IRAC

Insecticide Resistance Action Committee

Insecticide
Resistance
Action
Committee

Prevention and Management of Insecticide Resistance in Vectors of Public Health Importance

Prevention and Management of Insecticide Resistance in Vectors of Public Health Importance

Second Edition 2011

A manual produced by:

Insecticide Resistance Action Committee (IRAC)

IRAC, its aims and objectives

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 within 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 and Country or Regional Committees with information disseminated through conferences, meetings, workshops, publications, educational materials and the IRAC Website (www.irac-online.org).

---

*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.

Photo credits for front cover:
Syngenta

Acknowledgements

Contents

List of abbreviations used in this manual

AChE	Acetylcholinesterase
BMGF	Bill and Melinda Gates Foundation
Bs	*Bacillus sphaericus*
Bti	*Bacillus thuringiensis* serovar israelensis
CDC	Centers for Disease Control
CDNB	chlorodinitrobenzene
DCNB	dichloronitrobenzene
DEF	*S,S,S*-tributyl phosphorotrithioate synergist
FAO	Food and Agriculture Organization
GST	Glutathione S-transferase
IGR	Insect Growth Regulator
IRAC	Insecticide Resistance Action Committee
IRM	Insecticide Resistance Management
IRMS	Insecticide Resistance Management Strategy
IRS	Indoor Residual wall Spray
ITM	Insecticide Treated Material
ITN	Insecticide Treated Net
IVCC	Innovative Vector Control Consortium
IVM	Integrated Vector Management
kdr	knock-down resistance
LN	Long lasting insecticide treated Net
LSTM	London School of Tropical Medicine
MACE	Modified Acetyl Cholinesterase
MDA	Mass Drug Administration
METI	Mitochondrial Electron Transport Inhibitor
MFO	Mixed Function Oxidase
MGK-264	N-Octyl bicycloheptene dicarboximide synergist
MoA	Mode of Action
NMCP	National Malaria Control Programme
OCP	Onchocerciasis Control Programme
OP	Organophosphate insecticide
PBO	Piperonyl Butoxide synergist
PCR	Polymerase Chain Reaction
POP	Persistent Organic Pollutant
RFLP-PCR	Restriction Fragment Length Polymorphic Polymerase Chain Reaction
RNA	Ribonucleic acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
ULV	Ultra Low Volume
VC	Vector Control
WHO	World Health Organisation
WHOPES	World Health Organisation Pesticide Evaluation Scheme

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 trained in management principles in general, to ensure their proper implementation and surveillance. Of course new active ingredients with novel 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 the principles of resistance management.

Efficient communication, effective outreach processes, dissemination of information and advice are essential to good resistance management. The completely revised 2nd edition of 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 2nd edition of the manual either as authors or reviewers. The manual was completed and approved by the IRAC Public Health Team in November 2010.

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, the Bill & Melinda Gates Foundation web site at www.gatesfoundation.org and the website of the CDC www.cdc.gov.

Mr. Mark Hoppé   **Dr. Ralf Nauen**
IRAC Public Health   IRAC International
Team Chairman   Chairman

2. Introduction and objectives

2.1   Target audience and objectives of this manual

This manual is primarily targeted at managers of mosquito and other vector control programmes, operational staff, and policy makers. The key purposes of this manual are to inform the audience of the importance of insecticide resistance, why avoiding it is essential and to provide them the tools so to do. Also, to highlight the need to adopt and implement integrated vector control approaches in their programmes.

International agencies such as WHO and FAO, as well as academic institutions, in collaboration with insecticide manufacturers and distributors, should also utilise this manual in helping to mobilise resources to further develop and promote integrated vector management principles, including insecticide resistance management.

The objectives of this manual are:

- To offer basic information on insecticide resistance mechanisms.
- To provide a better understanding of the factors that may lead to the development of resistance in insect vectors of disease.
- To present the basic principles for maintaining susceptibility and avoiding the development of resistance.
- To effectively manage resistance where it has already developed.

2.2   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 southern Europe, and the worldwide spread of the vector *Aedes albopictus* (the Asian tiger mosquito) are serious and largely uncontrolled developments.

2.3 Vector control – a key component in managing vector borne diseases

There are currently no effective drugs or vaccines 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 due to 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 com-

plete elimination of infection if MDA is not supplemented by transmission control interventions in some areas. Many other examples that emphasise the need for vector control can be given for most tropical areas as well as developed countries.

2.4 The need for chemical control

Insecticides remain the most important element of integrated approaches to vector control. The recent restrictions 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. 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 larvicides. Newer technologies such as long lasting insecticide treated bednets (LNs) 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 to control malaria, especially in Africa, would be jeopardised by the widespread development of pyrethroid resistance.

Larviciding receives increasing interest as a component in vector control. For malaria control, the WHO especially focuses on larviciding during the elimination phase of the pest, when vector control concentrates on areas of active transmission. In other areas of mosquito control, larvicides are especially used in water reservoirs, containers with drinking water, drainage systems, sewerage lagoons, flooded fields, drains, and septic tanks.

2.5   The threat of insecticide resistance

Although public health uses account 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 and increased cost of the insecticide. The resistance genes in the vector population may also 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 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 may be exposed to the same or similar insecticidal compounds, which will select for resistance. This phenomenon is of particular relevance for malaria vectors. Moreover, many insecticides are also extensively used to control domestic pests, further exposing vector species that rest indoors. These so called "endophilic" vectors are of particular concern because of their close contact with humans.

It is common for a mosquito population to be exposed to a given class of insecticide at the larval stage, through agricultural spraying, and then again at the adult stage, through household pest control, as well as via vector control programmes.

2.6 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 understanding which target sites insecticides are acting upon, and identifying the mechanisms involved once resistance has appeared in a vector population.

2.7 Concerns about resistance development

Although there are no short term solutions to vector resistance problems, it is important for programme managers to better understand resistance issues and to promote good practices in insecticide 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 management issues. Vector control programmes need to meet this condition 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 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.

Syngenta

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 all populations of this species cannot be controlled. Thus a single report of resistance to an insecticide does not imply that an insecticide is no longer useful either within the local region or globally.

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 fulfil this function, namely esterases, monooxygenases and glutathione S-transferases. These enzyme systems are often enhanced in resistant insect strains enabling them to metabolise 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 activity, of esterases enzymes, which hydrolyse 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 overproduce 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 metabolises the OP malathion at a much faster rate than that in susceptible individuals. Metabolic resistance can therefore range from compound specific to very general resistance 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 currently used for vector control, including organophosphates, carbamates, pyrethroids and DDT (Figure 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 (e.g. 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. This results in the insects being unaffected, or less affected, by the insecticide than 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. Reduced susceptibility to pyrethroids conferred by *kdr* mutations has been confirmed in *Anopheles 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. Examples of reduced penetration mechanisms are limited, and are often considered a contributing factor to reduced susceptibility.

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 exposure to an insecticide. 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.

Figure 1. Major biochemical mechanisms conferring resistance to important classes of insecticides in adult mosquitoes.

(Circle size reflects the relative impact of the mechanism on resistance)

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, the voltage gated sodium channel. Past use of DDT has resulted in several insect species developing resistance to DDT due to 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 (Figure 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 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 need possess the characteristic 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 slows their spread within the population. If the resistance is genetically dominant, it can rapidly become established within the population and will be difficult to manage (Figure 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 are often found 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, selects for susceptible insects. 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 *Anopheles 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 in the following years.

It is this reversion to susceptibility which is the underlying assumption behind any effective resistance management strategy. However, reversion rates are 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

mechanism 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 *Anopheles culicifacies* and *Anopheles 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 in a mosquito population
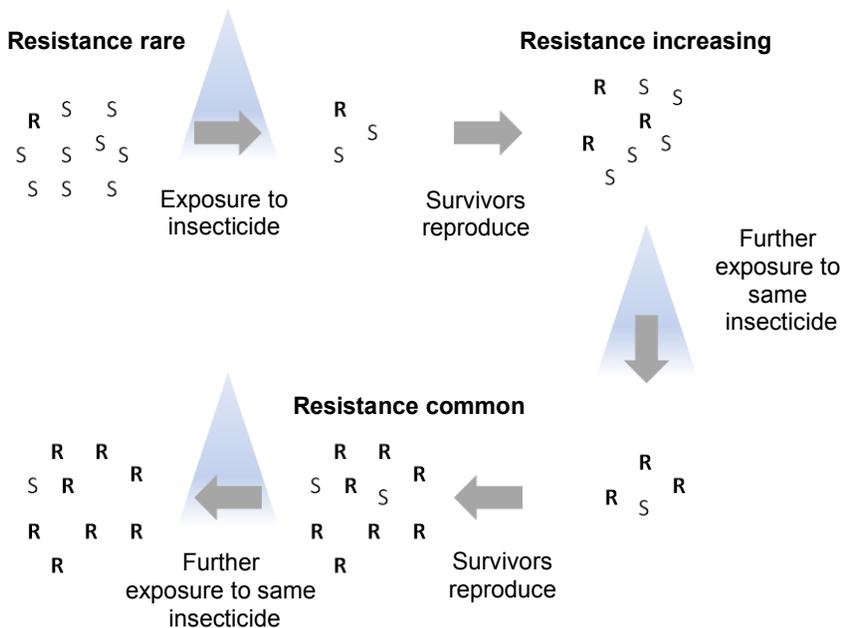
3.6 Major factors that influence resistance development

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

3.6.2 Dosage and persistence of effect

The length of time that an insecticide remains effective, also called its persistence, is dependent upon the physical chemistry of the insecticide, the type of formulation, and the application rate. Products which provide a persistent effect provide continual selection pressure in a similar manner to multiple treatments. 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 providing a selection pressure against many generations of the same insect. It is therefore important to always follow manufacturer and WHO recommendations when using such insecticides.

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, as any resistance genes can rapidly spread throughout the population. Mosquitoes have a history of insecticide resistance and are characterised by a relatively short life cycle and high fecundity, with females laying several hundred eggs during their reproductive life. In contrast, the tsetse fly is less likely to develop resistance to insecticides due to a longer life cycle and relatively low rate of reproduction, females producing in total fewer than 10 offspring.

3.6.4 Population isolation

With vectors of disease, the goal is often to eliminate all or the majority of the population, however the greater the selection pressure that is put on a population, the faster susceptibility may be lost. Immigration of individuals possessing susceptible genes from untreated areas will beneficially dilute and compete with the resistance genes in the overall population. An early step in a vector control programme should therefore be to estimate the significance of immigration of untreated insects. For example, an island where the entire area was treated would have a higher risk of developing resistance as few untreated mosquitoes would join the treated population. The risk of insecticide resistance developing should be considered when planning a resistance management programme. Awareness of, and coordination with neighbouring vector control programmes and agricultural activities should be encouraged, so that the regional effect on the target population is considered.

4. Resistance management – strategies and tactics

4.1 Approaches to resistance management

Insecticide resistance management can be undertaken 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 challenging 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. Rotation allows any resistance developed to the first insecticide to decline over time when the second insecticide class is introduced. The timeframe for rotation needs to be sufficiently short to prevent significant levels of resistance to develop to any one rotation partner. Whilst annual rotation is possible in most vector borne disease control programmes, in agriculture, the rotation of several classes of insecticides (with different modes of action) within a growing season is practiced.

4.1.2 Mixtures
In this context, a mixture is the co-application of two or more insecticides and can take the form of a single formulation containing more than one insecticide, two or more insecticide formulations being applied in the same spray tank, or an LN or ITM treated with two or more insecticides. In the widest definition it can also include the combination of an LN or ITM with an IRS application in the same dwelling.

The use of mixtures to avoid the development of fungicide resistance in plant pathogens is common in agriculture. Once again, the theory is that if resistance to each of the fungicide compounds within a mixture is rare then multiple resistance will be extremely rare. This approach will not be successful if resistance to one of the components 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, and is used in agricultural systems. However, for mixtures to work well in practice, both insecticides need to be used at their full application rate, and the efficacy and persistence of the two insecticides should be broadly similar. Mixtures of products are rarely adopted in vector control programmes on grounds of cost, logistics, safety

and because of the limited number of recommended compounds available. However, with the development of novel vector control insecticides, this approach may become viable.

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 dwellings 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. Larger scale mosaics have been shown to be effective, see section 9.2, *Anopheles albimanus* trial in Mexico. Whilst there are some practical difficulties implement ting a mosaic in a vector control programme, it offers the advantages of a mixture strategy with lower insecticide inputs and hence cost. Mosquito bed nets formed from panels treated with different insecticides achieves a similar mosaic effect to treating houses with different compounds but on a much finer scale.

4.1.4   IRM in an Integrated Vector Management context
Integrated Vector Management, IVM, can be defined as "a rational decision making process for the optimal use of resources for vector control". IRM is therefore an integral part of IVM, as only through the active management of insecticide resistance can the available resources be optimally and sustainably used.

Insecticide resistance develops in an insect population when individuals carrying genes that allow them to survive exposure to the insecticide pass these genes on. Thus, any activities that control the individuals with the resistance trait will delay the spread of the resistance genes in the population. IRM in the context of IVM therefore also includes activities such as habitat management, community education and mosquito larviciding. Mosquitoes with reduced susceptibility to an insecticide may still be controlled at the recommended label rate. However, exposure to sub-label rate applications may allow these individuals to survive and pass on the resistance genes. Sub-lethal exposure may arise in IRS due to poor choice of product, under dosing during application or poor application technique. In each case the residuality of the product may not be sufficient, delivering a sub-lethal dose before the next scheduled spray round. LNs may also deliver sub-lethal doses within their expected lifetime due to poor product choice, inappropriate storage, use or washing. These factors which reduce the efficacy of a vector control programme, can lead to a shift in the susceptibility status of the mosquito population, and should be avoided through informed product choice, effective IRS application and LN distribution, and education.

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 of an insecticide 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.

Syngenta

Although compounds within the same chemical class (e.g. 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 insecticide to another simply exposes the population to a single mode of action, and has no value in resistance management. It is therefore almost always better to rotate to a different mode of action, regardless of the mechanism of resistance.

Insecticides are applied against both adult and juvenile stages of a number of dipterous 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.

A resistance management programme can either be based on a planned or triggered rotation scheme. In a planned rotation scheme, the programme manager will decide at the beginning of the programme to rotate insecticidal modes of action at set intervals. In a triggered scheme one class of insecticides are only replaced with another after a trigger has been met. It is not uncommon for a vector control programme to consider changing the insecticidal intervention on the basis of epidemiological data, the number of cases of malaria has increased, or simply because there are many reports that the mosquitoes are no longer controlled. Whilst both these measures suggest that the intervention is losing effectiveness, other factors may also have lead to this outcome, and they cannot replace insecticide susceptibility monitoring assays.

Both planned and triggered rotation schemes require that susceptibility monitoring be undertaken. In the case of planned rotation, to identify which class of insecticide to change to. In the case of triggered rotation, to identify whether a change is required, and if so, to which class of insecticides.

Resistance management, through rotation of insecticidal mode of action, is most effective when resistance to the insecticide is present at low levels within the target population. The susceptibility monitoring assay therefore needs to be sensitive enough to identify those individuals with reduced susceptibility when they are infrequent in the population. Ideally, changes in susceptibility should be identified at levels well below that which would have an impact on programme effectiveness. Chapter 7 discusses insecticide susceptibility monitoring and highlights the importance of the choice of monitoring method and, in the case of bioassays, the discriminating or diagnostic dose chosen.

4.3 The IRAC Mode of Action Classification Scheme

IRAC has 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 ingredients 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 less 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 subclass which shouldn't). If rotation of insecticides from different classes is not possible, then rotation between subclasses is preferred to the use of the same insecticide.

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 manufacturers may 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. OPs). 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). 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 to insecticides 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, delaying insecticide resistance development:

- Avoid exclusive repeated use of insecticides from the same chemical subgroup.
- Employ an IVM approach, including other control methods (chemical, cultural, biological) into vector control programmes.

4.4 The role of synergists in public health

In the context of insect vector control, synergists can be defined as compounds that enhance the toxicity of some insecticides, although they usually have limited toxicity themselves. At non-toxic concentrations, insecticide synergists act by inhibiting certain enzymes naturally present in insects that would otherwise breakdown and detoxify insecticide molecules. For the purposes of this document, a synergist is considered as a chemical that, while not possessing significant inherent pesticidal activity, nonetheless promote or enhance the effectiveness of a particular insecticide.

Synergists, including piperonyl butoxide (PBO), *S,S,S*-tributyl phosphoro-trithioate (DEF), and *N*-Octyl bicycloheptene dicarboximide (MGK-264), enhance the effect of several classes of insecticide, including the pyrethroids, organophosphates and carbamates. This is achieved by inhibiting the enzymes that metabolise insecticides, P450s and esterases, within the insect. In susceptible insects, these metabolic enzyme systems are at a 'baseline level', whereas in resistant insects they are at an elevated level. Thus in susceptible insects, insecticides are already working at near maximum effect and the use of synergists may provide minimal enhancement. However, in certain types of resistant insects, synergists can significantly enhance insecticide performance due to the inhibition of the resistant insect's enhanced metabolic enzyme systems. Synergists have also been reported to be capable of delaying control failure, due to insecticide resistance, in an agricultural setting.

Little can be done to overcome altered target site resistance mechanisms, other than using an insecticide with an alternative mode of action. However the effect of metabolic resistance can be reduced with the use of synergists.

Synergists have been used successfully in mosquito control programmes for over 50 years, particularly to increase the efficacy of space sprays. They are primarily used to increase the killing effect of insecticides. The most well known example is the combined use of PBO with natural pyrethrins. The addition of PBO can provide increased mortality and efficacy at a reduced cost. In some situations, the addition of a synergist can reduce the required rate of insecticide by up to a half without a decrease in efficacy.

The use of synergists has a valuable place in increasing the activity of certain insecticides on insects with specific resistance mechanisms and prolonging the useful life of those insecticides where resistance is developing. However there is currently insufficient evidence to determine whether synergists can influence the frequency of resistance genes in a vector population and hence no recommendations relating to resistance management can be made at this stage.

4.5   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.
- Implementation of an IVM approach can reduce the overall selection pressure of insecticidal interventions.
- The IRAC Mode Of Action Classification Scheme is an up to date and accurate guide which may be used in formulating resistance management guidelines.
- Synergists have a valuable place in prolonging the useful life of insecticides were metabolic resistance is developing.

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

Primary Target Site of Action	Group	Sub-group	Chemical subgroup	Examples
Acetylcholinesterase (AChE) inhibitors	1	A	carbamates	bendiocarb, propoxur
		B	organophosphates[2]	fenitrothion, pirimiphos-methyl, malathion, temephos
Sodium channel modulators	3	A	pyrethroids and pyrethrins	allethrin, bifenthrin, *lambda-cyhalothrin, alpha-cypermethrin*, deltamethrin, cyfluthrin, permethrin, etofenprox, phenothrin, transfluthrin
		B	DDT	DDT
Nicotinic acetylcholine receptor (nAChR) allosteric activators	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 type 0	15		benzoylureas	diflubenzuron, triflumuron, novaluron

1. Including larvicidal and adulticidal insecticides. Insecticides with alternative modes of action may become available, always identify and consider all MoA classes locally approved for use in any Integrated Vector Management programme. The IRAC Mode of Action Classification Scheme is edited and updated frequently to include new products; please refer to www.irac-online.org for the latest table, and the complete MoA.

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 (Figure 1). Some of these metabolic resistance mechanisms may be 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. Practical approaches to resistance management

5.1 Lessons learned from agriculture

The most basic and fundamental lesson learned about resistance in both 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 extended exposure to a specific insecticide or chemical class of insecticide. In agriculture 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 is often a single product or chemical class used continually in an unsustainable manner. When resistance to the insecticide develops, the cost or benefits associated with switching 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 minimise 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

An analysis can be undertaken to determine the risk of the pest developing resistance. This analysis needs to be based on a range of factors, previously discussed, 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
- 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, an active IRM programme should be implemented such as introducing rotation of insecticidal class, or inclusion of a mosaic into the programme from the start. The principles of IVM should always be included. 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 for established insecticides to generate baseline data, and these should be used as a reference point for future monitoring. Other bioassays have been developed, and these are discussed further in Ch. 7. 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 a change in susceptibility is confirmed, tactics should be developed that result in the selection pressure caused by that insecticide (or class of insecticides) being reduced or removed all together. This is a key feature of a management strategy. Although, preferably these tactics should have been put into practice prior to the problem developing.

5.2.3 Complementary measures

A resistance management strategy should include complementary measures that can be 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, and form part of IVM programmes already discussed.

5.3   Implementation of a resistance monitoring programme

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 characterised 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 offer assistance in developing 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.

5.3.1   Monitoring after launch
Tracking efficacy in commercial use
- Follow 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.
- In areas where vectors have a high probability of developing resistance it is desirable to instigate selective monitoring during the season using diagnostic concentrations (see Chapter 7).
- All cases of confirmed resistance in the field should be documented, mapped and information made available to the relevant local authorities, WHO and the product manufacturer

5.4   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 in place, and may include further restrictions on frequency of use, rotation with insecticides with different modes of action, or restriction of product use, with the aim of encouraging susceptibility to return. This may allow for reintroduction of the product in the future. In general, the following actions should be considered:

- Use insecticides judiciously and preferably within a system of integrated vector 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, accepting this may be challenging in vector control
- Notify WHO and regional authorities
- Notify the manufacturer of the product
- If not all ready in place, develop a resistance management strategy in conjunction with national authorities, WHO and the manufacturer, see Ch. 4.

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.

5.5   Summary points

- Prevention of resistance is better than cure.
- Resistance management strategies are most effective when developed before control programmes are started.
- Ensure correct delivery of insecticide to target insect: dose, timing and technique.
- Insecticidal interventions should be part of a wider integrated vector management programme.
- If resistance occurs take immediate steps to contain it and reduce the selection pressure produced by the insecticide.

**Flow Chart 1:
Resistance
Management
Best Practice**

How do I prevent or delay the onset of insecticide resistance?

Conduct baseline susceptibility studies before commencing any large scale programme using WHO Test Kit or CDC Bottle Bioassay

Check susceptibility levels at least once a year in several set locations. Record and compare results with baseline study

Little or no change in susceptibility

High level of survivors in tests

Small increase in survivors, no field control failures

Continue with current strategy if impact on vectors and disease transmission is good

Conduct further tests, evaluate susceptibility against all approved MoA classes. Where possible, undertake assays to identify resistance mechanism

Increase monitoring. Rotate to alternative MoA insecticide if possible

34

Existing insecticide class is resisted but all other classes effective.

Other classes of insecticides also resisted. May indicate existing resistance or cross-resistance

All classes resisted.

Change MoA class of insecticide

Choose class of insecticide not resisted include larvicides with different MoA classes of insecticides where practical

Use MoA class that still provides best control. Ensure IVM is used, include larvicides with different MoA to adulticides where practical

Please note that this is a simple guide and with some products such as LN's there is currently no alternative insecticide, however nets may still give protection through physical means and repellency.

Flow Chart 2:
Product failure
Determining the Cause

The product does not seem to work – Why do you suspect this?
Complaints from population?   Disease rates increasing?
Large numbers of vectors still around after intervention ?

Adulticides: check for resistance using WHO cylinder test or CDC bottle bioassays.
Larvicides: WHO larvicide resistance monitoring kits.

Resistance indicated?
NO

Resistance indicated?
YES

Were manufacturers label recommendations followed exactly?

- Correct dilution rate?
- Correct application rate?
- Correct application equipment?
- Product within date?
- Product stored correctly?
- Was a good quality WHOPES recommended product used?

NO

YES

Re-apply appropriate product at correct rates and monitor effect

Failure may be due to other factors involving application techniques

Further tests using bio-chemical or molecular methodologies, where labs. are available to do this, can provide detailed information on resistance

Don't Panic!
How widespread is resistance? Conduct survey to check. Does resistance cause failure of product?

If resistance is confirmed refer to:
Flow Chart No 1
- Notify WHO & Regional authorities
- Inform manufacturer
- Identify likely cause of resistance e.g. has the same insecticide type been used locally for agriculture?
- Develop remedial programme in conjunction with National Authorities, WHO and manufacturer
- Undertake a monitoring program

36

Space sprays:

- Has machine been calibrated correctly e.g. flow rate, droplet size?
- Are applications made at the correct volume & dose rate per unit area? (Note: for ULV outdoor spraying the flow rate & vehicle speed must be correct to achieve dose/ha)
- Was application made at the right time of day for insect activity?
- Correct frequency of spraying?
- Meteorological conditions:
  - Is the wind speed <15kph?
  - Are the inversion characteristics correct if spraying outdoors?
  - Is it dry during application?
- Was the area surveyed properly and area calculated to ensure correct dose rates?

Indoor Residual Spray

<u>Equipment:</u>
- Has equipment been calibrated/ checked for the correct flow rate?
- Has the correct pressure been maintained in cylinder throughout spraying?

<u>Insecticide:</u>
- Was the correct dose used?
- Was it thoroughly mixed before spraying?

<u>Training:</u>
- Are all staff correctly trained?

<u>Coverage:</u>
- Were all houses sprayed or only partial coverage achieved?
- Has the population been instructed in what to do following IRS application; has the deposit been painted over/cleaned off etc.

Larvicides

<u>Equipment:</u>
- If using liquids, are the sprayers calibrated, flow rates determined relative to the area and volume of water to be treated?
- If using granules, has the weight per unit area been calculated correctly?

<u>Application:</u>
- Was the water depth considered when calculating application rate?
- Is the frequency of application correct?
- Delayed larval mortality may occur using an IGR - check pupal emergence rate.

Bednets – LN's
- Are nets being used?
- Has population been educated how to use the nets and care for them "correctly"?
- Is coverage complete?
- Are nets being washed as recommended?
- Do nets need replacing due to age or excessive damage

6. The economics of resistance management

6.1 The economics of adopting a resistance management strategy

The cost of insecticide, either formulated for spraying or as a delivered LN, represents a significant proportion of any vector control programme.

In order to be effective, resistance management programmes must result in the reduced use of insecticides from a given mode of action class over the short term. Depending on the situation and management programme, it has been estimated that by halving the number of applications of an insecticide from a given mode of action class (for example by reducing the treated area by half or by halving the number of treatments per season), the effective life of compounds from that class will be at least doubled. This obviously entails the use of alternative, possibly more costly, insecticides 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 products 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. A comparison of programme costs with or without resistance management is shown in Figure 3.

6.2 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 *H. 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 *Anopheles 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 (Figure 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 etc. 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.

6.3   Long term consequences of failing to address resistance

Reactionary approaches to resistance management are unfortunately common, particularly in the vector area (see Figure 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.

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.

6.4   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 to planning and budgeting.
- Failure to implement an IRM programme on financial grounds is a false economy and will lead to increased costs in the future.

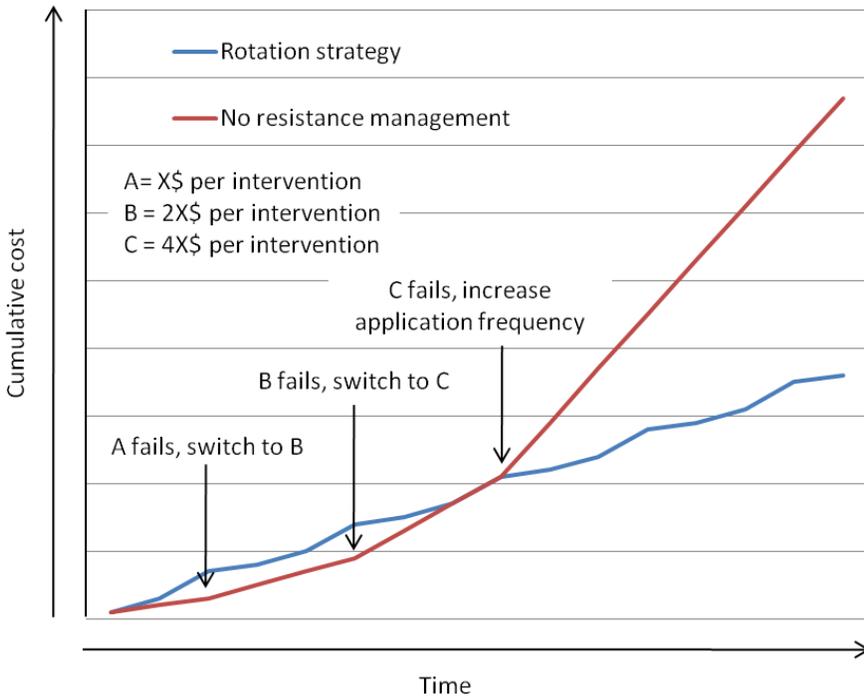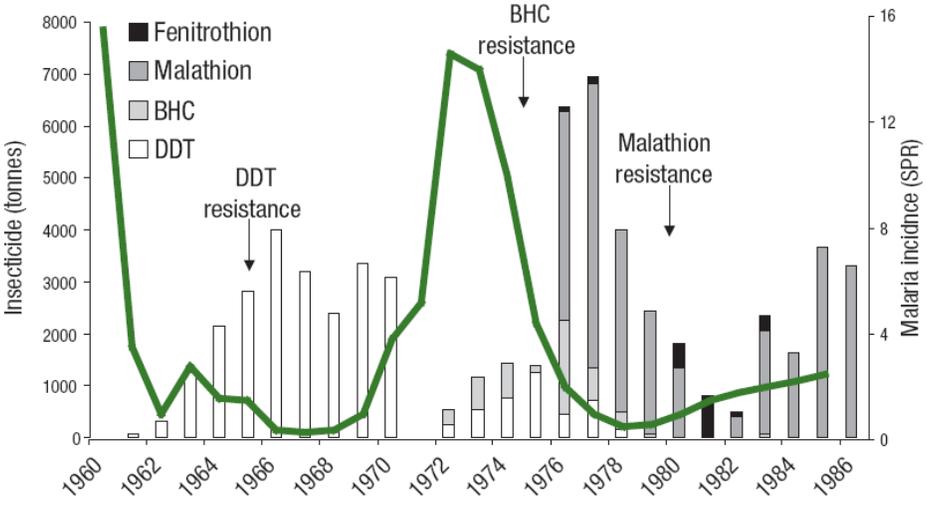**Figure 3: Hypothetical vector control programme cost with or without resistance management**

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

7. Monitoring of vector susceptibility and resistance detection

7.1 Monitoring objectives

Monitoring levels of insecticide susceptibility is essential to enable timely and rational decision making when substituting a product and or class of insecticide in favour of another, before insect control is compromised and hence the risk of disease transmission rises.

The switch in strategy can encompass a change of insecticide, a change from adulticide to larvicide, or implementation of alternative strategies, see Ch. 4. Monitoring must also include assessment of cross resistance, since changing from one product, which has been compromised, to another for which there is cross resistance would not be effective.

The monitoring of insecticide susceptibility in vector control programmes has the following three main objectives:

- **Baseline data collection:** Conducted prior to the start of a control programme in order to provide baseline data to inform planning and insecticide choice.
- **Monitoring of susceptibility over time:** To evaluate the proportion of susceptible mosquitoes in the population over time, comparing it with the pre-intervention baseline. Hence the impact of the control strategy on the proportion of susceptible individuals in the mosquito population can be evaluated.
- **Detection of resistance:** To detect resistant individuals when they are at a low frequency in the population so that resistance management can be effectively introduced. Detection of resistance when a large proportion of the mosquito population are already resistant limits the potential effectiveness of IRM strategies.

Figure 5: Detecting a shift in mosquito susceptibility at an early stage by monitoring LC$_{50}$ and calculating the Resistance Factor based on LC$_{50}$
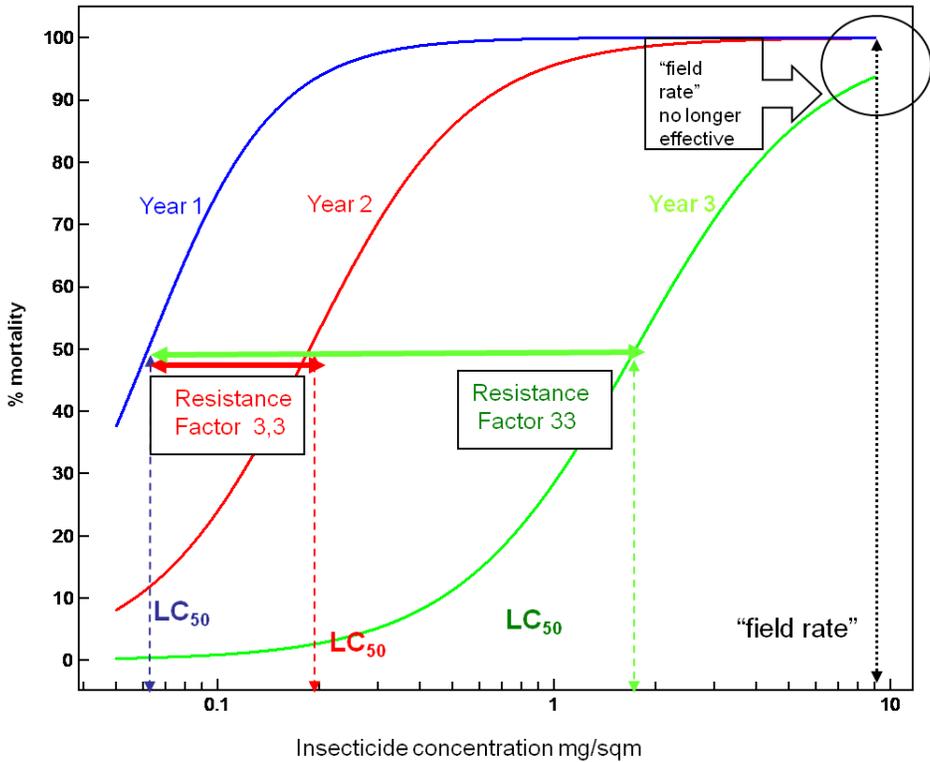
Year 1: 100% control at application rate. Strain fully susceptible

Year 2: Still 100% control at application rate. But build up of incipient resistance Resistance Factor 3,3

Year 3: Product failure = less than 100% control at application rate. Resistance Factor 33

7.1.1 Monitoring based on the Discriminating Dose (DD)

The Discriminating Dose is calculated as twice the LC_{99} of an insecticide. However, the LC_{99} may remain unchanged as long as the number of homozygous resistant individuals is still low, and the majority of individuals are still controlled by the insecticide. So, resistance may build up in a vector population before the LC_{99} is affected but an increase in the number of heterozygous resistant individuals will cause a shift in the LC_{50}.

7.1.2. Detecting early resistance based on LC_{50} and Resistance Factor

Looking at complete dose mortality data, including the LC_{50}, enables detection of a shift in vector susceptibility, an early sign of incipient resistance in the vector population, long before any sign of reduced insecticide efficacy in the field may occur. Calculating the Resistance Factor based on the LC_{50} allows a comparison of the susceptibility of a vector population over time, or to compare between strains.

7.1.3 Calculating the Resistance Factor

$$\text{Resistance Factor} = \frac{LC_{50} \text{ Resistant Population}}{LC_{50} \text{ Susceptible Population}}$$

The Resistance Factor should always be related to the method used, e.g. CDC bottle assay or WHO paper test etc.

7.1.4 Interpretation and benefit of resistance monitoring data

Careful use of the information generated from the LC_{50} and calculation of the Resistance factor will allow evidence based decisions to be made in the design of integrated vector management strategies in a specific locality.

The main problem associated with the onset of resistance is the failure to reduce vector populations and the potential for an increase in disease transmission. Monitoring will allow a timely change in strategy; however, in some cases such as with insecticide treated bednets (ITNs and LNs), where pyrethroids are the only class of insecticides currently approved for use by WHO, there is no ready alternative.

Recent work in experimental huts in southern Benin has shown reduced efficacy of pyrethroid treated LNs in an area with a high frequency of pyrethroid resistant mosquitoes. Insecticide resistance was also linked to a rise in malaria cases in South Africa following failure of pyrethroid based IRS.

Whilst monitoring and accurate assessment of the susceptibility of the vector population is fundamental to any programme, consideration must be given to factors, other than resistance, that can also lead to control failures. In many cases, poor application technique, under dosing, or application at the wrong time of day, in the case of space sprays, can cause control failure; these substandard practices must be addressed first.

Resistance may also be localised, therefore to ensure an effective regional IRM programme, the distribution of resistant individuals within the whole region should be assessed. Changes to the vector control programme can therefore be optimised to reflect local conditions.

Insecticide susceptibility monitoring and the detection of resistance alone, has little value unless a resistance management strategy has been defined and an action plan developed to respond to its discovery.

7.2   Monitoring methods

There are various bioassay, biochemical and molecular methods that can be adopted to test and monitor resistance development. These can be used together to maximise outputs from monitoring in a region. It is important to characterise resistance mechanisms present and also the level of susceptibility to other insecticide classes available for use in the region, as detailed later.

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 estimated to kill 100 % of mosquitoes of a susceptible strain. This approach has been designed to avoid spurious reports of resistance in the field where none may exist. The kit provides a simple test method which may be used in the laboratory or field to detect resistance in adult mosquitoes.

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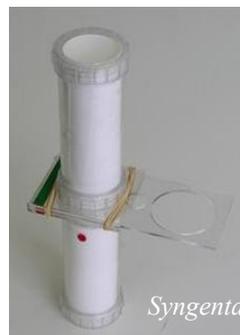
The kit and papers can be purchased with full instructions on their use. Supplier details can be found at: http://www.who.int/whopes/resistance/en/

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, and be either: a) emerged from field collected larval stages, b) F1 generation bred from field collected mosquitoes or, as the last choice, c) field collected mosquitoes. The use of laboratory emerged mosquitoes is preferable as it removes the variability due to the physiological status of mosquitoes i.e. age, blood fed status or stage of gonotrophic cycle.

The mosquitoes are exposed for a standard period of time to the papers before being removed and held in clean cups with net closures and sustenance for 24 hours before mortality is assessed.

Full details of the methodology are found in the document *Test Procedures for Insecticide resistance* (WHO/CDS/CPC/MAL/98.12) which can be viewed at www.who.int/whopes/resistance/en/*.*

For new insecticides a new diagnostic concentration has to be determined. The WHO recommended diagnostic concentrations for each group of vectors 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 result in greater than 98% mortality of individuals from 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	IRAC MoA group	Insecticide	Anophe-lines	*Aedes aegypti*	*Culex quinque-fasciatus*
Organo-chlorines	3 B	DDT	4%	4% [a]	4% [b]
Organo-phosphates	1 B	Fenitrothion	1% [c]		1% [d]
		Malathion	5%	0.8%	5%
Carbamates	1A	Bendiocarb	0.1%		
		Propoxur	0.1%	0.1%	0.1% [c]
Pyrethroids	3 A	*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%

* Diagnostic concentrations for *alpha*-cypermethrin and bifenthrin have been proposed at 0.05% and 0.2% respectively. These tentative concentrations await confirmation; refer to WHO website for further information.

 a - Half an hour exposure
 b - Four hours exposure
 c - Two hour exposure for *Anopheles sacharovi*
 d - 0.1% for *Anopheles sacharovi*
 e - Two hour exposure for *Anopheles sacharovi*

Control (blank) papers
The appropriate untreated papers should be included in assays, with selection based on the class of insecticide being tested:

- Organochlorine control, risella oil
- Pyrethroid control, Dow Corning® 556 silicone oil
- Organophosphate and carbamate control, 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 insecticides. Care should also be taken to follow the storage advice supplied with the papers as failure to do so can lead to premature degradation of the insecticide, with an increased risk of false positive results.

Note: Pyrethroid papers in particular should not be used more than 5 times, as with each exposure insecticide is removed and there is the risk with increased exposures that levels of insecticide will become depleted and false positive results may result.

Interpreting results
WHO Test Kit - Adult mosquitoes
Express the 24 hour mortality as a percentage. If the mortality in the control groups is over 5% but less than 20% a correction of mortality is made by applying Abbot's formula.

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

When the mortality in controls is $\geq$ 20% the test results are discarded and the test will need to be repeated. Calculate an average of the mortality obtained at the same concentration in at least three replicates.

Results are interpreted as follows:

98 – 100% mortality	Susceptible population
80 – 97% mortality	Resistant individuals within the population suspected, but verification/confirmation required
<80% mortality	Resistant individuals within the population present

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

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 collected from the wild. A wide range of concentrations is used to start with, so that an approximate level can be determined. Then a narrower range of 4-5 concentrations yielding 10% and 95% mortality in 24 hour 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:

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

The test kit stock solutions available do not include pyrethroids.

7.2.3 WHO Test Kit – Larvicides (Insect Growth Regulators)

Different tests are conducted with IGRs 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 (WHO/CDS/WHOPES/GCDPP/2005.13)

Insecticide	mg/ l				
Methoprene	20	4	0.8	0.16	0.032
Diflubenzuron	20	4	0.8	0.16	0.032
Control	Alcohol only				

Note that there is no stock solution for pyriproxyfen.

7.2.4 Bacterial larvicides
Larvicides such as Bti or Bs 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 WHO/ CDS/WHOPES/GCDPP/2005.13

7.2.5 CDC Bottle Test Kit - adult mosquitoes
The bottle bioassay method is a tactical surveillance tool for detecting and characterising changes in susceptibility to insecticides in vector populations.

The bioassay uses 250 ml glass bottles. The internal surfaces of the bottle are coated with the desired insecticide diluted in acetone or ethanol. Once the solvent has evaporated, between 10 and 20 adult mosquitoes are aspirated into the bottles and sealed in with the lid.

Assessments of knockdown or mortality are made at 10 minute intervals. Knockdown or mortality is then plotted against time. Changes in the slope of this graph over time are indicative of changes in the susceptibility of the mosquito population.

This method recognises the importance of the diagnostic dose and encourages those using it to calculate an appropriate diagnostic dose at the start of their monitoring programme using an insecticide rate range study. To guide this, the following doses are suggested:

Pyrethroid diagnostic dose	
Cyano-pyrethroids e.g. deltamethrin	25 µg/bottle
Non-cyano-pyrethroids e.g. permethrin	43 µg/bottle

Synergists, such as PBO and DEF, can be used with the insecticide in the bottle bioassay and can give a limited indication of metabolic resistance mechanisms, if present.

If there is a significant increase in the time to knockdown or mortality recorded in the mosquito population, further studies should be undertaken to identify whether the change in susceptibility is reflected in product performance, and where possible, to identify the resistance mechanism.

Likewise, if there is a significant decrease in the time to mortality of mosquitoes from the addition of synergists, a metabolic resistance mechanism can be suspected, and a sample of adult female mosquitoes from the area should be presented for further analysis.

Full details and a step by step method description are available from: www.cdc.gov/ncidod/wbt/resistance/assay/bottle/index.htm. Also the CDC will furnish, at no cost, premeasured amounts of WHOPES approved IRS and LLIN insecticides, sufficient to conduct approximately 100 bottle assays for each insecticide. The recipient is responsible for approval to import these insecticides into their country. Contact Dr. W. Brogdon (wbrogden@cdc.gov) for additional information.

7.2.6   Other monitoring methods

Test methods based on biochemical or molecular assays are also available for resistance monitoring. These techniques have several advantages over bioassays: they can detect resistance at very low frequency within a population, can indicate the presence of heterozygous individuals with recessive resistance genes that are not detected through bioassays and they require fewer mosquitoes than bioassays. This last point is of particular interest for species for which larvae or adults are not usually found in large numbers.

(c) M. Hallahan/Sumitomo Chemical-Olyset Net

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). However, specialist equipment is needed for these techniques. Whilst advances in the technology continues apace, they cannot yet be considered true field assays.

a) Biochemical tests methods

These methods rely on enzymatic action upon a model substrate which may, or may not, accurately reflect metabolism of insecticidal compounds. Typically P450 activity is measured using *O*-deethylation of 7-ethoxycoumarin, glutathione S-transferase activity is measured using chlorodinitrobenzene (CDNB) or dichloronitrobenzene (DCNB) and non-specific esterase activity using 1-naphthyl acetate. Using microplate technology these spectrophotometric/ fluorometric assays can become powerful, high throughput monitoring assays

CDC

b) Molecular test methods

Molecular assays can greatly complement bioassays, and are especially useful to monitor trends in resistance gene frequency over time. Their use is currently restricted to research labs since field test kits are still in development.

The following is a description of some molecular techniques, highlighting the information they can provide, and is not designed to be a guide to their use. Further advice should be sought on how to implement these increasingly valuable tools in a monitoring programme.

Species identification

In all monitoring surveys the mosquitoes first need to be identified as belonging to the *Anopheles* genus and to species level using morphological keys. The proportion of the morphologically identified *An. gambiae s.l.,* represented by each species, can be determined using a multiplex PCR assay. From this, the proportion of each species that were survivors and non survivors of bioassay susceptibility tests can be determined.

Identification of M and S molecular form

There is increasing support for the view that the M and S form of *Anopheles gambiae s.s.* are in fact separate species. It is therefore valuable to identify which form, or the relative proportion of each form, is present in the population under study.

Molecular M and S forms within *Anopheles gambiae s.s.* can be differentiated using a restriction fragment length polymorphic (RFLP) PCR assay.

The proportion of the *An. gambiae s.s.* test population, represented by M and S molecular forms, should be determined. From this, the proportion of each molecular form that were survivors and non-survivors of bioassay susceptibility tests can then be determined.

Detection of kdr by Polymerase Chain Reaction
Allele specific RT-PCR (Reverse Transcription Polymerase Chain Reaction) assays can be carried out on phenotyped samples using a restriction fragment length polymorphic (RFLP) PCR assay.
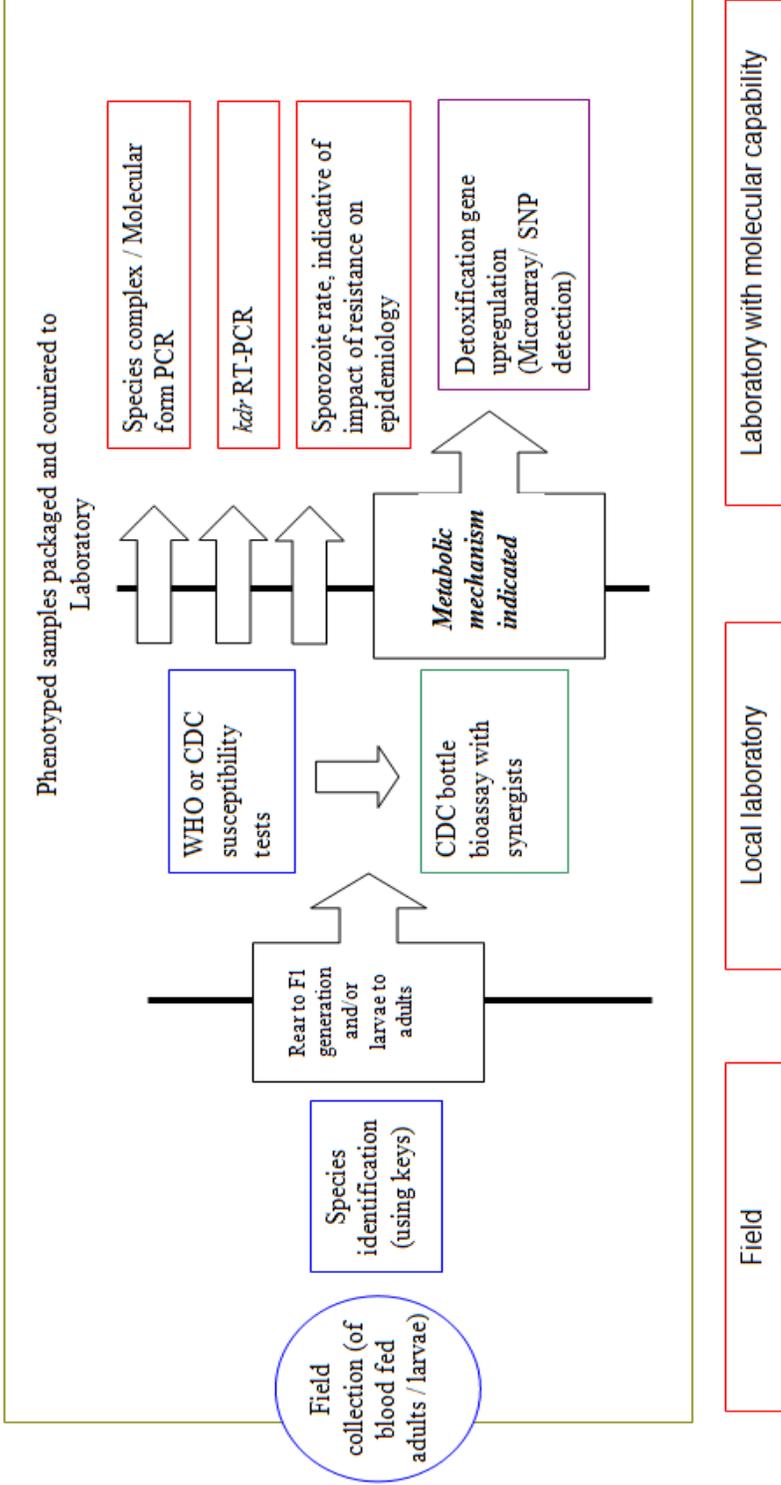
The frequency of *kdr* alleles can also be determined within each of the M and S molecular forms of *An. gambiae* s.s. and within survivors and non-survivors of bioassay susceptibility tests.

Microarray: Metabolic resistance mechanisms
Microarrays can be used to screen for metabolic resistance from field caught mosquitoes (e.g. *An. gambiae s.s.*, *An. arabiensis*, *An. funestus* and *Aedes aegypti*). Compared with biochemical assays they are highly sensitive and specific. Ideally, resistant and susceptible field mosquitoes should be co-hybridised however if no suitable field control is available lab colonised mosquitoes can be used as an alternative.

The *An. gambiae* and *Ae. aegypti* 'detox chips', which were developed at the Liverpool School of Tropical Medicine, can be used to identify increases in RNA levels associated with P450 oxidase, GST and esterase based resistance. The use of these detox chips requires access to a specialised laboratory and there is currently no field method to test for the presence of resistance associated P450 oxidases, esterases and GSTs, other than bioassays with synergists.

Flow Chart 3: Simplified diagram indicating possible steps in a resistance monitoring programme

Phenotyped samples packaged and couriered to Laboratory

Field collection (of blood fed adults / larvae)

Species identification (using keys)

Rear to F1 generation and/or larvae to adults

WHO or CDC susceptibility tests

CDC bottle bioassay with synergists

Metabolic mechanism indicated

Species complex / Molecular form PCR

kdr RT-PCR

Sporozoite rate, indicative of impact of resistance on epidemiology

Detoxification gene upregulation (Microarray/ SNP detection)

Field

Local laboratory

Laboratory with molecular capability

7.2.7 Mosquito bednets

Mosquito bednets have become a major intervention method for the control of malaria vectors in recent years. While the technologies described above can evaluate the susceptibility of mosquitoes to the insecticide incorporated in the bednet, different test methods are required for evaluating nets themselves. However, these must be treated as evaluations of the nets and not susceptibility tests, as the insecticide dosage received by the mosquitoes on the net may vary significantly. Results from such studies cannot be used to measure changes in insecticide susceptibility within a mosquito population. Care must be taken in the evaluation of such results to ensure that poor bioavailability of insecticide on the net has not compromised the assay. Loss of insecticide due to ageing, washing, inappropriate use or storage, etc. can lead to reduced mosquito control. However, this should not lead the tester to conclude that there is necessarily resistance in the mosquito population.

(c) M. Fallahan/Sumitomo Chemical-Olyset Net

7.3   Selection of monitoring sites

One challenge in monitoring changes in mosquito population susceptibility is establishing an adequate number of sentinel sites that will consistently sample the target population over time. Careful assessment should be conducted when determining collection sites, such that the abundance of the target species, the ease with which the site can be accessed, and the probability of it being available for multiple years are ensured. Selection of the specific site at each location should be based on advice from programme partners and recommendations for insecticide resistance monitoring.

The choice and number of sentinel sites is critical and should be carefully considered during the development of the vector control programme, with input from the appropriate NMCP, regional WHO advisors, etc. Key factors include:

1. Geographical size and population base of the intervention area
2. Pre distribution/application and post distribution/application coverage rates
3. Conditions of the location that may affect intervention usage/ uptake
4. Relative prevalence of malaria at the location (priority should be given to the most malarious regions)
5. Insecticide resistance status of local vector populations if known
6. Predominant species and densities of the local vector populations
7. Accessibility of the location
8. Environmental factors that may interrupt the programme
9. Local support, resources and stability

Ideally, an insecticide resistance monitoring program should cover a range of locations, including areas associated with agricultural use of insecticides.

8. Managing a vector control programme

8.1   Developing a long term plan is critical

The tools available for vector control are limited and new insecticides, against which there is no resistance, may be few in the near future. Therefore, once resistance to key insecticides has developed, it can have a major impact on mosquito control. Hence, the judicious use of insecticides is fundamental to any sustained effective vector control programme.

The current practice in many programmes has been to use an insecticide continuously until it fails. The result is a loss of a valuable mosquito control intervention. Instead agencies should develop plans that use multiple tools (Integrated Vector Management IVM). Additionally, only a few programmes regularly monitor susceptibility levels in the vector population, leaving many unaware of the effectiveness of their vector control interventions.

The widespread use of LNs, currently all treated with pyrethroid insecticides, is increasing selection pressure on this valuable class of insecticide.

8.2   Quality control of applications

In many cases resistance is blamed for control failure, when in fact there may be other 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) Poor quality product
- Use of products that fail to conform to recognised specifications, e.g. WHOPES
- Use of out of date or incorrectly stored product

c) Insufficient coverage
- Poor acceptance of control strategy by population.
- Failure to locate and treat all significant breeding sites when larviciding.
- Inadequate pre-spray survey to identify key breeding areas for space spraying.
- Failure to treat all relevant structures in IRS campaigns.

c) Incorrect dilution/application rate
- Failure by operators to correctly dilute the insecticide according to label recommendations.
- Failure to apply the correct dose per unit area.

Syngenta

d) Incorrect frequency of application
- Residual applications out of synchrony with transmission season.
- Space sprays not coinciding with peak vector activity.
- Inappropriate frequency of application.

e) LN specific issues
- Inappropriate use or failure to use LN as prescribed.
- Physical damage to LN, holes, rips, etc.
- Inappropriate washing with harsh detergents or bleaching agents.
- Inappropriate frequency of washing.

The above points must be checked before considering the possibility that insecticide resistance has developed. In addition poor application, such as under dosing, may accelerate the rate of resistance development as the vector population will be exposed to sub-lethal doses of insecticide.

8.3   What to do when resistance is suspected

The first question to ask 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 insecticide resistance or the product itself. Therefore the suspicions of resistance must be confirmed using bioassays, or as already noted, molecular 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 questions to consider before any action is decided:

a) How widespread is the resistance?
Surveys should be undertaken to identify the distribution of resistant mosquitoes.

b) Which species are resistant?
It is rare for resistance to occur in all mosquito vector species in the area and it may be only one species that is involved. Is the resistant species an important vector? If not the problem may be limited. In addition, sub-species may have different susceptibility profiles. This has been clearly identified in India and Africa where sibling species of malaria vectors have developed pyrethroid resistance while others, although closely related have not.

c) What is the proportion of resistant individuals in the population, and what is its impact on the control programme?
Is the level of reduced susceptibility causing control failure? If not, then the current programme may be continued in the short term with ongoing monitoring to determine if the level of resistance increases, whilst a resistance management strategy is formulated. Although an early shift to alternative insecticide or method is highly desirable, it is not always possible when such an alternative does not exist or are not locally available. This is particularly the case with LNs where there are currently no approved alternative modes of action available other than the pyrethroids.

However, it has been shown that pyrethroid treated bednets continue to give some protection even when resistance is present. If significant resistance is identified, the use of IRS with a non pyrethroid insecticide should be considered. Other actions in this case could be to control the larval stages with an unrelated compound, e.g. a bacterial larvicide or an insect growth regulator (IGR).

c) Identify the resistance mechanism(s) involved and the level of resistance of the target species.
Switching insecticides within the same MoA group is not recommended, see chapter 4; as cross resistance may be present and selection pressure is not removed. In addition, care must be taken to evaluate any potential cross resistance to other insecticide groups.

Identification of all resistance mechanisms involved gives an indication of which alternative compounds should be used. Figure 1 highlights which mechanisms may be acting to cause resistance and which other insecticides may be cross resisted. The susceptibility of the target mosquito population to insecticides from other MoA groups must be checked before changing products.

d) Identify source of insecticide pressure
It is important to understand from where the selection pressure is being applied. It may be through many years of repeated use of the same type of insecticide or 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 major source of selection, so that future strategies recognise the problems, and where possible, try to avoid using similar insecticides to those used in local agriculture, etc.

In addition, where practical, the relevant Vector Control and Agricultural Departments should work closely together, to avoid conflicts in the development of resistance management programmes.
.

9. Success stories in resistance management

The potential for managing the development of insecticide resistance has been modelled 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, still using 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 in the laboratory, thus further confirming the potential for rapid development of resistance under continuous use of a single chemical larvicide.

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 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, 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, 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   Integrated vector management in practice.

AngloGold Ashanti is a large gold mining corporation. At their Obuasi mine in Ghana, nearly 7000 man days were being lost per month to malaria in 2005. This had a significant impact on the mines productivity, and was a huge burden to the community at large. To address this, AngloGold Ashanti developed an integrated malaria control programme. Vector control plays a significant part in this programme. In combination with extensive medical interventions, the number of days lost to malaria fell to 282 per month in 2009. The increased productivity brought about by a reduction in the incidence of malaria more than outweighed the investment in the programme.

The malaria control programme is an ongoing commitment. With vector control playing a major part in the programme, insecticide resistance is a significant risk to the programme's future success. This was recognised and insecticide susceptibility monitoring was included from the start. Susceptibility monitoring guided the initial choice of insecticide and will be the basis for decisions on the rotation of insecticide mode of action class through time.

AngloGold Ashanti's vector control programme doesn't rely solely on LNs and IRS, but is augmented by other interventions. Where appropriate, focussed larvicide applications have been made and environmental management controls have been undertaken to reduce mosquito breeding sites.

This example shows that it is possible to implement an integrated vector management programme and have a significant positive impact on the burden of malaria. The sustainability of the vector control interventions used have been enhanced by considering insecticide resistance management throughout the programme, and by having a resistance management plan from the outset. As the London Financial Times newspaper wrote "The AngloGold Ashanti Malaria Control programme is the best example of a sustainable corporate social responsibility programme with a win – win for company and community".

9.4 Successful resistance management in agricultural

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) 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 minimise 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 recommended number of applications per season as indicated by the Cotton Catchment Communities Resistance Management Strategy.
- 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.

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.

9.5   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.
- Trials have demonstrated that a rotational strategy is both a technically sound and operationally acceptable means of managing resistance in vector management programmes.

10.   Concluding remarks

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 30 years, very few new insecticides have been developed for adult mosquito control; 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, further increasing the reliance of public health on a limited number of products. In some countries DDT has been reintroduced because of the lack of new viable alternatives and insecticide resistance to pyrethroids in some vector species. However international pressure continues for a complete ban on DDT for all uses, including vector control.

New classes of insecticides with novel modes of action are required, since they are less likely to be effected by existing resistance mechanisms. However, the prospect of accessing new public health insecticides with the ideal vector control characteristics in the near future appears limited.

The cost of developing a novel insecticide for vector control could exceed $200 million. Commercial companies, therefore, find it difficult to justify such investment when compared to the potential return. However, initiatives from organisations such as the Bill & Melinda Gates Foundation and IVCC are sponsoring the commercial sector to look through their libraries of compounds in the hope of finding new classes of public health insecticides.

It is essential that reliance on insecticides is reduced as much as possible by promotion the principles of Integrated Vector Management, using insecticides only when and where they are really needed, i.e. targeted applications. This is especially true in the management of diseases such as malaria and dengue where chemical mosquito control is such an important component.

10.2   Constraints and limitations to resistance management

In many vector control programmes there is currently a trend to shift from well planned vertical operations to community based interventions such

as the promotion of insecticide treated materials. Insecticide concentrations on these materials can vary depending on how they are used, and how frequently they are washed. Ensuring the presence of an effective dose on such materials is challenging, resistance management therefore becomes more difficult to implement. With the large scale introduction of Long Lasting Insecticidal Net (LN) programmes over recent years, which are wholly treated with pyrethroids, the selection pressure for this group of insecticides has increased. Alternative insecticides for use on nets are being sought, but in the meantime alternative measures need to be taken to protect the efficacy of the pyrethroids.

Many malaria endemic countries are currently involved in a process of decentralisation. As a result, regional 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 in many endemic countries, there is an urgent need for training and capacity development. There is also a need 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 to prevent the development of insecticide resistance. Many programmes managers may feel they are not able to cope with the financial and logistic challenges associated with the change of insecticides or vector control approaches because of the limited financial resources allocated to vector control. However, the consequences of not being proactive in resistance management 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 due to resistance.

10.3   The way forward

Realising these difficulties and constraints does not provide justification for opposition to progress. Agriculture has been confronted with similar problems to those encountered in vector control (though with different constraints and consequences) 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 their vector control activities and national pesticide management policies.

Institutions such as CropLife International, the industry federation, and IRAC are collaborating with organisations, such as the WHO, to provide practical help to public health 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, grouping agriculture, public health and domestic hygiene together. It is important for the producers of mosquito control products to be aware of the status of vector resistance, just as it is essential for vector control managers to increase their knowledge of the agricultural use of insecticides. It is hoped that this manual is a positive step in this collaboration.