The recent progress in malaria control, including in many of the countries receiving support from PMI, has been largely accomplished through a massive increase in vector control through long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Since both of these prevention measures depend on the ability of insecticides to kill or reduce the lifespan of female mosquitoes, understanding and monitoring insecticide resistance are critical to their continued effectiveness.

The World Health Organization currently approves twelve insecticides in four different classes for use in IRS programs. In contrast, because of its human safety and insecticidal properties, pyrethroids are the only insecticide class that can be used for treated nets. Pyrethroids are also now the cheapest, safest and, in the absence of resistance, the most effective insecticides used for IRS. Pyrethroids are also among the most popular insecticides for both agriculture and domestic use. While potent, safe and relatively inexpensive, genes that confer resistance to pyrethroids are spreading through some of the important malaria vectors in Africa, posing a significant threat to progress that has been made.

Responding to insecticide resistance is neither easy nor cheap; any alternative to the pyrethroids for malaria control will be both more complicated and more expensive, with decisions based on information that is often patchy and not definitive. Nevertheless, with the changing epidemiology, changing ecology and biology of the mosquito vector – as well as new chemicals and formulations soon becoming available – it is essential that we develop entomological capacity to monitor, adapt, and respond to emerging insecticide resistance.

The purpose of this document is to provide information and guidance to President’s Malaria Initiative teams on technical and programmatic issues surrounding entomological monitoring and insecticide resistance management. The core elements of this document are consistent with guidelines being developed by the WHO Global Malaria Program and the Roll Back Malaria Vector Control Working Group\(^1\), with some of the background and technical details expanded for the current situation in countries supported by PMI.

This guidance is divided into three sections:

1. A technical background and links to resources for further information on insecticides and their modes of actions; resistance and sources of selection; and the link between resistance and actual vector control failure. While this goes into some technical detail, it is important for PMI teams and partners to have a basic understanding of the biological and ecological basis of resistance, the terminology used and links to on-line resources available for insecticides and vector control;

2. A “Tactical” section, describing entomological collection techniques, analysis, interpretation and pesticide selection for deciding current vector control interventions, with specific PMI guidance on each of these areas. At the end of this section is a unit on “Frequently asked Questions”. While attempting to be as prescriptive and definitive as possible, many of the suggested steps and criteria for decision-making depend on the specific country-context, i.e. interpretation and judgement is required. To help

\(^1\) This draft was partly developed at an RBM Insecticide Resistance work stream drafting committee meeting, chaired by Prof Janet Hemingway.
with this interpretation and judgement, USAID/Washington and CDC/Atlanta have established a small core entomology team to assist country programs in these decisions;

3. A brief “Strategic” section, providing a longer-term view of insecticide resistance management through a re-orientation of national programs into an Integrated Vector Management framework. i.e. broader collaborations, evidence for better targeting and judicious use of insecticides and capacity-building. One of the most difficult steps in targeting and judicious use of insecticides is to help successful programs that have achieved a significant reduction in malaria transmission and burden to “graduate” to a more surveillance-driven and focal use of IRS applications. Strategies and indicators for these potentially “pre-elimination” situations are discussed in the linked WHO documents.
I. Technical Background

Insecticides and modes of action

While about twenty classes of chemicals are registered for use against agricultural or domestic pests, only four classes are registered for use against adult mosquitoes, with another three that can be used in larval control. A fifth class of insecticide, the pyrroles, is currently registered by the US Environmental Protection Agency for indoor use, e.g. commercial kitchens. One member of this class, the insecticide chlorfenyapyr, is being considered for development as an IRS chemical. There is also work with entomopathogenic fungi in the genus Beauveria as a potential product for IRS. Further background information on insecticides use in public health, their safety and efficacy can be found at the WHO Pesticide Evaluation Scheme web-site. http://www.who.int/whopes/en/ where the following table can be found. Information on USAID pesticide regulations and their use within PMI-supported operations can be found at www.pmi.gov/pesticides

The four classes of insecticides currently recommend by WHO for IRS are all neurotoxins that paralyze and kill the insect almost immediately. The oldest of these, is the organochlorine class to which DDT belongs, came in to widespread use in the 1940’s. The mode of action of the organochlorines, like that of the pyrethroids, developed in the 1970’s and 80’s, is to attach to the insect neuron sodium channel, keeping it open and not allowing the nerve impulse to recharge. The two other classes, the carbamates and the organophosphates inhibit acetylcholinesterase (AChE), the enzyme that terminates the action of the excitatory neurotransmitter, acetylcholine, at nerve synapses. Carbamates bind loosely and reversibly to AChE, whereas the organophosphates bind more strongly to both insect and human AChE. Thus, certain organophosphates can be relatively more toxic to humans and require weekly AChE monitoring of spray operators to assess any possible toxicity. A potential new class of public health pesticide, the pyrroles act by disrupting mitochondrial ATP, leading to cellular death and eventual insect mortality. Significantly, chlorfenyapyr which is being developed for IRS will not kill the insect as rapidly as the other four classes of neurotoxins. Understanding modes of action is essential for devising a strategy of switching or rotating insecticides. An excellent resource for learning more about the modes of action is the Insecticide Resistance Action Committee (IRAC) http://www.irac-online.org/.
Resistance to insecticides is defined as: *a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species* (IRAC). Entomologists classify insecticide resistance into two types:

**Behavioral** resistance is when the vector population evolves to an outdoor or more zoophilic feeding pattern (i.e. feeding on animals rather than humans) to avoid indoor insecticide application. This was initially thought to have happened in Northern Thailand in the malaria vector *Anopheles minimus* s.l., where vectors in villages with more DDT spraying showed a marked tendency for more outdoor resting and animal feeding. Later however, it was shown that what actually happened was that *An. minimus* is a species complex, and the more endophilic (house-entering) members of the complex were eliminated by spraying, leaving those sibling species more genetically pre-disposed to outdoor feeding. Likewise in Kenya, as shown in the chart above there has been a shift from *An. gambiae* to the somewhat earlier, outdoor biting *An. Arabiensis*. More recently, there have been some suggestions that *An. arabiensis* is shifting to earlier, outdoor feeding as LLIN coverage increases to high levels in Zambia. While this may not lead to complete control failure, these shifts may reduce the efficacy of the control measure. Overall, care should be taken when ascribing mosquito behavior change to insecticide exposure as it is often found that the underlying cause was a selection of particular members of a species complex.

**Physiological** resistance is conferred by two primary mechanisms. First, a detoxification mechanism, sometimes called metabolic resistance, involves a change or amplification in the enzymes that metabolize the insecticide, lowering the amount that eventually reaches the target site. There are three categories of metabolic resistance: a) esterases that work against the organophosphates and some of pyrethroids; b) mono-oxygenases (sometimes referred to as the p450’s) that work against all four classes of insecticides; and c) glutathion S-transferases, that work against DDT, pyrethroids and organophosphates.
Metabolic resistance can have a strong impact on vector control efforts. For example, it was the “mono-oxygenase” resistance in Kwa-zulu Natal that enabled the An funestus population there to become highly resistant to pyrethroids, forcing the national malaria control program to return to the use of DDT\(^5\). While still under investigation, this may also be the same mechanism we are beginning to see in the Malawian population of An funestus. Metabolic resistance may sometimes cost the mosquito energy to maintain higher levels of these particular enzymes. For an insect, such as a female anopheline, foraging for human blood and a place to lay her eggs, especially in settings with high ITN coverage and limited breeding sites, this extra energy expenditure may have a fitness cost. Thus, when the selection pressure of the insecticide is removed (through insecticide rotation) or if there are sufficient sites within the microhabitat where the vector will not be exposed to the insecticide as in mosaic spraying, these genes may die out.\(^6\) There are, however, exceptions to this finding, and this does not appear to have happened for An. funestus\(^7\).

The second major type of physiological resistance, “target site insensitivity” is related to the insecticide molecule no longer binding tightly to its target (e.g. sodium channel binding to DDT and the pyrethroids, and acetyl cholinesterase to the organophosphates and carbamates). Probably the best known of these is the change in the sodium channel detected through a genetic marker, known as the “knock-down resistance,” or kdr gene. As it is relatively easy to determine this resistance mechanism, kdr is widely reported; however in its heterozygote form, it is less associated with failure of vector control measures.

In addition behavioural and physiological resistance, there can be another biological form of resistance known as cuticular resistance, whereby in insects with thicker or waxier cuticles (the insect exoskeleton) there is less penetration of the insecticide. This was recently postulated as an auxiliary mechanism for the pyrethroid resistant An. funestus in South Africa\(^8\).

**Cross resistance**

Cross resistance for insecticides that share a similar mode of action is quite common. For example, it is believed that the common kdr target site resistance to pyrethroids in West Africa, actually arose as cross-resistance to heavy DDT use in commercial agriculture. Because all compounds within a single class share a common mode of action, there is a high risk that the resistance to one compound will confer cross-resistance to all compounds in the same subgroup. While there may be some differences between the detoxification mechanisms, and some differences shown by the different discriminating doses in the WHO Tube Assay, it is generally accepted that from an operational standpoint, resistance to one pyrethroid, to one carbamate, or to one organophosphate confers resistance to all members of that class. However, because of the different ‘discriminating doses’, one can sometime see ‘susceptibility’ to one pyrethroid and ‘resistance’ to another. Cross-resistance between pyrethroids and DDT, however, is not an automatic outcome. Pyrethroid-resistant An. funestus in southern Africa are fully susceptible to DDT\(^8\), but in this case kdr is not present and the resistance mechanism is metabolic. Cross resistance between and among carbamates and organophosphates is highly variable. Resistance to malathion often does not cross to the other organophosphates (notably in Sudanese An. arabiensis) and pirimiphos-methyl is also very different from the others.

**Source of Selection Pressure**

Resistance selection from non-public health pesticides, such as agricultural insecticides running off into breeding sites or oil pollutants contaminating the water table, may contribute to
selection pressure. There is clear evidence of situations where IRS drove selection of insecticide resistance (e.g. Bioko Island, Sri Lanka and Sudan) as well as evidence of agricultural pressure (e.g. Latin America, Cameroon, West Africa). There is also newer evidence from Benin and Nigeria that hydrocarbon pollution of ground water can confer insecticide resistance, including pyrethroid resistance, in *An. gambiae*. We don’t yet know how ITNs, especially LLINs, will drive resistance development, especially when universal coverage with LLINs is achieved.

**Detection and impact of insecticide resistance**

Resistance is assessed primarily by one of two roughly equivalent *in vivo* assays, the “WHO tube assay” and the “CDC bottle assay”. Both are limited by availability of mosquito specimens and the skills needed to conduct the tests and interpret the results. In addition to *in vivo* assays, there are laboratory techniques to determine the underlying mechanism of resistance, sometimes referred to as “molecular assays” or “genetic markers”. Although these can detect the two primary forms of resistance mechanisms noted above, two important caveats must be noted: molecular typing, especially detection of the kdr gene, does not always correlate with *in vivo* resistance; and while the *in vivo* results may be an indicator for growing resistance problems, the result by itself does not predict an operational failure of IRS or ITNs. Additional entomological and epidemiological indicators are needed to show that the resistance actually has an impact on transmission and warrants a change in insecticide. Entomological indicators, such as resting on freshly spray surfaces are described in more detail below. Epidemiological indicators, such as rising numbers of confirm cases, may be more difficult to attribute to resistance. There is a large, multi-country WHO project on the epidemiological impact of resistance addressing this issue. In terms of levels of resistance one cannot categorically state that a program should discontinue an insecticide when the mortality falls between 80 and 98% in an *in vivo* assay. But mosquito mortality of 80-98% does indicate that there should be follow-up investigations as described in more detail below. If below 80% mortality, the general recommendation has been to switch away from that insecticide class and carry out further investigation to confirm the mechanism and distribution of resistance.

**Mitigation of insecticide resistance through rotation of different classes of insecticides**

There are now sufficient data from control programs in both public health and agriculture to state that using carefully chosen rotations of insecticides (switching classes each round), mosaics (applying two different classes in the same area), or combinations of insecticides (analogous to combination therapy for drugs) work well in slowing down the rate at which operationally significant levels of insecticide resistance will be selected. While combinations of insecticides may be marginally more beneficial in reducing the rate of resistance selection, they have a large cost, that along with potential issues around length of efficacy of the different insecticides within the combination, make them economically and technically difficult to deploy.

Until further evidence becomes available, PMI does not support the use of combinations of insecticides. Likewise, mosaic spraying with the use of two different classes of IRS chemicals in the same village is difficult to manage and generally not supported by PMI. In the past some countries had deployed pyrethroids on “formal structures” with plaster-finished wall surfaces and DDT in “informal” houses with mud-surface walls. This should not be confused with “mosaic spraying” and was done to increase operational persistence of insecti-
icides on the sprayed surfaces and for homeowner acceptability, and not as a resistance management tool, as there is significant cross-resistance between these two classes.

Cross-resistance patterns between insecticides can be complex, but as a general rule, insecticides that share a common target site should not be rotated back-to-back. An ideal rotation would deploy insecticides with different modes of action rotated annually.
II: Entomological surveillance and insecticide resistance monitoring

As countries scale up LLINs and IRS programs there is increased insecticide selection pressure on the vector mosquito populations. One can expect to see changes in the species composition, as well as changes in susceptibility to insecticides and possibly changes in behavior. The large investments in LLINs and IRS made by Global Fund, PMI, and other donors, and our dependency on a limited number and classes of insecticides, make it imperative that national programs monitor and evaluate at least a few basic entomological parameters.

Following is guidance on primary and secondary entomological indicators, surveillance sites, reporting, capacity and infrastructure requirements.

Primary entomological indicators: these indicators are considered basic to any well-performing vector control program and should be measured in all PMI-supported IRS and LLIN programs:

1. **Species of malaria vectors in intervention areas**
   - Purpose: To determine which vectors exist in intervention areas.
   - Method: Mosquito identification is basic to all collections and analysis. Basic mosquito collection techniques described in more detail below, include, where appropriate, human landing collections, indoor resting collections, CDC light trap, exit traps and pyrethrum spray collections. Where feasible, larval collections may also be conducted, especially in the case, such as *An. arabiensis* where there may significant outdoor feeding. Where specimens are morphologically identified to the *An. gambiae* complex, a subsample will need to be sent to a reference laboratory for PCR identification to species level. The number of specimens in this subsample will be determined by the relative abundance of the sibling species and the capacity of the reference laboratory.
   - Reporting time frame: Beyond morphological identification of species, the PCR species identification should be conducted at the beginning and at the end of the transmission season, or as otherwise indicated by the relative frequencies of the sibling species.
2. **Vector distribution and seasonality**
   - **Purpose:** To determine vectors’ abundance, distribution and seasonality in the intervention area.
   - **Method:** As described above, the standard mosquito collection techniques will be used as appropriate. An excellent WHO “Manual on Practical Entomology for Malaria Control is available at: [http://whqlibdoc.who.int/offset/WHO_OFFSET_13_(part1).pdf](http://whqlibdoc.who.int/offset/WHO_OFFSET_13_(part1).pdf) and [http://whqlibdoc.who.int/offset/WHO_OFFSET_13_(part2).pdf](http://whqlibdoc.who.int/offset/WHO_OFFSET_13_(part2).pdf).
   - **Reporting time frame:** Collections will be made monthly during the transmission season, or before, if enough vectors are present.

3. **Insecticide susceptibility and mechanisms of resistance**
   - **Purpose:** To determine vectors’ susceptibility to insecticides currently in use or to be used in the future.
   - **Method:** Either the CDC bottle assay or the WHO tube bioassay depending on availability of materials, capacity and NMCP preference. The source of mosquitoes for testing is detailed below. Where possible, a known laboratory-reared, susceptible strain (e.g., KISUMU strain) should be used as controls. If resistance is detected (mortality below 98%), there should be further investigations to determine the mechanism.
   - **Reporting time frame:** Baseline insecticide susceptibility should be established before an intervention is initiated and then conducted annually as long as bioassays indicate 98-100% susceptibility. Testing frequency should be increased, expanded in geographic range, and resistance mode of action determined, if susceptibility falls below 98%. The frequency of testing should also be increased in order to confirm the presence and levels of resistance if there is an unexpected increase in the number of malaria cases in the area.

4. **Quality assurance of LLINs and IRS programs**
   - **Purpose:** To determine the quality of LLINs and IRS (e.g., decay rates taken immediately or one month after spraying can be used to assess sprayer performance) and the efficacy of the intervention (e.g., to determine how long insecticides last in killing or knocking down vectors).

   - **Method:** For IRS: Cone bioassays are currently the only way to measure insecticide decay on sprayed surfaces, although colorimetric tests are under development. Known susceptible laboratory-reared mosquitoes (e.g., KISUMU strain) should be used, if these are not available wild-caught female mosquitoes can be used as long as there is no demonstrated resistance. For LLINs: Similar bioassays and colorimetric tests can be performed on the netting material. For deltamethrin-treated nets there is
also a new field spectrophotometry method under development. Personnel should also be able to monitor physical durability of LLINs using standard inspection methods.

- Reporting time frame:

For IRS: Monthly decay rates should be measured. The first month’s data should be shared with the NMCP and implementing partners as soon as results have been collected in order to initiate immediate corrective action, if necessary. Subsequent results will be needed to determine needs for the next spray round. Obtaining monthly decay rate data is often difficult because of a shortage of susceptible mosquitoes for testing. Nevertheless, for shorter-acting formulations such as the current carbamates and organophosphates, every attempt should be made to conduct monthly testing. For longer-acting DDT and pyrethroids formulations, at least the baseline testing beginning in the 4th or 5th month after spraying should be attempted.

For LLINs the sampling time frame and methods will depend on the specific needs of the national program. PMI is currently supporting a study to monitor net insecticide longevity and physical durability in eight countries with about three monitoring sites per country. Additional studies are being supported by other groups. An informed decision on the utility and strategy for monitoring insecticide content on LLINs and their physical durability can be made when results from these studies are available.

5. Vector feeding time and location

- Purpose: To determine vector feeding locations, (i.e. outdoors versus indoors) and feeding times to understand where and when transmission is occurring.
- Method: Human Landing Collections is the preferred method. Where this is not possible, light traps or exit traps may provide some indication of indoor feeding but not on the time of feeding or the relative importance of outdoor transmission. The specimens collected should be subjected to secondary analysis to determine the source of the blood meal in fed mosquitoes.
- Reporting time frame: Collections should be conducted monthly during the transmission season.
Secondary entomological indicators

The following two indicators are additional to the basic entomological package listed above. As described here, they may be included if there is a specific need of the national program and capacity to perform these tests. However, inclusion of either of these indicators will need to be discussed with the PMI entomologists and agreed upon for each country program.

- **Identification of malaria infection in mosquitoes:** This can be undertaken in areas of high transmission by ELISA or molecular methods, and can be integrated with molecular assays for species identification and resistance. Sporozoite determination may serve three purposes. First, detecting differences in sporozoite rates in insecticide-resistant vs. susceptible individual vectors may be an indication of control failure. Second, sporozoite detection is necessary to determine the entomological inoculation rate (EIR) which describes the number of infectious bites an individual is exposed to in a given time period (typically a year). In theory the EIR is a good way to define transmission intensity. Unfortunately, EIR estimates may differ widely depending on the sampling tools used and sampling errors can be great in areas where mosquitoes are rare and/or rarely infected (as in areas with low parasite prevalence and low transmission). Therefore, in general, EIR determination is not considered part of the basic entomological monitoring package. Third, there may be situations, such as in the Mekong region, where there may be a need to determine the vector status of potential secondary vectors such as *An. maculatus*. In summary, inclusion of sporozoite determination will need to be discussed on a case by case basis with the PMI entomology team.

- **Age grading.** In special circumstances, and depending on the capacity of the entomological teams, age grading could be incorporated to monitor mosquito survivorship in the presence of IRS or LLIN interventions. Age grading is technically demanding and requires highly skilled technicians to dissect and examine mosquito ovaries, and like the EIR is fraught with sampling issues. Nevertheless, it can be a powerful indicator and warranted in very special circumstances as determined by the PMI entomologist working with the program.
Surveillance sites

Countries should do an initial stratification of their territory into eco-epidemiological zones and then establish at least one sentinel vector monitoring site per zone. As an approximate guide, one sentinel site per 500,000 nets distributed or 200,000 houses sprayed is recommended (the equivalent of one site per million people protected). Sites should be located in areas of greatest malaria incidence and pesticide use (including both agricultural and public health use). A country such as Nigeria, with a very large population, but with relatively limited eco-epidemiological strata may warrant less than one site per million people, where as a country such as Ethiopia with highly varied and isolated zones may warrant more. The exact number and location of sites should be discussed and approved by the PMI entomology team. Keep in mind that PMI works in collaboration with the national program and other partners and should therefore not be expected to be only source of funding for these sites. For example, a survey carried out by Awolola et al9 in Nigeria considered the following sites:

![Map of Nigeria showing the study sites in the different ecological zones.](image)

Reporting

Periodic reports of findings in a standardized format should be provided to the NMCP. The PMI Entomology team will work with the partners to develop this standard format and recommend the frequency of the reports. All susceptibility data from whatever source should be promptly shared with the NMCP and with district and regional malaria control staff. Entomological and epidemiological reports from local health facilities should be compared and shared by health officials. Some countries have a national Technical Advisory Committee that includes PMI and can review entomological monitoring data and make recommendations. PMI Resident Advisors should ensure that the USAID/CDC Entomology Team receives the information and are involved with these discussions. Standard reporting formats and a mechanism for relaying resistance data to international databases is currently under development.
Basic requirements of a national entomology monitoring program  

A national entomological monitoring program should include:

- Trained field technicians with supervisors having Master’s degree or equivalent level of training and experience.
- Reliable and available insecticide-free transport for mosquito collection teams when needed.
- Access to a laboratory with dissection microscopes, mosquito identification keys, mosquito traps and supplies (these should include entomological collection equipment, bioassay tubes and/or bottles).
- Access to WHO bioassay papers and/or technical grade insecticides for bottle assays.
- Where possible, an insectary with a colony of an appropriate (local species) insecticide susceptible mosquito strain, for cone bioassays, and to serve as controls in resistance monitoring.
- A written entomology monitoring and evaluation plan with a budget. This should be developed in collaboration with the PMI entomologist supporting the national program.
- Where these capacities do not exist in country, technical assistance, training and mentoring are needed. Local staff should develop needed skills while working with the technical experts. Promising personnel should be selected by the local government to receive long-term training to further bolster local capacity. Trained staff and technical resources available in neighboring countries, or countries sharing the same language, should also be utilized if possible.

Structure of entomological and insecticide resistance monitoring program

Correct performance of the collections and assays described above requires considerable skill and some basic laboratory and field equipment and supplies. Who actually performs the entomological collections, performs the insecticide resistance assays, interprets the data and makes recommendations will vary from country to country. In some cases it is the NMCP itself that is supported to perform these tasks. More often, however, data collection is contracted out to national universities or research institutions and done under the auspices of the NMCP. PMI support for entomologic monitoring should be directed to one or the other of these two groups but not to both, based on an estimation of the group most likely to be able to sustain such monitoring activities, if external support wanes. An NMCP/Ministry of Health Technical Advisory Committee, including PMI, should be supported to interpret data and make recommendations and decisions.

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Please note that PMI cannot be responsible to fund a national entomology monitoring program in its entirety but requires commitment from the national program and other partners.
Insecticide resistance monitoring and management

A core part of the basic entomological monitoring program is monitoring insecticide resistance. The objective of resistance monitoring is to assess the distribution, frequency, nature, underlying mechanisms and likely operational impact of any resistance observed. To do this, a number of basic monitoring steps should be performed, which are summarized in the steps outlined below. Note that the PMI country advisor should be involved with this process, especially if reduced mortality is detected in Step 2, and reconfirmation samples are tested.

<table>
<thead>
<tr>
<th>Resistance Monitoring Steps</th>
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</thead>
<tbody>
<tr>
<td>1. Collect mosquitoes</td>
</tr>
<tr>
<td>2. Bioassay with Discriminating Dose using insecticide(s) currently used in control programme</td>
</tr>
<tr>
<td>3. Check for Anopheles inside freshly sprayed houses or new holed LLINs</td>
</tr>
<tr>
<td>4. Cone bioassays on local wild caught mosquitoes</td>
</tr>
<tr>
<td>Alive &gt; 98 % Dead</td>
</tr>
<tr>
<td>Present Absent</td>
</tr>
<tr>
<td>Alive Dead</td>
</tr>
<tr>
<td>Change insecticide used in control programme immediately</td>
</tr>
<tr>
<td>Reconfirm resistance by increasing sample size.</td>
</tr>
<tr>
<td>Continue with control programme</td>
</tr>
<tr>
<td>5. Current Insecticide still probably operationally effective. Continue to incorporate in ongoing insecticide resistance management plan</td>
</tr>
</tbody>
</table>

It should be noted that while WHO susceptibility tests or CDC bottle assays form the basis of the initial tests, neither of these tests directly represents the insecticide concentrations or formulations used in the field. Resistance in either assay is not a direct confirmation of operational field failure of the vector control intervention. These assays should only be used as a good first indicator that resistance is present and being selected. Where there is evidence of resistance from initial testing (i.e. below 98% mortality), confirmatory testing should be undertaken, paying more attention to techniques and sample sizes. As stated earlier, when mortality in one of these test falls below 80%, PMI will probably recommend changing from the insecticide class to which resistance has been detected, and carrying out further investigations to confirm the mechanism, and distribution of resistance.

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3 This flow chart was developed by Maureen Coetzee and Janet Hemingway at the RBM Insecticide Resistance meeting held in Liverpool, October 2010.
Step 1. Sentinel site selection and mosquito collections

Sentinel sites should be established for mosquito collections and baselines determined at these sites before interventions are implemented. Selection of entomological monitoring sites will depend on the stratification of the eco-epidemiological zones and the capacity of local staff, costs, and resources. However to establish trends, multiple surveillance sites are needed with multiple data points from each site.

As stated above, a rough guide is to have one site per zone or per one million population protected, but the exact number and location will have to be determined by the national program and partners in consultation with PMI entomologist and country advisors.

The time lines for data collection throughout an annual cycle are given in the figure below:

Ideally, resistance testing should be done on adult mosquitoes reared from larvae, or an F1 (first) generation of mosquitoes raised from the eggs of field-caught females. Larval collections should cover multiple sites and eggs for an F1 generation should be from a large num-
ber of field-caught females to ensure adequate representation of resistance frequencies in the
field populations. Where F1 mosquitoes cannot be obtained and field-caught females them-
selves have to be used for testing, it is likely that resistance will be underestimated, as meta-
bolic resistance often declines dramatically with age of the mosquito. In contrast, if mosqui-
toes are collected resting indoors on sprayed surfaces, the F1 generation of mosquitoes from
these may provide an overestimate of the frequency of resistance.

Step 2. Conduct Bioassays.

Either the CDC Bottle Assay or the WHO tube tests can be used. While essentially equiva-
 lent, each has certain ad-
vantages and weakness that
are explained in more detail
in the linked references.
Bioassays should be per-
formed for all major vec-
tors at the sentinel site. All
mosquitoes tested should be
1-5 day old, non-blood fed
females. If males are tested
due to lack of female sam-
pies, the data for each sex
should be recorded sepa-
rately. Males are likely to
show somewhat more sus-
ceptibility in bioassays than
females. Tests should be
undertaken on at least one
representative insecticide
per class and on insecti-
cides that represent all available modes of action.\textsuperscript{10}

Protocols for the WHO test are available at:
CDC Bottle Assay are available at http://www.mr4.org/\textsuperscript{4}. A study showing equivalency of
the assays is available at: http://malariajournal.com/content/8/1/208

\textsuperscript{4} See also: http://www.mr4.org/Portals/3/Pdfs/Anopheles/4.3.3%20CDC%20Bottle%20Bioassays%20v%201.pdf
Sample processing.

While the exact structure will vary from one country to another, the above IRAC diagram illustrates an idealized flow diagram for sample processing. Specimens collected in the field could be morphologically identified using keys and reared to F1 generation, or reared from larvae to adults and tested with either the WHO or CDC assays. If resistance is detected in the in vivo assays (i.e. below 98% mortality), the mechanism of resistance should be determined through use of the CDC bottle assay using synergists or through molecular techniques, as this will assist with the decision on the best alternative insecticide. Capacity for such further testing will vary by country. Again, the PMI entomologist supporting the country will be able to provide guidance how these further tests should be performed.

**Step 3. Establish whether mosquitoes are resting in freshly sprayed houses or inside new holed LLINs.**

If the vectors survive in discriminating dose bioassays (i.e. less than 98% mortality) there is a need to investigate the operational significance of this resistance to vector control. The presence of live mosquitoes in sprayed houses can be assessed using a variety of collection methods, including pyrethrum spray collections or manual collections using aspirators from indoor resting sites or from inside new LLINs that have holes cut in them. If possible some mosquitoes should be preserved for molecular resistance analysis at a tertiary facility.

Mosquitoes found inside houses or in holed LLINs could indicate either a) operational problems with the spraying or with the insecticidal content of LLINs or b) that mosquitoes are able to survive the insecticide intervention. To distinguish between these two alternatives, a freshly sprayed wall or new LLIN should be used for the tests and insecticide activity of the nets should be verified by accurate insecticide diagnostic assays (colorimetric quantification kits or HPLC) or by cone bioassays with known susceptible mosquitoes.
If no mosquitoes are found inside sprayed houses, it still does not mean that control is working. We cannot provide definitive a priori guidance in this situation but will require more discussion and a more comprehensive understanding of the susceptibility profile of the local vectors to a range of insecticides.

**Step 4. Cone bioassays on local field caught mosquitoes.**

This step is recommended to ensure that the IRS is capable of killing local vector populations. This should be done even if no vectors are found resting inside houses or holed LLINs in Step 3 above. Local females collected from the field (e.g. resting catches from untreated houses or outdoor collections should be used. Testing should be undertaken on a freshly treated wall of typical local construction using a 30 minute exposure. Method:

1. Use bioassay cones and place on walls at different heights. Attach to walls using tape. Introduce batches of 10 female mosquitoes into the cones and expose to the wall surface for 30 minutes. After exposure transfer the mosquitoes to paper cups, provide with sugar solution. Record mortality 24 to 48 hours after exposure.

2. Control assays – either select houses of similar construction that have not been sprayed or cover sprayed wall with two layers of paper before attaching the cones. Introduce 10 mosquitoes per cone as above.

NOTE: this is not the same as the quality control assays for IRS described above. Those assays should be performed with colorimetric chemical assays or standard WHO cone bioassays using a laboratory susceptible strain of mosquitoes. Here local field caught females are used.

**Step 5. Interpretation of data.**

Decisions on operational control failure and changing insecticides should not be taken based on bioassay data alone. As defined, mortality below 98% in tube or bottle assays are an indication that further investigation as described in the steps above is required. Further field investigation of resistance provides a better indication of control failure or success. However, there is NO one test that can incontrovertibly demonstrate that insecticide resistance has resulted in a reduction of the efficacy of the control measure and in an increase in transmission.

However, indicators of resistance should never be ignored to the point where disease transmission rises substantially, as this is too late to maintain any long-term use of the particular insecticide. Ideally, resistance management should already have been initiated before resistance is detected. Once resistance is detected to an insecticide, the optimal use of that insecticide class within the resistance management strategy may change.

A national technical advisory committee may be in place that could help assess the information on behalf of the NMCP. The PMI Entomology Advisor supporting that country should be involved in these national discussions. There is also a recently established PMI Entomology Committee that will help interpret the data and make recommendations. Currently this Committee has been convening on an ad hoc basis, but in January 2011 the terms of reference for this Committee will be formalized.
Insecticide Choice

The range of insecticides that can be used for IRS is limited. Each as its own strengths and weaknesses as outlined below. Note the costs are approximate for 2010 prices and subject to change:

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Cost/sachet (200-250 m2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrethroids</td>
<td>Low toxicity&lt;br&gt;Low cost&lt;br&gt;&gt; 7 months duration</td>
<td>Resistance</td>
<td>$3.60 to $5</td>
</tr>
<tr>
<td>Carbamates</td>
<td>Medium toxicity profile&lt;br&gt;Less resistance</td>
<td>High cost&lt;br&gt;&lt; 4 month duration</td>
<td>$13</td>
</tr>
<tr>
<td>Organo-phosphates</td>
<td>Less resistance</td>
<td>Toxicity problems&lt;br&gt;Higher costs&lt;br&gt;Variable duration</td>
<td>$12</td>
</tr>
<tr>
<td>Organochlorines (DDT)</td>
<td>Low cost&lt;br&gt;&gt; 7 months duration</td>
<td>Management costs&lt;br&gt;Resistance&lt;br&gt;Supply</td>
<td>$4 to $6.70</td>
</tr>
</tbody>
</table>

Further specific information on the specific insecticides in each of these classes can be found at the WHOPES website: [http://www.who.int/whopes/en/](http://www.who.int/whopes/en/) with the names of approved manufacturers for each specific product at: [http://www.who.int/whopes/quality/newspecif/en/](http://www.who.int/whopes/quality/newspecif/en/)
Frequently Asked Questions Related to Insecticide Resistance Management

At what level of resistance (i.e. percent mortality in the in vivo assays) should we change insecticides?

As described above in Step 5, Interpretation of Data, the decision to switch insecticide cannot be made based on the level of resistance detected with the in vivo assay alone. Instead, a combination of different types of information should be considered in making the decision (see flow chart on page 10). Ideally, PMI supported national IRS programs should begin to rotate non-pyrethroid insecticides with the 2011 spraying season, especially in areas where there is high coverage of LLINs and where there is also agricultural use of pyrethroids. In areas with relatively short transmission seasons (i.e., four months or less) this can be done with a modest increase in program costs. In areas with transmission seasons of five months or more, this is a more difficult decision, as the formulations for carbamates and organophosphates are not as long lasting as the eight or more months one could expect from certain pyrethroids and DDT. However, it is expected that within the next year, new longer lasting non-pyrethroid insecticides will begin to become available and make this choice easier. For now however, the decision to change to a non-pyrethroid insecticide, especially in areas where pyrethroid resistance has not been detected, is not straight forward and requires a consideration of resources, coverage, and length of the transmission season. The newly established PMI Entomology Committee can help with this decision.

How do we know when we have enough information on which to base a decision?

Programs are often forced to make decisions on very sparse and patchy information. In an ideal situation, NMCPs should support about one entomologic monitoring site per 500,000 nets distributed or 200,000 houses sprayed (the equivalent of one site per million people protected). When the in vivo assay results fell below 98% mortality, the NMCPs should follow the steps outlined above to collect more information. In some cases, the decision is clear and unequivocal, as when mortality is confirmed to be below 80% and the vector is found resting on freshly sprayed surfaces. But in most situations the decision is less clear. The mortality data may be in the 90% to 95% range and vary geographically. This is when PMI and the NMCP need to weigh the options and reach a consensus on the way forward.

How do we interpret and deal with resistance in the same class?

For insecticide selection purposes, resistance to one insecticide within a class should be considered resistance to all (see page 4). Differences in the assays with two different pyrethroids may appear, for say, deltamethrin and alphacypermethrin, but these differences are largely due to the different discriminating doses for the two insecticides. Remember also that for procurement purposes, PMI can only tender by class and not by individual chemicals within the class. But insecticide choice should not be made on the results of the in vivo data alone, and as there is often “noise” and ambiguity in the results from limited samples; there should be other steps as described above.

If there is resistance to both pyrethroids and DDT how do we proceed with IRS?

If resistance, and control failure, is shown to both pyrethroids and DDT, programs will need to consider organophosphates and carbamates. While these are both effective insecticide classes, they present special issues for IRS programs. The carbamates are generally not as
long lasting as some of the newer pyrethroids formulations and DDT while two of the organophosphates, malathion and fenitrothion will require acetylcholinesterase monitoring of the spray operators

*If there is a suspected resistance to a given insecticide (below 98% mortality) should we follow up to determine the mechanism of resistance?*

Yes. Knowing the mechanism is important for deciding on the resistance management plan as described in Step 3 and later.

*Is there any level of resistance that would cause us to stop ITN distribution or IRS?*

While pyrethroids are the only class of insecticide that can be used on mosquito nets, even in the face of profound pyrethroid resistance, treated LLINs should still be deployed. The netting material itself provides some protection against mosquito bites. In addition, the cost of the insecticide on the net is marginal and may still, through irritancy to the vector help provide additional protection.

The same is true for IRS. At present, there is no place in Africa where the vectors are resistant to all four classes of insecticide. Therefore, we should choose an insecticide that works, not just for the transmission reduction, but as a strategy to help preserve pyrethroid susceptibility for the LLINs, remembering that the LLINs themselves can be selecting for resistance. There may be situations where there is year-round transmission of malaria, but only shorter-acting carbamates or organophosphates are efficacious, and the cost to the NMCP of two rounds of spraying may be very high. Here again, the PMI entomology team, with the NMCP will need to discuss if a single round, in conjunction with high LLIN coverage, would be expected to have an impact.

*What are the implications of changing to a carbamate or organophosphate insecticide?*

Changing from a pyrethroid or DDT to a carbamate or an organophosphate will marginally increase the costs of an IRS program where the transmission season is relatively short, i.e. less than four months. Overall, the costs of insecticides within an IRS program using pyrethroids may be 15% to 20%. For illustrative purposes, if the cost per structure sprayed is $10 per round with about $2 of this representing the cost of the pyrethroids, and if this tripled to $6 for a carbamate or organophosphate, then the cost per structure will rise to $14 – a 40% increase. If however the length of the transmission season is long (e.g., eight months) the program may need to do two rounds of spraying each year and the cost per structure per year will rise to $28.

With the use of organophosphate insecticides, there is an additional issue of acetylcholinesterase (AChE) monitoring of spray operators. As described above, both organophosphates and carbamates inhibit both insect and human AChE. Specific documentation and guidance can be provided, but in short, AChE monitoring is not required for carbamates or the organophosphate, primiphos-methyl, but is required for malathion and fenitrothion to identify proactively any possible toxicity.
III: Long-term strategy for Insecticide Resistance Management

It is recommended that National Malaria Control Programs develop long-term strategies for slowing down and mitigating the inevitable evolution of resistance in the local vector populations. Many programs are reorienting towards an Integrated Vector Management approach. It is important to clarify that IVM is not a separate program or activity, but simply a management approach that seeks to improve the efficacy, cost-effectiveness, and sustainability of vector control programs. The “IVM Framework” outlines five key elements to help re-orient national programs:

- Advocacy, social mobilization, regulatory control for public health and empowerment of communities.
- Collaboration within the health sector and with other sectors through the optimal use of resources, planning, monitoring and decision-making.
- Integration of non-chemical and chemical vector control methods, and integration with other disease control measures.
- Evidence-based decision making guided by operational research and entomological and epidemiological surveillance and evaluation.
- Development of adequate human resources, training and career structures at national and local level to promote capacity building and manage IVM programmes;

For more information, see: http://www.who.int/neglected_diseases/vector_ecology/ivm_concept/en/index.html

Within this “strategic” reorientation of programs towards IVM, there are a number of “tactical” actions that NMCPs and PMI-supported operations should undertake as part of their long-term resistance management plan.

The first of these is to ensure the quality of IRS. Haphazard, under-dosed spraying is a waste of resources and, like sub-lethal dosing of medications, will tend to select for the more tolerant individuals in the population. Guidelines for IRS management and supervision checklists are available elsewhere. PMI must remain committed to supporting quality spraying and supervision.

Second, is targeting IRS to those areas where it is most beneficial, and “graduating” or shifting to more focal spraying when transmission levels fall to a low level. Once IRS has begun in a particular location, it can be difficult, when indigenous cases have reached a very low level, to transition the program from large investments in blanket IRS coverage to more balanced investments into surveillance, health information systems, and targeted IRS for any remaining transmission foci. This “road to elimination” is outlined in a number of WHO manuals, including http://whqlibdoc.who.int/publications/2007/9789241596084_eng.pdf.

The WHO recommends that in theory, at least, NMCPs should consider transitioning to a pre-elimination strategy when “health facility data indicate that the monthly slide or rapid diagnostic test (RDT) positivity rate among febrile patients with suspected malaria is consistently less than 5%; and, a population-based surveys in the peak transmission season confirm a malaria parasite rate of less than 5% among people of all ages with current fever or a history of fever in the past 24 hours.”

In reality, many more factors should enter into the decision to transition to a pre-elimination strategy than parasite prevalence alone. At this stage, which may be met in some of the areas
under IRS currently supported by PMI, emphasis should be shifted to “strengthening the health information system, including entomological surveillance and immediate notification of all malaria cases; improving the effective coverage of good-quality curative and preventive health services in all transmission areas, and re-orientating public and private health service staff towards the new goals of malaria elimination.

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For questions or comments, please contact Michael Macdonald mmacdonald@usaid.gov

1 http://ipmworld.umn.edu/chapters/ware.htm
2 http://www.malariajournal.com/content/9/1/168
5 http://malariajournal.com/content/6/1/30
6 http://malariajournal.com/content/8/1/70
10 The tests should include ~100 females per insecticide. For susceptibility frequencies of 98 -100% or <80% this sample size is adequate. For values of 80-98% larger samples sizes would be beneficial. Where mosquito numbers are limited these should be tested even if a sample of 100 cannot be achieved. For WHO bioassays mortality should be recorded after a 24 hr holding period. For some slower acting insecticides mortality may need to be recorded at 48 hrs. Mortality is recorded over time with the bottle assays. A control paper/bottle should be used each day that tests are undertaken. If 24-hr mortality in controls exceeds 20%, all results from that days’ tests must be discarded. If mortality in the control is between 5-20%, results must be corrected for control mortality using Abbott’s formula. For WHO tests and CDC bottle assays each paper/bottle should ideally be used no more than 6 times before being replaced. The temperature in the room should be recorded for each test. Bioassays should ideally be carried out at 27±2°C and never at temperatures exceeding 30°C