teams and also provides a central coordination role to regional, country and technical groups around the world.

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* McCaffery A & Nauen R (2006). *The Insecticide Resistance Action Committee (IRAC): public responsibility and enlightened industrial self interest.* Outlooks on Pest Management 2, 11–14.

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1. Preface

Insecticide resistance is the selection of a heritable trait in an insect population that results in an insect-control product no longer performing as intended. Insecticides remain the mainstay of many tropical disease control programmes; therefore, the potential for such programmes to be compromised by insecticide resistance is of major concern. Although efforts are under way to develop new insect control products that will effectively control insect strains resistant to currently used insecticides, the need to protect and extend the useful life of current insecticides will remain. For this reason, resistance management must be given a higher priority in the decision-making process in vector-control programmes than is currently the case.

To establish effective long-term resistance management strategies it is necessary to consider many factors, for example, the regional availability of insecticides. This is not only achieved by making insecticides available but also by other factors, e.g., the development of monitoring programmes, training courses and educational material on disease prevention. In addition, it is essential that vector control programme managers are exposed to management principles in general, to ensure their proper implementation and surveillance. Of course new active ingredients with new modes of action would be most welcome in order to diversify the "tool-box" for vector control and to extend the life-cycle of all available insecticides, thus lowering the risk of the re-emergence of vector-borne diseases. Effective resistance management requires a sound understanding of the vector's biology and the monitoring of vector populations but also the detection, monitoring, and consequences of resistance as well as principles of resistance management. Efficient communication, effective outreach processes, dissemination of information and advice are essential to this understanding. This manual is a component of that process. It aims to introduce and reiterate the principles of resistance management to decision-makers and operators in the field of insect-vector control in a pragmatic rather than in a technical scientific manner.

IRAC International wishes to thank all colleagues for their valuable contributions to this manual either as authors or reviewers. The manual was completed and approved by the IRAC Public Health Team in Geneva, Switzerland during a meeting at the WHO Headquarters in August 2006.

For further information on the issues covered in this manual and a list of references, visit the IRAC web site at: www.irac-online.org, the WHOPES web site at: www.who.int/whopes and the Bill & Melinda Gates Foundation web site at: www.gatesfoundation.org.

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2. Introduction and objectives

2.1 Vector-borne diseases – a major public health problem

The socioeconomic burden associated with tropical diseases such as malaria, dengue, filariasis and trypanosomiasis is a serious impediment to development in many tropical countries, and most of these diseases are a major cause of poverty. It is estimated that malaria alone has reduced the gross national product of the African continent by more than 20% over the past 15 years. Vector-borne diseases account for a very significant part of total morbidity due to infectious diseases, and occur not only in the tropics but also in many temperate countries. For example, the recent progression of West Nile virus in North America, of Lyme disease in Europe, Chikungunya in the Indian Ocean and the worldwide spread of the vector *Aedes albopictus* (the "tiger mosquito") are serious and largely uncontrolled developments.

2.2 Vector control – a key component in managing vector-borne diseases

There is no effective drug or vaccine for important diseases such as dengue, dengue haemorrhagic fevers, and Chagas disease. The only way to control these diseases is to prevent transmission by insect vectors. Vector control, personal protection and community participation are the pillars of the WHO strategies for insect-transmitted disease control. Unfortunately, mass malaria chemo-prophylaxis cannot be implemented for technical and economical reasons, especially in Africa. The effective treatment of malaria cases is increasingly complex and expensive because of drug resistance. In high-transmission areas (which include most parts of Africa) malaria incidence cannot be reduced if, in parallel with early diagnosis and treatment, transmission is not controlled through very effective vector-control and/or personal-protection interventions. Vector control may also be important for diseases that are controlled primarily by preventive mass drug administration (MDA). The current strategy of the Global Alliance to Eliminate Lymphatic Filariasis is unlikely to achieve complete elimination of infection if MDA is not supplemented by transmission-control interventions in some areas. Many other examples that emphasize the need for vector control can be given for most tropical areas as well as developed countries.

2.3 The need for chemical control

Insecticides remain the most important element of integrated approaches to vector control. The recent restriction on the use of DDT by the Convention on Persistent Organic Pollutants (POPs) has dramatically underlined the high degree of reliance of malaria or leishmaniasis control programmes on residual insecticides such as DDT. To reduce this reliance, WHO is promoting integrated vector and pest management, including alternative measures such as biological control or environmental management when and where they are effective and applicable. WHO also promotes the safe and targeted use of insecticides when there is no alternative. For example, a very successful Chagas disease control programme in the Americas has been entirely based on indoor spraying of pyrethroid insecticides. Onchocerciasis (river blindness) has been successfully controlled for thirty years in eight countries of West Africa by weekly applications of safe larvicides. New technologies such as insecticide-treated bednets (ITNs) and insecticide-treated materials (ITMs) are now highly promoted and used to prevent diseases transmitted at night by mosquitoes and sandflies. Although applying insecticides on nets instead of walls is dramatically reducing the total amount of insecticide used for malaria prevention, ITNs remain highly dependent on a single class of insecticides; the synthetic pyrethroids. Most insecticides belonging to other chemical groups do not have all the required attributes in terms of efficacy and safety to be used on mosquito nets. The massive efforts currently developed to control malaria, especially in Africa, may be jeopardized by the widespread development of pyrethroid resistance.

2.4 The threat of insecticide resistance

Although public health accounts for only a very small fraction of overall insecticide quantities applied, many vector species of public health importance have already developed resistance to one or more insecticides. Development of resistance is a complex and dynamic process and depends upon many factors. Most commonly, when the frequency of resistant insects in a vector population increases, efficacy of the treatment decreases up to the point where the insecticide has to be replaced by another one. Increasing the dosages in an attempt to maintain efficacy is not a recommended option because of environmental and safety concerns, increased cost of the insecticide and the resistance genes can be driven to even higher frequencies. Replacing an insecticide with a new one has important cost, logistic and sociological implications that will be discussed later in this document. In addition, a significant reduction of morbidity and mortality can be achieved only if the efficacy of vector-control interventions is continuously maintained at a very high level. Almost all public health insecticides are also used in agriculture. When vectors breed within or close to agricultural crops, they can be exposed to the same or similar insecticidal compounds and develop resistance. This phenomenon is of particular relevance for malaria vectors. Moreover, many insecticides are also massively used to control domestic pests, and therefore, impact the vector species which are resting indoors. These so-called "endophilic" vectors are among the most dangerous ones because of their close contact with humans. It is common for a single vector-mosquito population to be exposed to a given insecticide (e.g. a pyrethroid) at the larval stage through agricultural spraying and then again at the adult stage through household pest control, as well as vector-control programmes.

2.5 A limited number of effective insecticides

Although there is a relatively long list of public health insecticide products that can be used to control adult vectors, these products are all members of a small number of chemical groups with discrete modes of action. The list is further shortened by similarities in the mode of action across some of these chemical groups and the phenomenon of cross-resistance. Cross-resistance explains why, in some situations, vector populations can develop resistance very rapidly to newly introduced insecticides. Furthermore, in some circumstances, resistance can persist in populations for very long periods after regular use of an insecticide has ceased. In these cases, resistance to new insecticides is inherited from the past as a result of the previous use of other insecticides. Such situations reinforce the importance of: i) understanding which target(s) insecticides are acting upon, and ii) precisely identifying the mechanisms involved once resistance has appeared in a vector population.

2.6 Need for concern about resistance development

Although there is no miraculous short-term solution to vector-resistance problems, it is important for programme managers to better understand resistance issues and to promote good practices in chemical-based vector control. It is essential to use public health insecticides in such a way that they are safe, effective, and affordable, while taking into account resistance issues. This is one of the conditions which vector-control programmes need to meet in order to be effective and sustainable. The relationship between vector resistance and the use of agricultural insecticides has been mentioned previously. It is very clear that closer collaboration between resistance experts in agriculture and public health is needed. Similarly, public health agencies can definitely benefit from the extensive experience gained by the agricultural sector in promoting integrated pest-management principles as well as developing and disseminating simple and pragmatic guidelines for insecticide-resistance management.

2.7 Target audience and objectives of this manual

This manual is primarily targeted at public health technicians, mosquito and other vector-control programme managers and policy-makers. Properly informing this audience on insecticide-resistance issues will strengthen their appreciation of why avoiding resistance is essential, and will increase awareness of the need to adopt and implement integrated vector-control approaches. At the same time, international agencies such as WHO and FAO, as well as academic institutions, in collaboration with state and commercial agricultural organizations and insecticide manufacturers and distributors, should mobilize resources to further develop and promote integrated vector-management principles, which include resistance management. A major focus of these efforts should be upon information dissemination and exchange, development of educational materials, training and capacity building.

The objectives of this manual are:

- to offer basic information on resistance mechanisms;
- to provide a better understanding of the factors that may lead to the development of vector resistance;
- to present the basic principles for avoiding the development of resistance.

3. What is resistance, and how does it develop?

3.1 Practical definition of resistance

There are many definitions of insecticide resistance – however the one promoted by the Insecticide Resistance Action Committee (IRAC) is probably the most pertinent to the management of a vector-control programme. IRAC defines resistance as the selection of a heritable characteristic in an insect population that results in the repeated failure of an insecticide product to provide the intended level of control when used as recommended. According to this definition, differences in susceptibility apparent in laboratory bioassays may not necessarily constitute resistance if the difference does not result in a change in the field performance of the insecticide.

In addition to the use of such a practical definition, it is also essential when considering resistance and its management to understand that resistance is a concept which applies to populations which are to a degree isolated from the remainder of the species concerned. In addition, resistance is a comparative term that relates the resistant population to a more susceptible normal population. Resistance does not imply that it is impossible to control the resistant population or to prevent disease transmission, or that the whole species becomes impossible to control. Thus a single report of resistance to an insecticide does not mean that the compound is no longer useful either within the local region or globally. This last point is exemplified by the situation in West Africa where, despite a very high frequency of a certain type of pyrethroid resistance among mosquitoes, malaria transmission can still be effectively prevented using pyrethroid-treated bednets.

3.2 Resistance mechanisms

The various mechanisms that enable insects to resist the action of insecticides can be grouped into four distinct categories:

3.2.1 Metabolic resistance

Metabolic resistance is the most common resistance mechanism that occurs in insects. This mechanism is based on the enzyme systems which all insects possess to help them detoxify naturally occurring foreign materials. Three categories of enzymes typically fulfill this function, namely esterases, monooxygenases and glutathione-S-transferases. These enzyme systems are often enhanced in resistant insect strains enabling them to metabolize or degrade insecticides before they are able to exert a toxic effect. One of the most common metabolic resistance mechanisms is that of elevated levels or activities of esterases, enzymes known to hydrolyze ester bonds or sequester insecticides. Nearly all of the strains of *Culex quinquefasciatus* which resist a broad range of organophosphate (OP) insecticides have been found to possess multiple copies of a gene for esterases, enabling them to over-produce this type of enzyme. In contrast, strains of malathion-resistant *Anopheles* have been found with non-elevated levels of an altered form of esterase that specifically metabolizes the OP malathion at a much faster rate than the normal ones. Metabolic resistance can therefore range from compound-specific resistances to very general resistances, affecting a broad range of compounds. Similarly, the level of resistance conferred can vary from low to very high and may differ from compound to compound. Metabolic-resistance mechanisms have been identified in vector populations for all major classes of insecticides including organophosphates, carbamates, pyrethroids and DDT (Fig. 1).

3.2.2 Target-site resistance

The second most common resistance mechanism encountered in insects is target-site resistance. Insecticides generally act at a specific site within the insect, typically within the nervous system (for OP, carbamate, and pyrethroid insecticides). The site of action can be modified in resistant strains of insects such that the insecticide no longer binds effectively at that site. The result is that these insects are unaffected, or are less affected, by the insecticide than are susceptible insects. For example, the target site for OP and carbamate insecticides is acetylcholinesterase (AChE) in the nerve cell synapses. Several mutated forms of AChE (also called MACE, modified acetylcholinesterase) have been found which result in reduced sensitivity to inhibition by these insecticides – resistance to OPs in *Culex* spp. e.g. typically results from this mechanism. Similarly, a mutation (known as *kdr*) in the amino acid sequence in the voltage-gated sodium channels of nerve cell membranes leads to a reduction in the sensitivity of the channels to the binding of DDT and pyrethroid insecticides. Resistance to pyrethroids conferred by *kdr* mutations has for example been confirmed in *An. gambiae* in West, Central and East Africa.

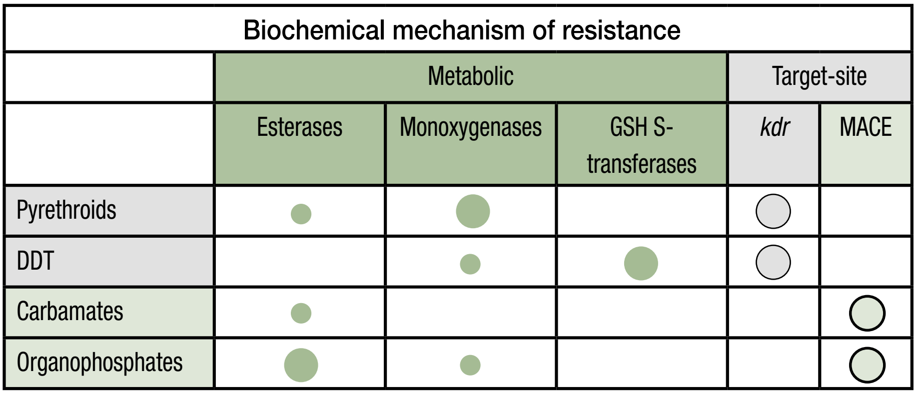
3.2.3 Reduced penetration

Modifications in the insect cuticle or digestive tract linings that prevent or slow the absorption or penetration of insecticides can be found is some strains of resistant insects. This resistance mechanism can affect a broad range of insecticides. Reduced-penetration mechanisms have been identified in houseflies but no other insect vectors, and are often considered a contributing factor rather than a powerful mechanism of resistance on its own.

3.2.4 Behavioural resistance

Behavioural resistance describes any modification in insect behaviour that helps to avoid the lethal effects of insecticides. Insecticide resistance in mosquitoes is not always based on biochemical mechanisms such as metabolic detoxification or target-site mutations, but may also be conferred by behavioural changes in response to prolonged spraying programmes. Behavioural resistance does not have the same importance as physiological resistance but might be considered to be a contributing factor, leading to the avoidance of lethal doses of an insecticide. A behavioural response is either dependent or independent on a stimulus. If mosquitoes avoid a treated place due to sensing the insecticide it is considered to be a behavioural change dependent on a stimulus, whereas the selective and sustained occupation of an untreated area can be considered as stimulus independent response.

Figure 1. Major biochemical mechanisms conferring resistance to important classes of insecticides in adult mosquitoes (dot size gives the relative impact of the mechanism on resistance)



Biochemical mechanism of resistance					
	Metabolic			Target-site	
	Esterases	Monoxygenases	GSH S-transferases	*kdr*	MACE
Pyrethroids	•	●		○	
DDT		•	●	○	
Carbamates	•				○
Organophosphates	●	•			○

3.3 Cross-resistance

Cross-resistance occurs when a resistance mechanism, that allows insects to resist one insecticide, also confers resistance to compounds within the same class, and may occur between chemical classes (depending on mechanism). The phenomenon of cross-resistance is a relatively frequent one in vector populations. For example, DDT and pyrethroid insecticides are chemically unrelated but both act on the same target site (sodium channel). Past use of DDT has resulted in several insect species developing resistance to DDT by the *kdr* mutation at the target site. Where these mutations have been retained in the population, the insects have some resistance to all pyrethroids in addition to DDT. Cross-resistance can also occur between OP and carbamate insecticides when resistance results from altered AChE (Fig. 1).

3.4 Multiple resistance

Multiple resistance is a common phenomenon and occurs when several different resistance mechanisms are present simultaneously in resistant insects. The different resistance mechanisms may combine to provide resistance to multiple classes of products. It is also quite common for the contribution of different mechanisms to change over time as selection processes evolve.

3.5 Genetic basis of resistance

The use of insecticides *per se* does not create resistance. Resistance occurs when naturally occurring genetic mutations allow a small proportion of the population (typically around 1 in 100 000 individuals) to resist and survive the effects of the insecticide. If this advantage is maintained by continually using the same insecticide, the resistant insects will reproduce and the genetic changes that confer resistance are transferred from parents to offspring so that eventually they become numerous within the population. This “selection” process is the same as that which drives other evolutionary changes. The process will take longer if the gene conferring resistance is rare or present at a low frequency. Resistance should not be confused with tolerance that can occur after sub-lethal exposure to insecticide and is not passed on to offspring.

Resistance genes can range from dominant through semi-dominant to recessive. If dominant or semi-dominant, only one parent must possess the trait for it to be fully or partially expressed in the offspring.

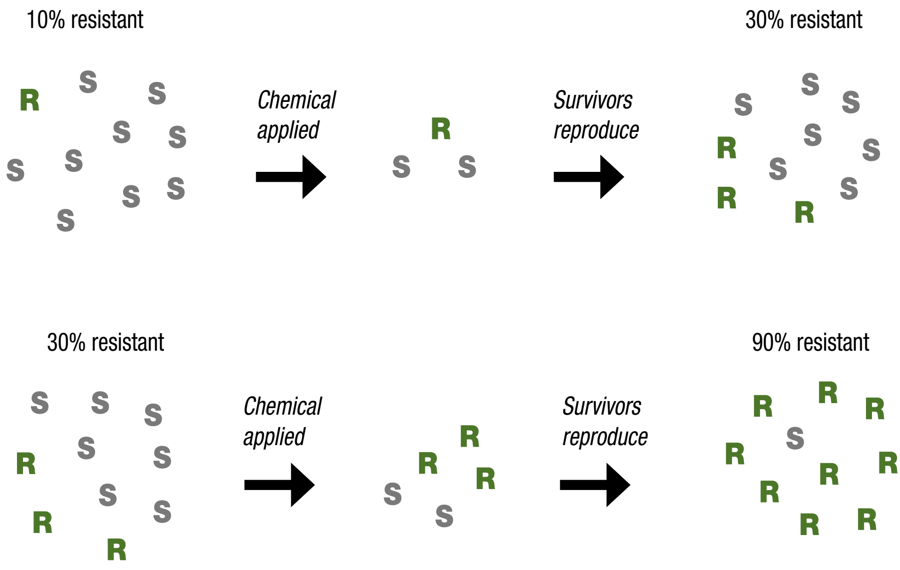
If recessive, both parents must possess the trait. Fortunately, most resistance mechanisms (for example *kdr*) are controlled by recessive or semi-dominant genes, which increases the chance of managing resistant populations. If the resistance is genetically dominant, it can rapidly become established within the population and be difficult to manage (Fig. 2).

3.5.1 Fitness cost

Populations of insects that have never been exposed to insecticides are usually fully susceptible, and resistance genes within those populations are very rare. This is usually due to a "fitness cost", which means that insects possessing the resistance gene lack some other attribute or quality such that it gives an advantage to the susceptible insects in the absence of the insecticide. Differences in the number of offspring, longevity or overall robustness can often be measured in resistant insects. There is good laboratory and field evidence to suggest that the absence of selection pressure (in the form of insecticide treatment) in most cases operates against resistance. Resistant colonies in the laboratory often revert to susceptibility if the insecticide selection pressure is not maintained. Similarly once resistance in the field has been selected it often rapidly reverts once the insecticide treatment regime is changed. A good example of this occurred in *An. arabiensis* in Sudan, where malathion-specific insecticide resistance was selected in the early 1980s through antimalarial house spraying. The development of resistance prompted a switch of insecticide treatment to fenitrothion and the malathion resistance rapidly reverted over the next few years.

It is this reversion to susceptibility which is the underlying assumption behind any effective resistance-management strategy. However, reversion rates are potentially variable and may be very slow, particularly when an insecticide has been used for many years. If there is no fitness cost for the resistance there is no reason for the resistance genes to be lost in the population and for resistance to fully revert. For example, DDT was used extensively for malaria control over a 20-year period up to the 1960s in Sri Lanka to control *An. culicifacies* and *An. subpictus*. DDT was replaced by malathion in Sri Lanka in the early 1970s when a total and effective ban on DDT use was implemented. Subsequent regular monitoring has shown that DDT resistance has reverted very slowly towards susceptibility. Around 80% of the adult mosquito population was resistant in the 1970s compared to about 50% in the 1990s. This rate of reversion is clearly too slow to establish any effective resistance-management strategy involving the reintroduction of DDT.

Figure 2. Possible scenario for resistance development



3.6 Major factors that influence resistance development

3.6.1 Frequency of application

How often an insecticide or control tactic is used is one of the most important factors. With each use, an advantage is given to the resistant insects within a population. The rate of increase of resistance on any population will be faster in the presence of a lower fitness cost.

3.6.2 Dosage and persistence of effect

The duration of effect or persistence of an insecticide is affected by the physical chemistry of the insecticide, the type of formulation, and the application rate. Products which provide a persistent effect can be considered to act in a similar manner to multiple treatments in that they provide continual selection pressure. For example, a space spray will persist for a very short time and will select only against a single generation of mosquitoes. In contrast, a residual wall application or a bednet treatment will persist for months or years and therefore can potentially select against many generations of the same insect. It is therefore important to always follow WHO or manufacturer recommendations and to use products at full recommended rates.

3.6.3 Rate of reproduction

Insects that have a short life-cycle and high rates of reproduction are likely to develop resistance more rapidly than species which have a lower rate of reproduction because more generations and more insects may be rapidly exposed to an insecticide application. Mosquitoes have a history of insecticide resistance and are characterized by a relatively short life-cycle and high fecundity, with females laying several hundred eggs during their reproductive life. In contrast, the tsetse fly does not typically resist insecticides and has a longer life-cycle and relatively low rate of reproduction, with females producing in total fewer than 10 larvae.

3.6.4 Population isolation

With disease vectors, the goal is often to eliminate all or most of the population but the more selection pressure that is put on a population, the faster resistance will evolve. The immigration of individuals possessing susceptible alleles from untreated areas will beneficially dilute and compete with resistant-insect alleles in treated areas. An early step in vector-control programmes should therefore be to identify the source of the vectors and to estimate the significance of immigration of untreated insects. For instance, an island where the entire area was receiving treatment would be at very high risk of developing resistance. Awareness of, and coordination with, other vector-control programmes and agricultural activities should occur so that the regional effect on the target population is considered.

4. Different approaches to resistance management

4.1 Approaches to resistance management

Resistance management can be attempted using insecticide-based approaches in conjunction with other non-insecticidal vector-control methods (integrated vector and pest management; see also chapters 5.2 and 10.3). In practice, many integrated control programmes work well in experimental trials, but become inoperable when scaled-up into long-term control programmes. Operationally, the simplest form of resistance management is likely to be insecticide-based, and this could take several forms.

4.1.1 Rotation

Rotational strategies are based on the rotation over time of two or preferably more insecticide classes with different modes of action. This approach assumes that if resistance to each insecticide is rare then multiple resistance will be extremely rare. Hence, any resistance developed to the first insecticide will decline over time when the second insecticide class is introduced. The time frame for rotation needs to be sufficiently short for resistance to still revert rapidly after it has been selected for. Although with most vector-borne disease-control programmes annual rotation is practical, the rotation of several classes of insecticides (with different modes of action) within a growing season is practised in many agricultural cropping systems.

4.1.2 Mixtures

The use of mixtures to avoid the development of antibiotic (and plant-pathogen) resistance is common. Once again, the theory is that if resistance to each of the two insecticide compounds within a mixture is rare then multiple resistance to both will be extremely rare. This approach is unlikely to be successful if resistance to one of the insecticides used is already present at a detectable level. The use of tank mixes is a relatively easy resistance-management tactic to implement and can have other benefits in terms of an improved spectrum of activity, particularly in agricultural systems. However, for mixtures to work well in practice both insecticides need to be used at their full operational target dose, and the efficacy and persistence of the two insecticides should be broadly similar. Tank mixing of products is rarely adopted in vector-control programmes on grounds of cost, safety and the limited number of recommended compounds. However, this should not preclude further investigations of the use of mixtures as a means of managing resistance in vector populations in future.

4.1.3 Fine-scale mosaic

Spatially separated applications of different compounds against the same insect constitute a “mosaic” approach to resistance management. Fine-scale mosaics can be achieved in vector-control programmes, for example, by using two insecticides in different houses within the same village. This creates the potential for insects within a single generation to come into contact with both insecticides, and would reduce the rate of resistance selection – provided that multiple resistance within the vector population was extremely rare. If such a fine-scale mosaic is to be used, careful records of which insecticide was used in each house are essential. Research is currently under way looking at mosquito nets treated with two insecticides with differing modes of action. This achieves a similar mosaic effect to treating houses with different compounds but on a much finer scale.

4.2 Resistance management and mode of action

In order to successfully develop and implement rotation, mixture or mosaic resistance-management strategies, knowledge of the mode of action and/or chemical class of the available insecticide products is essential. Although legislation generally requires the specific and common chemical name to be included on product labels, the chemical class and mode of action are not usually provided. More typically, the information is provided in commercial technical bulletins. One way to determine the mode of action, is to look up the chemical name in the IRAC MOA Classification Scheme which can be found on the IRAC website (www.irac-online.org). An online eTool is also available and further details are given in section 4.3 below.

Although compounds within the same chemical class (such as carbamates, OPs, or pyrethroids) will all have the same mode of action, there may be many different commercial products within a single chemical class. Thus all pyrethroids have the same mode of action and belong to the same chemical class. Rotating from one pyrethroid compound to another simply exposes the population to a single mode of action, and has no value in resistance management. In fact, selection of resistance will be increased. Theoretically, rotation within a chemical class or mode of action could take place in a situation where a single compound was unaffected by a resistance mechanism that affected all other compounds in that class or mode of action. However, these situations are not common and require a detailed understanding of the resistance mechanisms. It is therefore almost always better to rotate to different modes of action regardless of the mechanism of resistance.

Insecticides are applied against both adult and juvenile stages of a number of dipteran public health and vector pests. Where this is common practice, a rotational system should be established to avoid exposure of both life stages to the same mode of action.

4.3 The IRAC mode of action classification scheme

IRAC has recently worked with several government agencies to develop a comprehensive mode of action classification system (shown in Table 1 and available on IRAC´s web site at: www.irac-online.org) with the eventual goal of including such information on all product labels. The system lists all of the current known insecticide modes of action (designated by a unique number) along with the chemical classes in use, and examples of the active chemicals that belong to each class. By searching on the chemical name of the compound it is therefore possible to determine its mode of action (this can easily be done on the IRAC web site by using a tool called eClassification). There may be chemical subgroups (designated by letter) that have the same mode of action but are chemically different and so are not likely to lead to cross-resistance. For example, the OPs (1A) and the carbamates (1B) have the same mode of action, but there is not always cross-resistance to the two groups, especially when metabolic resistance is involved. In certain circumstances class 1A and 1B products could be rotated (as opposed to products in the same class which couldn't). If rotating entire classes is not possible, then subclasses within a mode of action class are preferred instead of using the same chemical.

4.3.1 IRAC mode-of-action label statement

It is proposed that product labels will at a minimum show the chemical group and type of material as shown – individual companies can choose to add more detail.

CHEMICAL GROUP	1A	INSECTICIDE

For resistance-management purposes, each insecticide product (X) will belong to one chemical group (e.g. OP´s). A given insect population may contain individuals naturally resistant to X (and to other OP-group insecticides) and these individuals will become the dominant type if such insecticides are used repeatedly. Eventually these resistant insects may not be controlled by X (or any other OP insecticides), and local experts and commercial distributors should be consulted for local resistance management recommendations. Although the classification scheme shown in Table 1 is based on mode of action, resistance in insects and mites to insecticides and acaricides, respectively, can also result from enhanced metabolism, reduced penetration or behavioural changes as outlined in chapter 3. These are not linked to any site-of-action classification, but are specific to chemical classes and sometimes even to individual chemicals. Despite this, alternation of compounds from different chemical classes remains a viable management technique, and to delay insecticide resistance:

- avoid exclusive repeated use of insecticides from the same chemical subgroup;
- integrate other control methods (chemical, cultural, biological) into insect-control programmes.

4.4 Summary points

- Successful resistance management depends upon reducing the selection pressure exerted by a particular mode of action or chemistry on a population.
- Selection pressure can be reduced through a number of strategies, including rotation, the use of insecticide mixtures, and mosaic applications.
- The IRAC mode of action classification scheme is an up-to-date and accurate guide which may be used in formulating resistance-management guidelines.

Table 1: IRAC Mode of Action (MoA) Classification for active ingredients useful in vector control[1]

Primary target site of action	Group	Subgroup	Chemical subgroup	Examples
Acetylcholinesterase inhibitors	1	A	carbamates	bendiocarb, propoxur
		B	organophosphates[2]	fenitrothion, pirimiphos-methyl, malathion, temephos
GABA-gated chloride channel antagonists	2	B	fiproles	fipronil
Sodium channel modulators	3	B	DDT, pyrethroids and pyrethrins	allethrin, bifenthrin, lambda-cyhalothrin, alpha-cypermethrin, deltamethrin, cyfluthrin, permethrin, etofenprox, phenothrin, transfluthrin
Nicotinic acetylcholine receptor agonists	5		spinosyns	spinosad
Juvenile hormone mimics	7	A	juvenile hormone analogues	methoprene, hydroprene
		C	pyriproxyfen	pyriproxyfen
Microbial disrupters of insect midgut membranes	11	A1	*Bacillus thuringiensis var. israelensis*	
		A2	*Bacillus sphaericus*	
Inhibitors of chitin biosynthesis	15		benzoylureas	diflubenzuron, triflumuron, novaluron

1. Including larvicidal and adulticidal insecticides. This mode-of-action classification is edited and updated yearly to include new products; please refer to www.irac-online.org for the complete mode of action list.

2. Not all compounds within the OPs are cross-resistant. Different resistance mechanisms that are not linked to target site of action, such as enhanced metabolism are common for the OPs (Fig. 1). Some of these metabolic resistance mechanisms are sometimes specific to a particular subgroup or particular compounds within the OPs. As a result, there are proven examples of the successful management of resistance to a particular compound or subgroup of compounds within the OPs using OP compounds from a different subgroup.

5. Resistance-management basics

5.1 Lessons learned from agriculture

The most basic and fundamental lesson learned about resistance in agriculture and public health is the need to carefully manage the selection pressure exerted by the insecticide on the insect. Resistance arises where insect populations are subjected to high selection pressure resulting from excessive exposure to a specific insecticide or chemical class of insecticide. Most growers base their choice of insecticide on grounds other than resistance-management concerns. Decisions are instead frequently based upon (short-term) economic interests, while worker safety, ease of use, supply, and concerns about the environmental impact can also influence product choice. The end result of applying such criteria is often similar with a single product or chemical class used continually in an unsustainable manner. When resistance to the compound develops, the cost or benefits associated with choosing a replacement product may be much less attractive to the grower. It is nearly always true that sustainable approaches to pest control are more cost-effective in the long term, although they may appear slightly more expensive in the short term. Prevention is better than cure and it is better to have a strategy to minimize the chance of resistance occurring rather than leaving it to chance.

Many factors contribute to the speed at which resistance can arise:

- Insects with multiple generations per year and high reproductive capacity represent a higher risk than those producing single generations per year.
- The chemistry of the insecticide, the type of formulation and its usage pattern will also affect the rate at which resistance develops. For example, resistance will generally develop more rapidly to products which have a persistent effect, or which require repeated application, than to those which are not persistent and are applied infrequently.
- The resistance history of an insect species also gives a reliable indication of the potential for future resistance problems. History shows that aphids, whiteflies and mites have a higher capacity for developing resistance than other insect groups. Characteristically, they have many generations per season, a high reproductive capacity, often a narrow host range, and in many agricultural situations they

develop as local populations with limited opportunities for gene mixing. In public health both mosquitoes and flies have similar characteristics to these agricultural pests and are able to develop resistance to frequently used products and insecticide classes.

5.2 Resistance-management tactics

5.2.1 Pre-launch tactics

A risk analysis can be undertaken to determine the risk of the pest becoming resistant. This analysis needs to be based on a range of factors including the mode of action of the product, the chemical properties of the product and its formulation, the past history of resistance in the target pest, the biology of the pest and the proposed usage pattern of the product. Based upon the outcome of this exercise and the degree of conservatism taken in its interpretation, an appropriate management strategy for the product can be developed. If the assessment suggests that there is a high risk of the pest developing resistance, it is best to design a management programme that incorporates various chemical and non-chemical methods of control. It is important to stress that resistance management programmes are most effective if implemented before resistance develops or when resistance-gene frequency is still very low.

5.2.2 Monitoring and baselines

Where resistance is likely to occur it is desirable to define dose-response relationships between the pest and the product at an early stage, especially before introducing a new mode of action or chemistry. WHO has developed bioassays to establish a baseline, and this should be used as a reference point for future monitoring. Once the baseline is established, regular monitoring of field performance should be carried out. If any change in performance occurs, tests should be made and results compared to the baseline to confirm that resistance is the problem and that other factors have not influenced the result. Once resistance is confirmed, then tactics should be developed that result in the selection pressure caused by that insecticide (or family of insecticides) being reduced or removed all together. This is a key feature of a management strategy. (To be really effective the tactics to be used once resistance is detected should have been put into practice prior to the problem developing.)

5.2.3 Complementary measures

A resistance-management strategy includes complementary measures or “modifiers”. A modifier is any type of practical measure used to reduce the risk of resistance occurring. These can be based on chemical measures such as changes in usage pattern (e.g., restricting the number of applications per season, or alternating with other modes of action) or non-chemical measures such as environmental management (e.g., the removal of mosquito breeding sites, or conservation of refugia).

5.3 Implementation

Communication and education are probably two of the most important factors in the successful implementation of a resistance-management programme. Information must be available to the people who make the choice of product in order to influence and inform this decision. Successfully implemented management schemes in agricultural systems have been characterized by well-established and efficient infrastructures through which information can be disseminated. In vector management, WHO, government agencies, and manufacturers should be able to offer technical support, training and information through workshops, meetings and literature to ensure that operators and local officials fully understand the principles and practice of resistance management with regard to insect vectors. A network of trained staff from the product-manufacturing companies should also be able to provide professional advice on the correct use of the product and to define resistance-management programmes. Product manufacturers should ensure that product labels are available in local languages and are clear and simple irrespective of application method or usage pattern. Similarly, literature containing technical information on resistance management, with examples of treatment programmes, should also be available from manufacturers.

5.4 Monitoring after launch

This stage consists of a number of elements:

- Tracking efficacy in commercial-use trials.
- Following up on reported poor performance and field failures. When other factors which might have caused product failure or reduced effectiveness have been eliminated (see Chapter 8), resistance should be investigated as a possible cause. For example in the case of mosquitoes they should be collected and tested using the same WHO-recommended methods used to establish the baseline susceptibility.
- In areas where vectors have a high probability of developing resistance it may be possible to instigate some selective monitoring during the season using diagnostic concentrations (see Chapter 7).

- Reporting: all cases of confirmed resistance in the field should be documented, mapped and information made available to the relevant local authorities and WHO.

5.5 What to do if resistance is found

The course of action to be taken will depend upon the circumstances. Where appropriate, modifications can be made to the resistance-management strategy and may include further restrictions on frequency of use, rotation with different products or restriction of product use to maintain efficacy and to allow resistance to regress. This may allow for reintroduction of the product in the future. In general, the following actions should be considered:

- Use products judiciously and preferably within a system of integrated pest management.
- If resistance is detected, confirm the data with subsequent tests and rule out misapplication or other causes of treatment failure.
- Assess the extent of the problem area, even though for vector control this may be difficult for many reasons.
- Notify WHO and regional authorities.
- Notify the manufacturer of the product.
- Determine the root cause of the resistance.
- Develop a remedial programme in conjunction with national authorities, WHO and the manufacturer.

In agricultural cropping systems the source of selection pressure on the insect population is generally clear. However, the situation is much more complex in vector control where vectors may encounter insecticide used not only for disease control but also against agricultural or domestic pests. A good understanding of vector behaviour is needed to allow the relative importance of public health and agricultural selection to be calculated. For example, in Sri Lanka, *An. culicifacies* (an indoor-resting, non-rice-field breeder) is unaffected by insecticides used for the control of rice pests, while in *An. subpictus* and *An. nigerimus* (indoor- and outdoor-resting rice-field breeders respectively) resistance is primarily selected for by agricultural insecticides. In this scenario, resistance management aimed at *An. culicifacies* could be undertaken purely within the public health sphere, while management of resistance in the latter two species would need a collaborative effort between the vector-management and agricultural sectors.

5.6 Successful resistance management in the agricultural sector

One of the most successful examples of resistance management can be found in the major cotton-growing areas of Australia. Over the years, the cotton bollworm, *Helicoverpa armigera*, developed resistance to many insecticides. An intensive programme of research resulted in the identification of the parameters involved in resistance build-up, and the development of management principles that are reviewed annually and updated by the local departments of agriculture. The key recommendations of their Insecticide Resistance Management Strategy (IRMS)[1] are shown below, and include both chemical and non-chemical modifiers:

- Plough in cotton and alternative host-crop residues as soon as possible after harvest to destroy over-wintering pupae.
- Use recommended larval thresholds to minimize pesticide use and reduce resistance selection.
- Avoid using broad-spectrum sprays such as OPs or pyrethroids early in the season in order to preserve beneficial arthropod populations.
- Rotate chemistries to avoid continuous sprays of any one chemical group. Do not exceed the maximum acceptable number of applications per season as indicated on the Cotton Resistance Management Strategy chart 1.
- Do not respray an apparent failure with a product in the same mode-of-action group – unless the failure is clearly due to factors such as poor application or timing, etc.
- Comply with any use restrictions placed on insecticides used on crops other than cotton for the purposes of managing resistance.

Resistance-management guidelines developed by IRAC are also intended to provide a technically sound foundation for local resistance-management/IPM (Integrated Pest Management) programmes. A good example of this is provided by the guidelines developed for resistance management in spider mites in top fruit that have now been adapted and integrated into regional IPM programmes in Europe. The guidelines were based upon product rotation for a number of reasons, including cost and the requirement for mixture components to have equal efficacy and persistence – a factor that commonly rules out the use of mixtures as an effective resistance-management tool.

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[1] Details can be found at www.cotton.crc.org.au

Groupings of compounds not subject to cross-resistance were proposed following extensive literature searches, consultation with independent experts and the combined experiences of the companies represented on IRAC. Subsequent amendments were made following an IRAC-sponsored research programme at Cornell University and following the introduction of the mitochondrial electron transport inhibitor (METI) acaricides.

The guiding principles to be used in conjunction with the product groupings are:

- Not more than one compound from any group should be applied to the same crop in the same season.
- Any one compound should be used only once per season on any one crop, and although mixtures of acaricides from different groups may be used, the use of mixtures of products from the same group is not recommended.

These relatively simple principles were effectively communicated through advisory services, product literature and product labelling and were implemented in a number of European fruit-growing regions.

5.7 Summary points

- Prevention of resistance is better than cure.
- Resistance-management strategies should be developed before control programmes are started.
- Deliver the "correct" dose to the target insect.
- Use both chemical and non-chemical methods for control.
- If resistance occurs take immediate steps to contain it and reduce the selection pressure produced by the product.

6. The economics of resistance management

6.1 Short-term economics

Choosing the most cost-effective vector-control solution is not a simple process, even when resistance management is not a prime consideration. The cost of insecticide frequently represents the single greatest cost associated with a vector-control programme, regardless of the control tactic adopted. For this reason, the considerations governing product choice are most frequently economic rather than biological.

The wide price range of different products does not help this process. For example, some carbamate products can be up to 15 times more expensive than DDT on a cost-per-kg basis. However, cost calculations need to take into account more factors than just the cost per kilogram of formulated product. For example to calculate the insecticide requirements of an indoor residual spray programme, it is necessary to take into account the application rate, size of house, number of houses, formulation strength, persistence of effect, and the number of applications required. Short-term costs can be minimized by optimizing product selection and control strategy using a detailed understanding of the local situation with respect to disease epidemiology, vector biology and prevalence, product behaviour on local surfaces, and importantly the resistance status of the vector population.

The fact remains however that cost is one of the key factors affecting product choice, especially when decisions are made where the tenure and performance appraisal of officials (and planning and funding cycles) are relatively short-term. The future benefits of maintaining susceptibility within the vector population are not readily apparent in the face of the immediate budgetary and logistic considerations facing the managers of vector-control programmes. In a situation where the consequences of choosing inappropriate products are unlikely to be apparent for a number of years, a longer-term approach to decision-making is essential in order to increase the chance of success.

6.2 The economics of adopting a resistance-management strategy

In order to be effective, resistance-management programmes must result in the reduced use of at least one compound over the short term. Depending on the situation and management programme, it has been estimated that by halving the number of applications of a given compound or chemistry (for example by reducing the treated area by half or by halving the number of treatments per season), the effective life of that compound will be at least doubled. This obviously entails the use of alternative, possibly more costly, compounds in order to maintain the required level of insect control.

Any potential short-term financial advantages of relying on a single compound or chemistry will inevitably be lost when resistance necessitates switching to more costly resistance-breaking compounds. This cycle will continue, until all effective chemistries are exhausted. Although a rotation strategy may have higher immediate costs, as the more costly compounds are integrated into the programme at an earlier stage, such a strategy will be sustainable for a longer period. Long-term expenditure is ultimately lower than when no resistance-management strategy is adopted, and the effectiveness of the compounds is preserved, avoiding the massive financial implications of repeated control failures. In effect, the cost of any insecticide used against today's susceptible insects should be increased to account for the increased costs associated with future failures due to resistance. A comparison of programme costs with or without resistance management is shown in Figure 3.

6.3 The economics of failing to manage resistance

One of the principal reasons for engaging in resistance management is that insecticide resistance reduces the effectiveness of insect control. The economic consequences of failing to address this are readily seen in agricultural situations where the commercial value of the food or fibre makes calculations of the cost/benefit of inputs relatively straightforward. The consequences of reduced yields or increased costs arising from the failure to effectively control resistant insects are both immediate and apparent.

This is well illustrated by the failure of the cotton industry in the Ord River valley region of Australia to address rising DDT resistance in *Helicoverpa armigera* in the 1970s. Over a period of 4 years the cost of insecticide applications for *H. armigera* control increased by more than 3 fold. As a result, cotton production was not economically viable and was abandoned. Interestingly, the collapse of the industry and the disastrous effects on the local economy were subsequently a major influence in the successful implementation of pyrethroid resistance management programmes in the Australian cotton industry during the 1980s – these programmes contributing significantly to the expansion of the industry during this period.

Concurrent with the Australian experience, a very different situation arose in the Thai cotton industry. A programme to manage pyrethroid resistance in *H. armigera* was not effectively implemented and growers were forced to abandon cotton production, resulting in the collapse of the country's cotton industry at a time of increasing demand. Other, similar events occurred in Mexico and in Texas, USA.

Similar situations have also occurred in public health. For example, DDT was spectacularly successful in controlling malaria transmission by *An. stephensi* in Pakistan during the early 1960s. Although resistance was detected within 5 years, the use of this single product continued, resulting in an exponential rise in malaria transmission rates over the next 5 years (Fig. 4). Similarly, a resurgence of malaria in India in the 1970s could also be attributed to insecticide resistance in the vectors.

The economic consequences of failing to effectively control insect vectors of disease are not as apparent as in agricultural situations, although they may be every bit as great or even greater. Insect-vectored diseases can present both a direct economic burden at the personal (drugs) and public (clinical services) levels as well as indirect costs in terms of productivity losses, lost education, absenteeism and so on. Even though these losses are harder to quantify than tangible losses (such as reduced yield) they should always be taken into account when considering the economics of vector-resistance management.

6.4 Long-term consequences of failing to address resistance

Reactionary approaches to resistance management are unfortunately common, particularly in the vector area (see Fig. 3). Such approaches were possible in the early years of chemical insecticides, but are now no longer sustainable and could eventually lead to a complete absence of effective products. Effective insecticides should be considered as a valuable and non-renewable economic resource which should be preserved – just as insect susceptibility is.

New insecticides with modes of action required to control resistant populations of vector pests are not on the horizon. Although insecticides with novel modes of action have recently been introduced into agricultural markets, few of these new compounds appear to have the biological or physical properties required for space spray, residual wall spray, or bednet treatments. In addition, the increased costs associated with developing and registering new insecticides mean that products generally appear in the more profitable agricultural markets before consideration is given to their public health potential.

The most recent "new" compound made available for vector use is etofenprox which was commercialized in 1986, and even this did not possess a distinct mode of action. It is therefore very important to delay the spread of resistance and to preserve the long-term viability of currently available control measures.

6.5 Summary points

- Insect susceptibility and effective products are both non-renewable valuable economic resources which should be preserved.
- The future costs of losing insect susceptibility should be considered when making a choice of which products to use.
- Failure to successfully manage resistance has well-documented financial implications in both agricultural and vector situations.
- Successful resistance management is dependent upon long-term approaches to planning and budgeting.

Figure 3. Hypothetical programme costs with or without resistance management

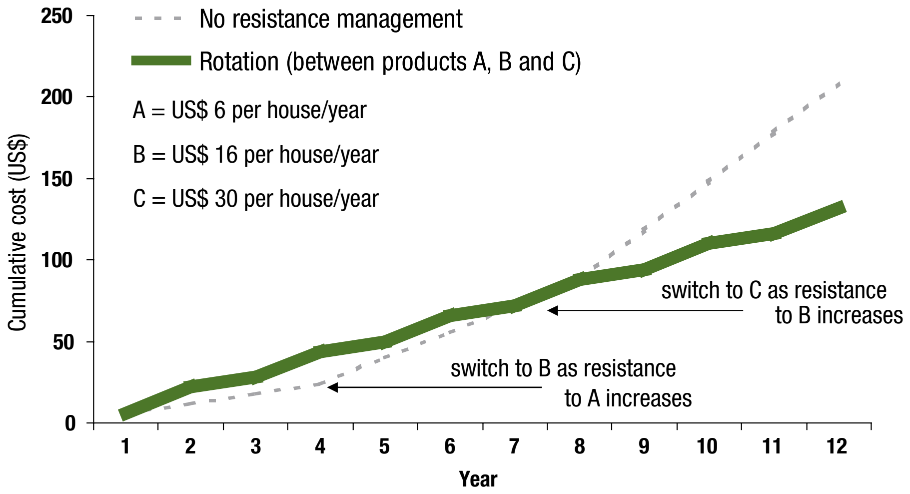
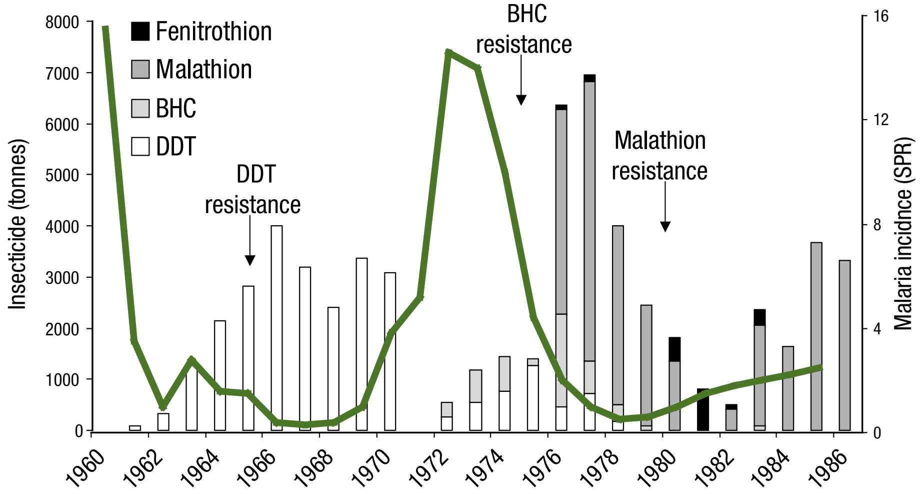


Figure 4. Types and quantities of insecticides applied annually for malaria-vector control in Pakistan



7. Monitoring and resistance detection for mosquito control

7.1 Monitoring objectives

Monitoring levels of resistance is an essential tool in enabling a decision to be made when it is wise to change the product and class of insecticide in favour of another before insect control fails and the risk of disease transmission rises.

The switch in strategy may not only be a change of insecticide but a change from adulticide to larvicide or implementing other strategy based methodologies.

Monitoring must also include cross resistance assessment since changing from one product which has failed to another which is cross resisted would a waste of both time and money.

The monitoring of insecticide resistance in vector control programmes has three important objectives:

- Before the start of a control programme to provide baseline data for programme planning and choice of insecticide.
- To detect resistance at an early stage so that resistance management can be introduced. If resistance is only detected at a late stage when control failure has occurred, it only defines the problem and is not a strategy for management.
- To monitor the levels of resistance over time and compare data with the baseline data before intervention and therefore evaluate the effects of control operations on resistance.

The main problem associated with the onset of resistance is the failure in the control of the vector insects and therefore prevention of disease transmission. Monitoring will allow a change of strategy, however in some cases such as with insecticide-treated bednets (ITN`s) there is not a ready alternative, but it has been shown that despite high frequency of pyrethroid-resistant mosquitoes, effective prevention of malaria transmission can still be achieved using pyrethroid-treated bednets where they act through repellency more than through killing action.

While monitoring and accurate assessment of the susceptibility of the vector population is fundamental to any programme, there are a wide range of reasons other than resistance why failure may occur, in many cases poor application technique, under dosing, application at the wrong time of day (space sprays) can cause control failure, these must be eliminated first.

Resistance can be very localized so before a panic reaction is made to the discovery of resistance, its distribution should be first assessed. Monitoring and detection of resistance has little value unless a management strategy has been defined and an action plan developed to react to the build up of resistance.

7.2 Monitoring methods

7.2.1 WHO Test Kit – Adult mosquitoes

The principle of this test is to expose mosquitoes for a given time in a specially designed plastic tube lined with a filter paper treated with a standard concentration of insecticide. The dose rate on the paper (diagnostic concentration) is 2x the lethal dose required to kill 100 % of mosquitoes of a susceptible strain to avoid spurious reports of resistance in the field where none may exist. The kit provides a simple to use test method, which may be used in laboratory or field to detect resistance in mosquito adults.

The kit and papers can be easily purchased with full instructions on their use. Supplier details can be found at found at: www.who.int/whopes/resistance/en.

There are a range of treated papers available and the diagnostic dose rates should give at least 98% mortality in a normal susceptible population. The mosquitoes used should preferably be 2 to 5 days old, emerged from field collected larval stages, F1 generation bred from field-collected mosquitoes or, as the last choice, field collected mosquitoes. The use of laboratory emerged mosquitoes is better as it removes the variability due to the physiological status of mosquitoes (age, blood feeding status or stage of gonotrophic cycle).

The mosquitoes are exposed for 1 hour to the papers before being removed and held in clean cups with net closures and sustenance for 24 hours before mortalities are assessed. The standard papers should give results, which are interpreted as follows:

- > 98% mortality = susceptible;
- 80–97% mortality = resistance suspected but verification/ confirmation required;
- < 80% mortality = resistant individuals present.

When < 95% mortality occurs in tests that have been conducted under optimum conditions with sample size of > 100 mosquitoes, then resistance can be strongly suspected.

It is recommended that the full details of the test technique and methodology are read from the document *Test procedures for insecticide resistance* (WHO/CDS/CPC/MAL/98.12) which can be viewed at www.who.int/whopes/resistance/en, monitoring malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces

For new insecticides a new diagnostic concentration has to be determined. The WHO-recommended diagnostic concentrations for each group of vectors (Table 2) are chosen so that exposure for a standard period of time (usually 1 hour) followed by 24 hours holding period, can be relied upon to cause 100% mortality of individuals of susceptible strains. Full details on the development of diagnostic concentrations can be found at: www.who.int/whopes/guidelines/en – *Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets* (WHO/CDS/NTD/WHOPES/GCDPP/2006.3).

Table 2: Diagnostic dose rates of insecticide impregnated papers available from WHO

Class	Insecticide	Anophelines	*Aedes aegypti*	*Culex quinquefasciatus*
Organochlorines	DDT	4%	4%[a]	4%[b]
Organophosphates	Fenitrothion	1%[c]		1%[d]
	Malathion	5%	0.8%	5%
Carbamates	Bendiocarb	0.1%		
	Propoxur	0.1%	0.1%	0.1%[c]
Pyrethroids	Alpha-cypermethrin			
	Bifenthrin			
	Cyfluthrin	0.15%		
	Deltamethrin	0.05%	0.025%	
	Etofenprox	0.5%		
	Lambda-cyhalothrin	0.05%[e]	0.03%	0.025%
	Permethrin	0.75%	0.25%	0.25%

[a] half an hour exposure

[b] 4-hour exposure

[c] 2-hour exposure for *Anopheles sacharovi*

[d] 0.1% for *Anopheles sacharovi*

[e] 2-hour exposure

Control (blank) papers

Control in risella oil
Control in silicone oil
Control in olive oil

Other concentrations are available on request. It should be noted that WHO test kit papers have a shelf life of 1 year for most insecticide and approximately 6 months for those containing deltamethrin and permethrin.

Note: papers should not be used multiple times as with each exposure insecticide is removed and there is the risk after a few exposures that levels are depleted and false positives of resistance may result.

Interpreting results

WHO Test Kit – Adult mosquitoes

Read the 24-hour mortality for each test and calculate in percentages. If the mortality in the control groups is over 5% but less than 20% a correction of mortality is made by applying Abbots formula:

$$\frac{100 \text{ x } (\% \text{ test mortality} - \% \text{ control mortality })}{100 - (\% \text{ control mortality})}$$

When the mortality in controls is ≥ 20% the test results are discarded. Calculate an average of the mortality obtained at the same concentration in at least three replicates.

7.2.2 WHO Test Kit – Larvicides (Chemical)

This methodology aims to determine resistance in mosquito larvae based on diagnostic concentrations developed from dose-response lines against susceptible species. The test may assess the resistance to the insecticide used but also may be used to determine if cross resistance is present.

Details for the test method may be found at: www.who.int/whopes/guidelines/en – *Guidelines for laboratory and field testing of mosquito larvicides* (WHO/CDS/WHOPES/GCDPP/2005.13).

Briefly the technique requires the testing of 3rd and 4th instar larvae taken from the wild using a wide range of concentrations to start, so that an approximate level can be determined. Then a narrower range of 4–5 concentrations yielding 10% and 95% mortality in 24 or 48 hours are used to determine LC_{50} and LC_{90} values.

Test kit from WHO

The kit comes with all the equipment required such as pipettes, bottles, report forms, etc.

The range of insecticides available at present are:

Malathion	781.25 mg/l	156.25 mg/l	31.25 mg/l	6.25 mg/l
Temephos	156.25 mg/l	31.25 mg/l	6.25 mg/l	1.25 mg/l
Bromophos	31.25 mg/l	6.25 mg/l	1.25 mg/l	0.25 mg/l
Fenitrothion	31.25 mg/l	6.25 mg/l	1.25 mg/l	0.25 mg/l
Fenthion	31.25 mg/l	6.25 mg/l	1.25 mg/l	0.25 mg/l
Chlopyrifos	6.25 mg/l	1.25 mg/l	0.25 mg/l	0.05 mg/l

Control: alcohol only
The test kit stock solutions available do not include pyrethroids.

WHO Test Kit – Larvicides (Insect Growth Regulators)

Tests conducted with IGR`s are different as mortality may be slower or not take place until the pupal stage. Therefore mortality is assessed every other day or every three days until the completion of adult emergence. The result is expressed in terms of the percentage of larvae that do not develop into successfully emerging adults, or adult emergence inhibition.

Details for the test method may be found at: www.who.int/whopes/guidelines/en – *Guidelines for laboratory and field testing of mosquito larvicides.*

Methoprene	20 mg/l	4 mg/l	0.8 mg/l	0.16 mg/l	0.032 mg/l
Diflubenzuron	20 mg/l	4 mg/l	0.8 mg/l	0.16 mg/l	0.032 mg/l

Control: alcohol only
Note that there is no stock solution for pyriproxyfen.

Bacterial larvicides

Larvicides such as *Bti* or *Bsph* may be tested in the laboratory to determine resistance in the same methodology as for chemical larvicides except in the preparation of stock solution.

Details for the test method may be found at: www.who.int/whopes/guidelines/en – *Guidelines for laboratory and field testing of mosquito larvicides.*

7.2.3. Other monitoring methods

Test methods based on biochemical or molecular assays are now also available for resistance monitoring. They have several advantages over bio-assays: they can detect resistance at very low frequency, can indicate the presence of heterozygous individuals with recessive resistance genes that are not detected through bio-assays and, finally, they can be used with much fewer mosquitoes than bioassays. This last point is of particular interest for species of which larvae or even adults that are not usually found in large numbers. These biochemical or molecular assays detect the presence of a particular resistance mechanism/gene and, for some, are able to identify genotypes (heterozygous or homozygous for resistance).

These assays are an ideal complement to bioassays, and are especially useful to monitor trends in resistance gene frequency over time. However, they do not replace bioassays, particularly when several resistance mechanisms are involved in the same insect. Their use is currently restricted to research labs since resistance test kits using these test methods have not yet been developed. The full descriptions and methodology can be found at: www.who.int/whopes/resistance/en – *Techniques to detect insecticide resistance mechanisms (field and laboratory manual* (WHO/CDS/CPC/MAL/98.6).

7.2.4 Mosquito bednets

Mosquito bednets have become perhaps the biggest intervention method for the control of malaria vectors over the last few years. While the above technologies can check the susceptibility of mosquitoes to the insecticide incorporated in the bednet there are several test methods for evaluating nets, however these must be treated as evaluations of the nets and not susceptibility tests as the actual dose of insecticide that mosquitoes are exposed to on the net may vary enormously. Results will not be a measure of the factor of resistance. Care must be taken that a poorly performing net does not automatically lead the tester to interpret the results as resistance in the mosquito population.

7.3 Selecting resistance monitoring sites

One challenge in monitoring resistance is establishing an adequate number of sentinel sites that will consistently sample the target population over years. Careful consideration has to be given to collection sites considering not only the abundance of the target species but also the ease with which the site can be accessed and the probability of it being available for multiple years. The role of agricultural insecticides in the selection of vector resistance has been clearly established for some important malaria vector mosquitoes. Hence, priority should be given, especially in the case of malaria vectors, to areas where insecticides are heavily used, either for agriculture or domestic hygiene or both since in many instances, these insecticides are the same as used for public health.

8. Managing a vector control programme for the long term

8.1 Developing a long-term plan is critical

The tools we have for vector control are limited and availability of new molecules to which there is no resistance will be few in the near future. Therefore once resistance to most key insecticides has developed, options become very limited. Hence, the judicious use of insecticides is fundamental to any sustained effective vector control programme.

The current strategy in most countries is to use an insecticide continuously until it fails. The result is the loss of the most cost effective tool resulting in more costly and less effective programmes in the future. Instead countries should develop plans that use multiple tools (integrated vector and pest management) and do not induce too much selection pressure through any one intervention. Additionally, only a few countries regularly monitor susceptibility levels in the vector population and therefore are unsure if their programmes are as effective as expected.

8.2 Quality control of applications

In many cases resistance is blamed for control failure when there are several reasons why control is not being achieved some of which are listed below:

a) Poor application

- Lack of training of spray personnel
- Badly maintained equipment
- Incorrectly calibrated equipment
- Failure to follow manufacturers recommendations
- Incorrect spraying
- Spraying at wrong time

b) Insufficient coverage

- Poor acceptance of control strategy by population (treated bednets, space spray, indoor residual spray)
- Failure to locate and treat all significant breeding sites when larviciding

- Inadequate pre-spray survey to identify key breeding areas for space spraying

c) Incorrect dilution or application rate

- Failure by operators to correctly dilute the insecticide according to label recommendations is common.
- Failure to apply the correct volume per hectare – space spraying or volume per m^2 for residual applications.

d) Incorrect frequency of application

- Residual applications out of synchrony with transmission season
- Space sprays not coinciding with peak vector activity

The above points must be checked before considering the possibility that insecticide resistance has developed. In addition poor application such as under dosing will accelerate the rate of onset of resistance because the vector population will be exposed to sub-lethal doses of insecticide enabling selection to take place, hence all the above points are very important in delaying resistance.

8.3 What to do when resistance is suspected

The first question is: "Why is resistance suspected?"

There can be several reasons:

- decreased susceptibility detected during monitoring,
- complaints from local users,
- disease transmission rates increasing,
- vectors seen in large numbers in treated areas and evidence of breeding.

In many cases, control failures might be due to reasons other than resistance or the product itself. Therefore the suspicions of resistance must be confirmed using bioassays or biochemical assays. A survey of the area must be made and mosquitoes collected and tested. If resistance is confirmed then the survey should be expanded so that the extent of the problem can be assessed.

8.4 What to do when resistance is confirmed

There are several points to consider first before any action is decided and these are as follow:

a) How widespread is the resistance?

Resistance can be very localised and therefore decisions that are not necessary could be taken prematurely. Surveys should be conducted to see how widespread the resistance is. Then a map can be drawn up to see the problem area. The action may be only to adapt the intervention in that area.

b) Which species are resistant?

It is rare for resistance to occur in all species in the area and it may be only one species that is involved. Is the resistant species an important vector? If not there may not be a problem. In addition, sub-species can have different risks of building up resistance. This has been clearly identified in India and Africa where some sibling species of malaria vectors have developed pyrethroid resistance while others although closely related have not.

c) What is the level of resistance and its impact of the intervention?

Is resistance causing control failure and if so, is urgent action required? If not, then the current programme may be continued in the short term with ongoing monitoring to determine if the level of resistance increases so a strategy can be formulated. It has been above mentioned that important pyrethroid resistance mechanisms in malaria vectors did not reduce the protective efficacy of insecticide treated mosquito nets. Although an early shift to alternative insecticide or method may be desirable, this is not always possible when such an alternative does not exist or are not locally available. With bednets there is no alternative available on nets at present other than pyrethroids.

Therefore there is not an alternative strategy. However it has been shown that pyrethroid treated bednets continue to give some protection even when resistance is present. The only action in this case is to attack the larval stages with a totally unrelated compound, e.g. a bacterial larvicide or an insect growth regulator (IGR).

d) Identify the resistance mechanism(s) involved and the level of resistance of the target species.

There is no gain in switching insecticides within the same MoA group; target insects will be cross resistant in most cases. In addition care must be taken that the compound used has not selected a mechanism that will cause cross-resistance to another insecticide group. Identification of all resistance mechanisms involved gives an indication of which alternative compounds should be used. It is useful to refer back to Figure 1 to see which mechanisms may be acting to cause resistance and which other insecticides may be cross resisted. The susceptibility to these other insecticides must be checked before changing products.

e) Identify the origin of resistance and the source of insecticide pressure

It is important to understand why resistance has arisen in the insect population. It may be through many years of repeated use of the same type of insecticide or it may be selection pressure from similar insecticides being used on crops grown in the area e.g. cotton. It may even occur through the heavy use of domestic products such as aerosols, mosquito coils, vape mats, etc.

It is important to identify the cause so that future strategies recognise the problems and try to avoid using similar insecticides to local agriculture, etc.

In addition, the Vector Control Department should work closely with the Agricultural Department to avoid these conflicts of interest.

9. Success stories in resistance management

The potential for managing resistance has been modeled for many years, but there are few good field-based data to substantiate any of the various strategies for managing resistance for insect vectors of disease.

9.1 Onchoceriasis Control Programme in West Africa

In West Africa, the Onchocerciasis Control Programme (OCP) managed by WHO was almost entirely based on vector control, through weekly application of larvicides in rivers to kill the larvae of the blackfly vector. Continuous weekly spraying was maintained for at least 15 years over 8 countries, thus exerting a very high selective pressure on vector populations. Having rapidly faced very serious temephos resistance problems (temephos was the only larvicide used at early stages of the OCP), the Programme strengthened resistance monitoring and developed a very efficient resistance management scheme. Instead of continuous use of a single OP larvicide, a pre-planned rotation of unrelated products was implemented using still OPs for limited periods complemented by a microbial larvicide (*Bacillus thuringiensis israelensis*), a pyrethroid and a carbamate insecticide. *Bti* and chemical larvicides have been applied strategically, based on resistance status and trends, vector population dynamics, environmental impact, cost and logistical factors.

This strategy has been highly successful over the 17 years of its implementation: temephos resistance regressed to the point it was possible to re-introduce it in the rotation scheme and never developed in areas where it was not previously present. No resistance developed to any of the other insecticides used. However, artificially selected resistance in the Simulium vectors developed rapidly to a new insecticide, thus further confirming the potential for rapid development of resistance under continuous use of a single chemical larvicide. Extensive use of the microbial larvicide *Bti*, itself a biological means of co-treating the insects with multiple toxins, has allowed successful resistance management, using continuous weekly larviciding, without any measureable medium or long term detectable impact in the biological equilibrium of the treated rivers.

9.2 *Anopheles albimanus* trial in Mexico

9.2.1 Background and objectives

Models of resistance management come to variable conclusions depending on the assumptions that are made, although most suggest that resistance selection is slowed but not completely stopped by the management tactics described previously in this document. To test the fine scale mosaic and rotation strategies directly, and compare the results to single, long-term insecticide use under field conditions, a large-scale programme was set up over several years in Mexico funded by the Insecticide Resistance Action Committee under the auspices of WHO. Mexico was chosen as the field site, as the vector, *An. albimanus* had a history of intense insecticide selection through cotton crop spraying in the 1960s and early 1970s. This resulted in multiple resistance mechanisms being selected in this vector. A programme was established in 1995 to intensively monitor baseline resistance levels for a year and then use replicate districts to spray a single insecticide (a pyrethroid or DDT), an annual rotation of organophosphate, pyrethroid, carbamate, pyrethroid, organophosphate, etc., or a fine scale within-village mosaic of an organophosphate and a pyrethroid. This allowed the following questions to be answered:

- How fast does DDT resistance revert once the DDT selection pressure from anti-malarial activities is removed?
- How quickly does pyrethroid resistance emerge when it is used continuously for malaria control?
- Is the rate of pyrethroid resistance selection reduced in the rotation and mosaic areas compared to the single use districts?
- Are the rotations and mosaics acceptable at an operational level?
- Is the rotation or the mosaic more beneficial?

9.2.2 Results from the trial

Initial monitoring showed that resistance to organophosphates, carbamates and pyrethroids was present in the *An. albimanus* field population, although at a low frequency. Use of different monitoring techniques (bioassays, biochemical and molecular assays) showed that, as expected, the WHO diagnostic adult mosquito bioassay was the least sensitive method for early detection of resistance when resistance genes are at low or very low frequency.

Operationally, implementing either the rotation or the fine scale mosaic posed no significant problems. Acceptability of different treatments by householders was similar for all insecticides, as judged by treatment rates and directly by questionnaires administered to the householders at the beginning and end of the programme.

Pyrethroid resistance rose rapidly in the areas under pyrethroid treatment alone to levels significantly above those in the rotation and mosaic areas. However, there was an increase in pyrethroid resistance in all areas, and data had high variances, possibly due to the effect of pyrethroid use on the local banana crops, which may have reduced, but did not negate the beneficial effects of both the rotation and mosaic strategies.

DDT resistance did not revert towards susceptibility over the six-year intervention period in any district, and was stable in the areas under DDT treatment. Hence, as in Sri Lanka, this resistance appears to have been selected to the point where it no longer has a negative fitness associated with it. Over the six-year time frame of the intervention with different treatments, there was no major difference in the performance of the mosaic and rotation strategies. Hence a decision on which of these strategies should be used in practice can be made on operational factors.

The biochemical and molecular assays for resistance detection gave a more accurate measure of the true resistance gene frequencies within the field population than traditional bioassays. The WHO diagnostic assays (using a single robust dosage to detect resistance in a bioassay), although the simplest system to interpret conceived to point out resistance when installed in a population, gave underestimates of the underlying resistance problem.

Throughout the intervention more than 80% of susceptible mosquitoes were killed on all treated surfaces with all insecticides.

9.3 Summary points

- Insecticide resistance management in vectors follows the principles developed for other areas, with rotations and mosaics offering value.
- Rotations or mosaics of unrelated insecticides have been more efficient in managing insecticide resistance than continuous use of a single insecticide.
- Two trials demonstrate that a rotational strategy is both a technically sound and operationally acceptable means of managing resistance in vector management programmes.

10. Future needs and the way forward

10.1 Protecting our current tools

Almost all insecticides used for public health have been developed for agriculture and are (or have been) used for this purpose. The development of new molecules is an increasingly complex, long and costly process that cannot be justified by vector-control alone, which currently represents less than 1% of the total pesticide market. Over the last 20 years, very few new insecticides have been developed for indoor residual spraying; all of these being pyrethroids. In addition, because of new re-registration procedures and environmental constraints, a number of insecticides have been or may soon be withdrawn by the industry, thus dramatically increasing the reliance of public health on a limited number of products. The prospects of having access to new public health insecticides in the coming years appear extremely limited. It is therefore essential that reliance on insecticides is reduced as much as possible by the promotion of integrated vector- and pest-management principles, using chemicals only when and where they are really needed. This is especially true in the management of diseases such as malaria, dengue where chemical control is an important component. These vector-borne diseases are the most globally significant, and it is vital to promote methods and strategies to avoid the development of resistance, or to limit its increase and geographical spread once it has developed.

10.2 The need for a good understanding of resistance

Vector resistance can be understood and eventually managed only through a close monitoring of vector populations in the field. Entomological factors are essential to understand appearance, evolution and spread of vector resistance. Among these factors are the dynamics of vector populations (size, growth, isolation…) and introgression of resistance genes among sibling species that are commonly found in disease vectors (e.g. malaria vectors). Once resistance has been detected, it is essential for Programme Managers to understand its operational significance (impact on the efficacy of on-going or planned interventions). Also important is the need to identify the origin of resistance since it can result from applications other than public health (e.g. in agriculture or household pest-control). It would be very difficult for a vector control programme to manage an insecticide resistance that would result from agriculture unless close collaboration is established between agriculture and public health.

10.3 A reminder of the basic principles of resistance management

- Insecticides should be applied only when and where needed and where no other effective control intervention can be implemented.
- Insecticides have to be applied at the concentration recommended by WHO and by the manufacturers (label instructions), avoiding over-dosages which are costly and potentially hazardous, as well as under-dosages which are not effective enough and may accelerate the development of resistance.
- Insecticides of the same chemical group, acting on the same target site, should be considered as a single product as far as resistance is concerned.
- The use of one chemical class against both larval and adult life stages should be avoided.
- The combined use of unrelated insecticides (for example in rotation) should be preferred to the continuous use of a single insecticide for extensive periods of time.
- Soon after resistance is detected in a target vector population, another unrelated insecticide should be introduced, either alone or in combination.
- If necessary, an insecticide can still be used for some time when resistance is at a low frequency, especially when resistance is recessive and individuals are mostly heterozygous. However, such an insecticide should preferably be replaced by a non-related one when vector populations are seasonally expanding or at their peak density (for example at the beginning and during the rainy season for tropical mosquito species). A seasonal or annual rotation of unrelated insecticides, taking into account vector-population dynamics, is therefore a good option to consider where and when it is feasible.

10.4 Constraints and limitations to the implementation of resistance management in public health programmes

In most vector-control programmes there is currently a clear tendency to shift from well-planned vertical operations to community-based interventions such as insecticide-treated materials. Insecticide concentrations on these materials are very variable as they depend on how they are used and how frequently they are washed. Controlling these concentrations is almost impossible and resistance management therefore becomes more difficult to implement.

Many developing countries are currently involved in a decentralization process. As a result, provincial public health services increasingly have responsibility for the selection, planning and implementation of vector-control interventions, including the choice and purchase of insecticides. Considering the current lack of qualified vector control specialists at peripheral level in most endemic countries, there is an urgent need for training and capacity development, and for the production and dissemination of simple guidelines and educational materials related to good pesticide-management practices, including resistance management.

When an insecticide is still effective in preventing disease transmission, it is difficult to convince health programme managers to replace it, usually by a more costly product, or to change vector-control strategies and procedures just to prevent the development of insecticide resistance. Many programmes claim they are not able to cope with financial and logistic constraints associated with the change of insecticides or vector-control approaches because of the very limited financial resources allocated to vector control. However, the consequences of not being proactive in resistance-management programmes are likely to be much more costly over the longer term and potentially catastrophic if the limited arsenal of vector-control tools still available is further depleted because of resistance.

10.5 The way forward

Realizing the difficulties and constraints does not provide justification for opposition to progress. Agriculture has been confronted with relatively similar problems to those encountered in vector control (though with different constraints) and has developed and promoted appropriate corrective measures and educational materials. Public health should benefit from this experience and adopt resistance-management principles as part of vector-control activities and national pesticide-management policies. Major institutions such as CropLife International, the industry federation, and IRAC are collaborating with WHO to provide practical help to public heath programmes. Exchange of information and experience sharing will be an important component of such collaboration. Vector-resistance monitoring has to be strengthened and results rapidly and widely disseminated through easily accessible web sites grouping agriculture, public health and domestic hygiene together. It is important for pesticide producers to be aware of the status of vector resistance, just as it is essential for vector-control managers to know more about the agricultural use of insecticides. It is hoped that this manual developed for vector-control programme managers is a positive step in this collaboration.

Resistance Management for Sustainable Agriculture and Improved Public Health

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