The Uganda National Academy of Sciences (UNAS) is an autonomous body, bringing together a diverse group of scientists from the physical, biological, social and behavioral sciences, working together in an interdisciplinary and trans-disciplinary manner. The UNAS main goal is to promote excellence in science, with a vision to evolve into an eminent body of scientists offering independent, evidence-based advice for the prosperity of Uganda.

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Reviewers

All presenters at the Open Session have reviewed and approved their respective sections of this report for accuracy. In addition, this report has been reviewed in draft form by independent reviewers chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the UNAS Council. The purpose of this independent review is to provide candid and critical comments that will assist UNAS in making the published report as sound as possible and ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential, to protect the integrity of the deliberative process.

The Uganda National Academy of Sciences thanks the following individuals for their participation in the review process:

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Lawrence Slutsker, Chief, Malaria Branch, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Prof. Patrick Rubaihayo (Department of Crop Science, Makerere University) and Dr. Enriqueta Bond (The Burroughs Wellcome Fund), who were responsible for ensuring an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with individual paper authors and the authoring committee.
*In Memoriam*

The Uganda National Academy of Sciences and the Committee on Assessing and Managing Malaria Vector Resistance to Insecticides Used for Indoor Residual Spraying in Uganda wish to acknowledge the life of Professor Chris Curtis, of the London School of Hygiene and Tropical Medicine, who died on May 14, 2008. Prof. Curtis was a medical entomologist and a great malaria scientist whose field and laboratory research advanced malaria control greatly. His contribution to the integrity of this report and to the science of malaria control is acknowledged and greatly appreciated.
Preface

The mission of the Uganda National Academy of Sciences (UNAS) is to advance the ability of Uganda to address its most serious health challenges by (1) engaging in a series of scientific activities designed to elucidate potential evidence-based solutions to pressing national and regional health concerns; (2) enhancing the general capacity of UNAS to provide relevant and useful scientific policy advice; and (3) building Uganda’s appreciation of and demand for advice from the academy.

Like many other academies of science, the Uganda National Academy of Sciences is an autonomous body that brings together a diverse group of scientists from the physical, biological, and social and behavioural sciences. These scientists work together in an interdisciplinary and trans-disciplinary manner to achieve their main goal of promoting excellence in science by offering independent, evidence-based advice for the prosperity of Uganda. The success of any academy lies in the strength and expertise of its membership and its ability to mobilise scientific experts to continually advise government policymakers. Given that UNAS is now two hundred members strong and growing, the academy is well positioned for success.

In November 2005, UNAS convened a stakeholders’ meeting in Kampala to accomplish two goals. One was to identify the most critical health issues in the country that could be subjected to closer scrutiny through the relevant Academy activities and the other was to introduce the concept of a UNAS Forum on Health and Nutrition. In one particularly telling presentation, it was revealed that the incidence of malaria peaked in 2001 with over 5.6 million reported cases of malaria. At the end of the meeting, the UNAS Council selected malaria as a major topic of concern for the Academy, due to the burden it places on Uganda and its people from the extraordinarily high number of deaths in its most vulnerable populations. Furthermore, the
Council decided that the first Forum workshop would address malaria. This decision was based on the strong reactions of their stakeholders and the Academy members’ desire to assist the nation during this major health crisis.

The Uganda National Academy of Sciences was asked to convene an expert committee to help foster a successful implementation strategy for indoor residual spraying (IRS) in Uganda. This committee came together for 4 days of face-to-face discussions. Following an open information gathering session, committee members entered three days of closed door deliberations where they drafted recommendations that were later peer-reviewed for accuracy and appropriateness. The result of these efforts is the following report that contains the consensus committee’s conclusions, recommendations and supporting text that have undergone a rigorous review process.

The report is made up of 2 parts. Part I, “Responding to a National Need,” sets the context in which the committee was asked to review the evidence and respond to the statement of task; and it lays out the actual recommendations of the committee along with text and references supporting their recommendations. Part II of the report entitled, “Current State of Knowledge and Policies” is a written account of the presentations the committee heard during the open session. Because the presenters are the authors of the papers, it is important to understand that the views presented in Part II are those of the individual authors, and not necessarily those of the Uganda National Academy of Sciences. Reviewers were therefore asked to focus primarily on the conclusions and recommendations found in Part I.
Acknowledgements

The Uganda National Academy of Sciences and the Committee on Assessing and Managing Malaria Vector Resistance to Insecticides Used for IRS in Uganda wish to express their warmest appreciation to the individuals who gave valuable time to provide information and advice to the Committee through their participation in the framing of the study topic through to the open session workshop of the study. The biographies of the presenters can be found in Appendix E.

The Committee is indebted to the UNAS staff (in particular Franklin Muyonjo and Solome Mukwaya) who contributed during the course of the study and the production of this study report. UNAS gratefully acknowledges the sponsors of this study, the US National Academies (USNAS), through the African Science Academy Development Initiative (ASADI). Special thanks are extended to Dr. Patrick Kelley (the ASADI Board Director); the USNAS staff (especially Patricia Cuff and Katherine McClure – for providing guidance to the UNAS staff); Prof. Patrick Rubaihayo and Dr. Enriqueta Bond (for coordinating and overseeing the review process); and to the reviewers (see page iii) who volunteered their time to provide candid and critical comments to ensure that the report is accurate, effective, and credible.

Dr. James Tibenderana, Chair, Committee on Assessing and Managing Malaria Vector Resistance to Insecticides Used For IRS in Uganda

Prof. P.E. Mugambi, President, Uganda National Academy of Sciences
Contents

PART I
RESPONDING TO A NATIONAL NEED

1. Background
   Technical Context ................................................................. 1
   Policy Context ................................................................. 3
   Statement of Task .............................................................. 6
   Expert Committee and Staff ................................................. 7

2. Conclusions and Recommendations
   Introduction ................................................................. 10
   Pre-implementation Phase of IRS Spray Round ...................... 10
   Implementation Phase of IRS Spray Round ........................... 23
   Post Implementation Phase of IRS Spray Round .................... 25
   Closing Remarks .............................................................. 29

PART II
CURRENT STATE OF KNOWLEDGE ANDPOLICIES

3. Framing the Issues
   Current State of Malaria Vector Resistance in East Africa and
   Uganda.................................................................................. 36
   Martin James Donelly
   The Role of IRS in Contemporary Malaria Control ............... 42
   Allan Schapira
   Insecticide Resistance in Malaria Vector Mosquitoes in a Gold
   Mining Town in Ghana and Implications for Malaria Control .... 60
   Maureen Coetzee et al
4. Monitoring Resistance and Maximising Insecticide Effectiveness

Monitoring DDT in the Environment ........................................... 73
Patrick Isagara Kamanda

Maximising the Effectiveness of DDT and other Insecticides........... 79
Jacob Williams

5. Technical Aspects of a Malaria Control Programme 83

Monitoring Insecticide Resistance in Malaria Vectors using the
CDC Bottle Assay ........................................................................ 84
Josephine Birungi and Louis G. Mukwaya

Evaluation of the Residual Efficacy of Lambda-cyhalothrin 10CS
Field Use of Indoor Residual Spraying........................................... 88
Kate Kolaczinski, James Kirunda and Juma Mpima

An Ecosystem Approach Towards Developing Policies Appropriate
for Sustainable Pesticide Use in Agriculture and Malaria Control.... 98
Peter Mohloai

GLOSSARY OF TERMS................................................................. 109

ACRONYMS ................................................................................. 111

APPENDICES................................................................................. 113

A. The Insecticide Resistance Status of the Main Malaria Vectors in
Uganda ....................................................................................... 113
Van Bortel et al

B. Open Meeting Agenda ............................................................ 125

C. Biographies ............................................................................. 128
The Uganda National Academy of Sciences (UNAS) responded to its nation's need for evidence-based advice concerning the current state of knowledge and policies pertinent to monitoring malaria vector resistance in the context of an effective national program for indoor residual spraying that could include dichlorodiphenyltrichloroethane (DDT) for controlling malaria. Currently there are twelve insecticides recommended by the World Health Organisation for use in indoor residual spraying (see Annex 1, page 49).

**TECHNICAL CONTEXT**

Indoor residual spraying (IRS) will only be effective if the target vectors are susceptible to the insecticide in use. The development of resistance to insecticides constitutes a major threat to the chemical control of malaria vectors, as it compromises the insecticide's efficacy. In the past, countries deploying IRS have often been forced to switch to alternative and more expensive insecticides due to the development of vector resistance. Outside Africa, the prevalence and distribution of insecticide resistance in malaria vectors have not, so far, been a major impediment to insecticide-based interventions, except in some areas of India, the Middle East and Central America (WHO, 2006). However, in Africa, the potential threat of resistance to insecticides used for public health appears to be significant.
Resistance to DDT (an organophosphate) and pyrethroids in major malaria vectors has been found throughout West and Central Africa, in some areas at a high level, as well as in several parts of Eastern and Southern Africa (WHO, 2006). Resistance to carbamates has been found in countries of West Africa, with a mechanism that also induces cross resistance to organophosphates (WHO, 2006). The selection of resistance in most malaria vectors is thought to be largely the result of past and present use of insecticides in agriculture although previous indiscriminate use of insecticides for IRS during the “malaria eradication era” of the 1950s and 1960s also contributed to resistance. The precise operational implications of insecticide resistance are not yet fully understood.

Specific interactions occur between insecticides and malaria vectors. Some insecticides tend to repel rather than kill vector mosquitoes. Such changes in vector behaviour induced by insecticides may have important operational implications, and it is important to take them into consideration when selecting insecticides for IRS. In the case of DDT, it is the only insecticide used exclusively for public health purposes, and therefore, unlike with other insecticides, the development of resistance to DDT should no longer be due to its use in agriculture. However, because DDT was previously used widely in agriculture, resistance to DDT may still be found as a result of past use; and since DDT remains in the environment longer, the possibility of DDT resistance is higher than with other shorter-acting pesticides.

There is also the question of how insecticide choice for IRS should or should not be linked or considered in the context of the extent and location of the insecticide-treated nets (ITN) program in the country. Although this document is focused on resistance to insecticides used for IRS, it seems reasonable to acknowledge the risk of resistance to pyrethroids due to wide-scale deployment of pyrethroid treated bed nets. The basic question – should pyrethroids be used as a first choice in IRS programs given wide-scale ITN distribution? Cost considerations and the lack of a plethora of other choices for IRS suggest that pyrethroids may frequently be selected, but the consequences and potential downsides to this merit recognition in light of recent findings from Benin providing persuasive evidence that

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1 A pyrethroid is a synthetic chemical compound (similar to the natural chemical pyrethrins) produced by the flowers of pyrethrums. Pyrethroids are common in commercial products such as household insecticides and insect repellents.
pyrethroid resistance is capable of undermining IRS control measures based on ITN (N’Guessan et al., 2007).

POLICY CONTEXT

Malaria is one of the most severe public health problems worldwide and is a leading cause of death and disease in Africa, particularly among pregnant women and young children. With a death toll of over one million people a year, it is second only to tuberculosis in its impact on world health. In Uganda, malaria accounts for almost one-fourth of deaths in children under five years and for 25-40 percent of all out-patient visits at health facilities (UNAS, 2007). It is the leading cause of morbidity and mortality in the country and places a major burden on the population. The burden is not only from the extraordinarily high number of deaths due to malaria but also because of missed school days and missed work days resulting in personal and national economic losses when employees are too ill to report to work. Clearly, malaria is one of the country’s primary public health concerns.

The use of IRS to control and eliminate malaria is one of the strategies of the current Uganda Malaria Control Strategic Plan, Health Sector Strategic Plan and Poverty Eradication Action Plan. To support the appropriate use of IRS in Uganda, the Ministry of Health has adopted certain policy statements noted in Box 1. The committee is supportive of these statements and in particular, discussed the need for a national advisory committee with a mandate to provide technical and administrative advice within a multi-sector framework on all aspects of IRS and resistance management; and the need for all companies involved in spraying of insecticides, such as those involved in fumigation, to be licensed and registered/accredited by the National Drug Authority (NDA). In addition, the committee drafted conclusions directly related to statements 8 and 9.
Policy Statement Guidelines to Support Appropriate Use of IRS in Uganda

1. The objective of indoor residual spraying (IRS) is to reduce malaria transmission and to eliminate it from certain areas of Uganda. IRS will be implemented as part of a package of other malaria control interventions such as case management, use of insecticide treated nets (ITNs) and intermittent preventive treatment of malaria in pregnancy (IPT).

2. Indoor residual spraying shall be used in areas where malaria transmission occurs as appropriate; however, in Uganda, priority will be in the following areas:
   - **Congested areas**: IRS will be applied in selected areas (e.g., in high-density slum settlements).
   - **Institutions**: IRS to be used at these at specific sites (e.g., boarding schools, barracks, prisons, agricultural and industrial estates).
   - **Emergency situations**: IRS may be used following population displacement (e.g., in internally displaced people’s (IDP) and refugee camps).
   - **Malaria Epidemic-prone areas**: IRS will be deployed in districts that are prone to epidemics.

3. The Ministry of Health (MOH) through the National Malaria Control Programme (NMCP) will establish structures and systems for managing IRS activities at Sub-county, County (Health Sub-district), District and National levels to ensure annual and bi-annual campaigns of IRS.

4. Indoor residual spraying, using approved and registered insecticides and compression sprayers, is an integral part of the malaria vector control strategy for Uganda.

5. Insecticides and relevant equipment for IRS should be registered by the National Drug Authority (NDA) in consultation with the NMCP and the Vector Control Division of the MOH. Registration shall conform to World Health Organisation (WHO) specifications and standards (see Table 1 for the list of insecticides used most extensively for vector control).

6. Private companies and nongovernmental organisations (NGOs)
offering IRS services to the public shall be registered with the NMCP and Vector Control Division of the MOH and should offer these services according to NMCP guidelines. NGOs should work within the national IRS structure and systems.

7. All insecticides and equipment that are donated for IRS should comply with national guidelines and WHO specifications.

8. Malaria vector surveillance and research will be conducted at designated sites to provide regular information about the types, distribution, resting densities and resistance of the malaria vectors in the country. IRS will be adjusted according to the vector types and resistance patterns using techniques of insecticide rotation for management of insecticide resistance.

9. Importation, distribution, storage, use and disposal of insecticide residues will supervised by the NMCP and monitored by the Environment Health Division in line with NDA and National Environment Management Authority (NEMA) guidelines and procedures.

10. MOH shall establish a Multi-sectoral Monitoring and Evaluation Task Force to ensure the safe and correct application of residual insecticides and safe disposal of residues and expired insecticides in order to limit human & environment exposure to residual insecticides. The Team will also ensure that IRS is done in time in the months of January to March before the first rains and in June to July before the second rains.

11. Adequate regulatory control and enforcement measures will be put in place to prevent an unauthorized and un-recommended use of DDT in agriculture and thus avoiding contamination of agricultural products, with stiff penalties for the culprits.


In August 2007, Uganda's Ministry of Health (MOH) was authorized to begin indoor spraying with the pesticide DDT. These efforts are being supported by the United States through its President’s Malaria Initiative (PMI) with monitoring by the US Agency for International Development (USAID, 2006) as well as the Ministry of Health. Currently, PMI is

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2 This is in addition to the other malaria prevention and treatment strategies including the use of insecticide-treated nets (ITNs), home-based delivery of anti-malaria medicine to children under 5 years and the shift to the new, more effective malaria treatment using Artemisinin-based Combination Therapy (ACT) (Ministry of Health, 2005).
supporting Uganda in developing and expanding its indoor residual spraying campaigns that use synthetic pyrethroids. In Year 1, PMI sprayed over 100,000 households in the Kabale district. In Year 2, PMI significantly expanded IRS to cover eight (Kabale, Kanungu, Kitigum, Pader, Amuru, Gulu, Apac and Oyam) districts, both in the epidemic-prone southwest and the highly endemic conflict-ridden north. To date, the IRS campaign and associated activities has covered 600,000 households and trained over 1,500 local spray personnel (PMI, 2007b). This number is said to have recently increased to 4,062 trained local spray personnel that includes 2,938 supervisors/sprayers, 123 clinicians, and 1 environmentalist.

Building on experience from the first two years, PMI will support spraying in the high malaria transmission areas of three epidemic-prone districts (Kabale, Kanungu, and Rukungiri). This will include spraying of approximately 33,000 households in nine subcounties in Kabale, 50,000 households in nine subcounties in Kanungu, and 50,000 households in nine subcounties in Rukungiri (PMI, 2007a). PMI will spray a second round in the northern conflict districts of Kitgum, Gulu, Pader, Amoro, Apac, and Lira in 2008 that covers over 330,000 households. Finally, PMI will expand its spraying efforts to include 350,000 households in the neighboring northern districts of Dokolo, Amolatar, Kabermaido, Amuria, and Soroti. PMI expects to achieve at least 85% coverage of the 800,000 households targeted for spraying.

**STATEMENT OF TASK**

To help foster a successful implementation strategy for IRS, UNAS was asked to convene a consensus committee that would identify, review and assess the current state of knowledge and policies pertinent to monitoring malaria vector resistance in the context of an effective national program for IRS with DDT for controlling malaria. The convened committee would also review issues and guidelines on the use and selection of IRS insecticides used against malaria vectors that should be considered in national decision-making.

More specifically, over the course of three months, it was proposed that the committee would:
1. Use a comprehensive review of the published literature and other data sources to conduct a background assessment to frame the issues involved with potential insecticide resistance;
2. Review the optimum approach for monitoring malaria vector resistance to insecticides in Uganda; and
3. Recommend best practices for the effective use of DDT and other malaria vector insecticides for use in Uganda that take into consideration insecticide susceptibility as well as vector and human behaviour and might include such issues as: insecticide rotation, length of insecticide use, implementation of multiple interventions, and community outreach and education.

The committee was also asked to: report on the assessed strengths and weaknesses of the proposed “best practices” with respect to maximizing the effectiveness of DDT and other insecticides; and to identify contextual issues that would have a bearing on successful implementation of the “best practices.”

EXPERT COMMITTEE AND STAFF

To effect the task, a consensus committee of nine experts was formed; however, late in the process it was revealed that three committee members were unable to attend the meeting and had to be quickly substituted in order to maintain the appropriate balance of expertise on the committee. These three individuals were designated consultants to the committee in order to avoid any question of bias or conflict of interest. As consultants, these individuals could inform and advise the committee but could not approve or disapprove of the final recommendations. For a full listing of the consensus study committee members and their consultants, see page ii of this report.

The committee (along with their consultants) had domestic and international expertise in the areas of entomology, malaria, vector control, epidemiology, health economics, community health and public health. It was anticipated that with this diversity, the committee members would be able to adequately reflect the relevant and diverse viewpoints and experiences needed to respond to the charge. To obtain the necessary expertise and experience for this consensus study, some committee members were from countries
outside of Uganda. However, the majority of members on the committee were Ugandan.

Background data came from a variety of relevant sources including the Uganda Ministry of Health, the World Health Organisation and relevant US government agencies. The committee’s work plan included a 4-day meeting. The first day featured an open workshop with invited speakers who addressed topics that covered such areas as efficacy of IRS, current levels of pesticide resistance among malaria vectors and ways to prevent the development and spread of resistance, the potential for harm due to likely human and environmental exposure to insecticides, a comparative analysis of insecticide alternatives, insecticide rotation, length of insecticide use, and implementation of multiple interventions. The remaining 3 days comprised closed sessions for committee members to deliberate the charge and to draft evidence-based conclusions and recommendations in response to the charge. For the purpose of this report, conclusions are statements summarizing the committee’s decisions on specific topics and recommendations are statements about next steps and delineate the committee’s feelings about what action a specific agency or organization should engage in that would help support appropriate use of IRS in Uganda to avoid malaria vector resistance to insecticides used for this purpose.

Follow-up with the Committee was conducted by email and telephone. All committee members signed-off on the recommendations within four months of the close of the meeting and signed-off on the entire report following the peer-review process. Reviewers of the report possess similar expertise to the committee to assure a fair and just report based on the best available evidence. The outcome of this process is the following report. Conclusions and recommendations were sectored into the pre-implementation phase, the implementation phase, and the post implementation phase of indoor residual spraying and are presented in the next section following a brief introduction.

REFERENCES


Chapter 2
Conclusions and Recommendations

INTRODUCTION

The recommendations in the following sections are meant to build upon previously drafted policies and strategies – noted in Chapter 1 - in order to assist the Ministry of Health in obtaining its goals. To do this, the committee separated the recommendations into 3 sections based on the implementation phase of the IRS spray rounds (i.e., pre-implementation, implementation and post-implementation phases). Each of these sections is then divided into subsections where specific conclusions and recommendations are proposed. It is the hope of this committee, that through these recommendations, a clearer vision can be achieved of how the Ministry of Health can duplicate the success of other countries that effectively utilized IRS and can bring down the enormous death toll from malaria in Uganda.

PRE-IMPLEMENTATION PHASE

Susceptibility Testing

Conclusion 1: The selection of sites for IRS is a programmatic decision. Criteria for selection of vector resistance sentinel sites will be based on factors unique to Uganda but may include available resources, disease risks, previous and current use of insecticides in health and agriculture (WHO, 2006a). Sentinel sites should also be dispersed in each major eco-epidemiological zone in Uganda such as the forests, highlands, and wetlands.

Recommendation 1a: Previously conducted susceptibility tests carried out in 2005 and 2006 should be confirmed and new

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3 A measure of vector susceptibility to a given insecticide based on standard bioassays
susceptibility tests should be conducted in additional sites in order to get more comprehensive information on the current resistance distribution (2007) throughout the country. The country should be divided into ecological zones, based on broad characteristics of the mosquito vectors responsible for malaria transmission and on malaria transmission intensity. Susceptibility tests should be carried out within each ecological zone represented. As a next step, if resistance is detected in any ecological zone, the districts targeted for IRS within that zone must be further investigated.

Recommendation 1b: The Ministry of Health should set up sentinel sites for monitoring vector insecticide susceptibility, using the four classes of insecticides and locate them in areas that maximise the species variation of mosquitoes responsible for transmission, by taking into consideration the geographic distribution of specific mosquito populations in the country. In deciding the location and number of sites, other considerations include the prevalence of agricultural pesticide use and presence of mosquito breeding sites. Where applicable, the Ministry of Health should use the existing sentinel sites for drug efficacy monitoring.

Limited data from various areas of Uganda indicate low levels of resistance to pyrethroids and Dichlorodiphenyltrichloroethane (DDT) in Anopheles gambiae s.l. (see Donnelly; and Birungi and Mukwaya in Part II). In Kabale district, pyrethroid resistance was 14%, while in other districts it ranged from 3-30% (USAID, 2006). Pyrethroids are commonly used in Kabale for agricultural purposes, but the extent of their use and potential impact on Anopheles resistance is unknown. Insecticide resistance testing was conducted in seven districts of Uganda in 2005 and 2006 (see Tables 1, 2 and 3 and Figures 1 and 2 in the paper by Van Bortel et al. in Appendix A). The data from Van Bortel et al on insecticide resistance in Uganda shows that mortality ranges are consistent with sensitive or tolerant responses in the main vectors and that tolerant populations can be found throughout the country.

To evaluate the susceptibility of malaria vectors to the four classes of chemical insecticides (organochlorines, organophosphates, carbamates and pyrethroids noted in Table 1) with a reasonable degree of confidence,
tests need to be conducted in a number of well-chosen sentinel sites located within ecological zones in the country.

Table 1. Insecticides used most extensively for vector control reported to The WHO Pesticide Evaluation Scheme (WHOPES)

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organochlorines</td>
<td>DDT</td>
</tr>
<tr>
<td></td>
<td>Chlorpyrifos</td>
</tr>
<tr>
<td></td>
<td>Chlorpyrifos-methyl</td>
</tr>
<tr>
<td></td>
<td>Fenthion</td>
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<tr>
<td></td>
<td>Fenitrothion</td>
</tr>
<tr>
<td></td>
<td>Malathion</td>
</tr>
<tr>
<td></td>
<td>Pirimiphos-methyl</td>
</tr>
<tr>
<td></td>
<td>Temephos</td>
</tr>
<tr>
<td>Organophosphates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bendiocarb</td>
</tr>
<tr>
<td></td>
<td>Propoxur</td>
</tr>
<tr>
<td>Carbamates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alpha-cypermethrin</td>
</tr>
<tr>
<td></td>
<td>Bifenthrin</td>
</tr>
<tr>
<td></td>
<td>Cyfluthrin</td>
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<tr>
<td></td>
<td>Cypermethrin</td>
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<tr>
<td></td>
<td>Deltamethrin</td>
</tr>
<tr>
<td></td>
<td>Etofenprox</td>
</tr>
<tr>
<td></td>
<td>Lambda-cyhalothrin</td>
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<td></td>
<td>Permethrin</td>
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One challenge to monitoring resistance is establishing an adequate number of sentinel sites that will consistently sample the target population over years. Careful consideration has to be given to collection sites, considering not only the abundance of the target species, but also the ease with which the site can be accessed and the probability of it being available for multiple years. The role of agricultural insecticides in the selection of vector resistance has been clearly established for some important malaria vector mosquitoes. Hence, priority should be given, especially in the case of
malaria vectors, to areas where insecticides are heavily used, either for agriculture or domestic hygiene or both since in many instances, these insecticides are the same as those used for public health (IRAC, 2006).

**Characterisation of Mosquito Species and Behaviour**

**Recommendation 2:** The Ministry of Health should carry out a baseline assessment of key entomological variables such as mosquito species and biting behaviour, including ecological mapping to collect relevant information that can inform indoor residual spraying (IRS) implementation. Data collection should be of sufficient detail to allow conclusive mapping of the country which would facilitate informed decision making, even in the case of an emergency.

Effective indoor residual spraying against malaria vectors depends on whether or not the mosquitoes rest indoors (i.e., endophilic behaviour; Pates and Curtis, 2005). This varies among species and is affected by insecticidal irritancy (Grieco et al, 2007; Roberts et al., 2000). Exophilic behaviour has evolved in certain populations exposed to prolonged spraying programmes. For example, endophilic mosquitoes have been shown to switch to exophilic behaviour, in response to indoor residual spraying with DDT (Bruce-Chwatt, 1985).

The major malaria vectors in Uganda, *Anopheles gambiae* and *Anopheles funestus*, are both highly endophagic and endophilic (NEMA, 2006). Biting activity for these vectors is nocturnal, with the maximum period of biting occurring between midnight and sunrise. The peak activity for *Anopheles funestus*, occurs just before sunrise (Haddow and Senkubuge, 1973) and closer to midnight for *Anopheles gambiae* (Aniedu 1993). DDT resistance in *Anopheles gambiae* has been shown to decline with mosquito age inferring that the older, genetically resistant mosquitoes may be susceptible to DDT (Lines and Nassor, 1991). Therefore, tests on adult insects may give the impression of a lower level of resistance in the population than is actually the case.

By use of detailed ecological maps, mosquito control activities (that include spraying with insecticides) can be accurately targeted leading to efficient control, reduced costs and minimal likelihood of insecticide resistance (Gabinaud, 1987). A number of experts emphasize the importance of
assessing the resistance status of the local vector mosquitoes before deciding on strategies for malaria vector control (see Coetzee et al.; Birungi and Mukwaya in Part II). Such observations were drawn from a recent malaria epidemic in South Africa caused by the presence of insecticide resistance, as well as from susceptibility testing of mosquito populations that were wrongly assumed to be DDT naïve (Hargreaves, et al., 2000; Hargreaves, et al., 2003).

Selection of Insecticide

Conclusion 2: Vector insecticide resistance will inevitably develop against the insecticide(s) used for IRS especially if one class of insecticide is used for a prolonged period.

Conclusion 3: The Ministry of Health’s policy on IRS should include guidance on detection and monitoring of vector insecticide resistance. The guidance policy should be prepared in partnership with key stakeholders such as the Ministry of Agriculture and the National Environment Management Authority (NEMA).

Conclusion 4: There is geographic heterogeneity in insecticide resistance and this will necessitate appropriate vector resistance management to ensure that appropriate insecticides are being used.

Conclusion 5: The safe use and disposal of all insecticides including DDT must follow guidelines set by the World Health Organisation (WHO), the Basal Convention, the Stockholm Convention and NEMA/National Drug Authority.

Recommendation 3: In selecting insecticides for IRS, the Ministry of Health should be guided by the IRS policy and the strategy for insecticide resistance management. In addition the following should be considered:

a) The results of susceptibility tests carried out using the WHO bioassay tests for comparability and since they have already been used extensively both in country and in neighbouring states; Centers for Disease Control and Prevention (CDC) bottle assays can be carried out alongside or for research purposes
b) Insecticides that have been recommended by The WHO Pesticide Evaluation Scheme (WHOPES)

c) Cost of implementing IRS must be determined on a case-by-case basis because they vary so much over time and space, and depending on the implementation approach. Cost analyses should as much as possible be based on context-specific data and should take into account differences in costs of storage, transport and disposal of remains for different insecticides. If cost of implementing IRS is not readily available from existing literature, it should be generated.

d) The insecticide’s reliability of supply

e) Acceptability to the community

As malaria vector control activities in Africa increase, the careful selection of the appropriate insecticide will become more critical in order to optimally deploy the scarce resources available. Insects generally exhibit wide variability in susceptibility to insecticides. Insecticides exert selection pressure on target populations promoting the least susceptible individuals over time. This gives rise to phenotypically resistant strains that survive even higher doses of insecticides. Resistance to one insecticide can confer cross resistance to chemically similar compounds and even to families of compounds that are chemically unrelated (Reiter, 2001). Thus, this knock down resistance (kdr) to DDT (an organophosphate) confers kdr to pyrethroids.

The traditional method for dealing with resistance has been to use a new pesticide when the current one is no longer effective—however this does not address the problem of resistance (NRC, 1986). At best, it simply delays the recognition of the problem and may exacerbate it through cross-resistance. For these reasons and because further discovery and development of new and better pesticides is uncertain, efforts are needed to conserve existing materials and to react to resistance in a timely manner (See Donnelly in Part II).

An important consideration to choosing insecticides for malaria vector control programmes is proven effectiveness on the target species, which in Uganda would be the major malaria vectors, *Anopheles gambiae* and *Anopheles funestus*. The effectiveness of a pesticide is driven by a number
of factors, which may be broadly categorized as either intrinsic or external (see Williams in Part II). Intrinsic drivers relate to the internal properties of pesticides that elicit the desired goal of killing the targeted mosquito and external drivers impact on the overall effectiveness of a pesticide used for IRS. An important external driver is vector resistance to pesticides and a vital first step to evaluating the effectiveness of any insecticide is susceptibility testing. Thus simple assays are required to monitor and evaluate insecticide resistance and its underlying mechanisms.

A number of biochemical and molecular assays have been developed, some of which, like the CDC and WHO methods, can be carried out in the field (see Box 2). Bioassay data generated by either the CDC or WHO method are good indicators of the presence of resistance in mosquito populations; but neither method provides information on the mechanisms of resistance, gene frequencies or epidemiological impact of resistance (Coleman and Hemingway, 2007).

### Box 2

**WHO Diagnostic Assays and CDC Bottle Assays**

WHO Diagnostic Assays are the most widely used assays in the field. To carry out this test, insects are exposed to filter papers impregnated with insecticide in a carrier oil formulation. The insecticide dosage is set at double the amount that which kills 100% of three-day-old, non-blood fed adult females of known susceptible laboratory colonies for a range of anopheline mosquitoes. This is a robust dosage that susceptible individuals are unlikely to survive by chance. The guidelines from WHO suggest that resistance is only indicated if over 5% of insects survive the exposure, thereby reducing the risk of false positives. However, many heterozygous resistant individuals may be killed by the dose. Hence, resistance levels may need to be very high before they can be detected using this methodology.

CDC Bottle Assays are similar to the WHO discriminating dose assays. Insecticide impregnated bottles are prepared by coating glass bottles with an acetone or alcohol based formulation. Insects are then exposed to the insecticides in the bottles. This assay has the advantage over the WHO test
kit in that the rate of insecticide knock down is easier to score during the course of the exposure period. With rapid acting insecticides, such as pyrethroids, this can be predictive of a kdr-type resistance mechanism within the population. Care should be taken not to over-interpret such data, however, as several effective metabolic resistance mechanisms also produce a reduced knock-down phenotype without any accompanying change in sensitivity at the sodium channel target site. This method is, however, a good way of testing actual insecticide formulations that will be sprayed on the walls of houses to assess the efficacy on wild populations of mosquitoes regardless of their resistance profiles.

CDC bottle bioassay data has not been shown to be comparable with the more widely used WHO diagnostic assays although recent data from South America may refute this statement (unpublished data, Colombia).


An important part of the implementation strategy is selecting which insecticide to apply. WHO recommends particular insecticides for use in IRS, as listed in the table found in Annex 1, *The twelve insecticides recommended for IRS by WHO’s Pesticide Evaluation Scheme* (see Schapira in Part II). It should be noted that new formulations of some insecticide compounds have shown longer residual activity than represented in this table (Bayer, 2007; Bayer, Accessed October, 2007; Kolaczinski et al., 2007a). The final decision as to which insecticide(s) to use needs to consider such issues of safety, efficacy and residual effect, insecticide formulation, community acceptability and cost, as well as the susceptibility data mentioned above (Malaria Control Programme, 2004). The use of DDT is covered by the Stockholm Convention on ‘Persistent Organic Pollutants’ (or POPS Treaty), which Uganda ratified in July 2004 (Stockholm Convention on POPS, 2001). By signing onto this agreement, Uganda is required to comply with the requirements surrounding the use and safe disposal of DDT and other insecticide products used in public health. WHO guidelines on safe use and disposal are designed to protect the health of the populations concerned as well as the environment, food, and other consumer products.
In 2006, WHO announced its support for the continued use of DDT for disease vector control, under the POPS Treaty (WHO, 2004; WHO, 2006b). Similarly, signatories to the Stockholm Convention recognize the tremendous public health burden of malaria in developing countries and thus support the use of DDT in accordance with the limiting provisions agreed to by the more than 150 nations in the Stockholm Convention (Sierra Club, 2006; Environmental Defence, 2006).

Published information on the relative costs of DDT and other insecticides used for malaria control is scarce and the choice of insecticides is hampered by the lack of economic costing data (Kolaczinski et al., 2007b; Yukich et al., 2007). One available study compared recent price quotes from manufacturers and WHO suppliers on the cost of DDT and formulations of nine other insecticides (two carbamates, two organophosphates and five pyrethroids) commonly used for residual house-spraying in malaria control programmes (Walker, 2000). Based on these ‘global’ price quotes, detailed calculations show that DDT remains the least expensive insecticide on a cost per house basis\(^4\). At the same time, the prices of pyrethroids are declining, making some only slightly more expensive than DDT at low application dosages.

A global cost comparison may not realistically reflect local costs or effective application dosages at the country level. Data on insecticide prices, paid by the health ministries of individual countries, showed that prices of particular insecticides can vary substantially in the open market (Walker, 2000); therefore, the most cost-effective insecticide in any given country or region must be determined on a case-by-case basis. Regional coordination of procurement of public health insecticides could improve access to affordable products. In the past, spraying has been done in Uganda using the pyrethroid lambda-cyhalothrin (PMI, 2007b). Should it be decided to continue with this pesticide a possible approach for cost savings might be to consider purchasing several pyrethroids through an open tender system. This would enable lower prices to be paid for the insecticides and the savings could be used elsewhere. Box 3 provides a rough indication of the cost implications of implementing recommendations found in this report.

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\(^4\) The potential for changes in prices being dependent on levels of production. For example, prices are likely to decline if larger amounts of insecticide have to be produced.
Box 3

Estimated Costs of President’s Malaria Initiative (PMI) for IRS in Uganda

PMI’s estimated costs of proposed activities for indoor residual spraying of 795,000 households in Uganda in 2008 (total expenses approximately 8,520,000 USD):

1. **Support second round of spraying in four IDP districts in northern Uganda:** The PMI will support the second round of spraying in IDP camps, urban areas and newly settled villages in Pader, Kitgum, Gulu and Amoro districts of northern Uganda. ($2,000,000)

2. **Support one round of IRS in six highly endemic districts:** PMI will assist NMCP/MOH to conduct one round of IRS in Lira, Dokolo and Amolatar districts in northern Uganda and three additional districts categorized as highly endemic in eastern Uganda, namely Kabermaido, Amuria and Soroti. ($4,400,000)

3. **Support a third round of targeted IRS in Kabale and Kanungu District:** The PMI will assist with a third round of IRS in 9 sub counties affected by malaria in Kabale (approximately 35,000 of households) and 6 sub counties in Kanungu district covering approximately 75,000 of households. ($800,000)

4. **Support one round of targeted IRS in Rukungiri District:** The PMI will conduct one round of spraying in this highland area, which has both high transmission and epidemic-prone transmission. It is estimated that nine sub counties in the district fall within a malaria risk zone. ($750,000)

5. **Support entomologic monitoring and evaluation:** PMI will support the IRS baseline and post intervention entomologic surveillance and conduct susceptibility, bio assays and vector bionomic studies related to IRS ($70,000)

6. **Support the MOH in IEC/BCC/community mobilization:** Continue IEC/BCC activities specific to IRS, and support campaigns to mobilize and educate communities on what IRS is, its benefits and risks, and proper procedures for safety and community participation. ($500,000)

Source: PMI, 2007b.
Long-term use of IRS as a Highly Effective Intervention

Recommendation 4: The Government of Uganda should prepare to implement IRS, in the long term in all areas where it is started, most especially in areas of stable transmission because once the human population loses its malaria immunity, IRS should not be stopped until other proven methods to maintain low levels of malaria transmission are in place.

People residing in malaria-endemic regions acquire immunity to malaria through natural exposure to malaria parasites (Pombo et al., 2002; Aponte et al., 2007). This naturally acquired immunity that is protective against parasites and clinical disease, results only after continued exposure from multiple infections with malaria parasites over time. In areas of low malaria endemicity, both children and adults suffer disease and high parasitemia since exposure is less frequent (CDC, 2004). In 1969 for example, a resurgence of malaria occurred in many areas because people lost their immunity following the end of a global malaria eradication campaign involving worldwide spraying with DDT (Najera et al., 1998; see Schapira in Part II).

According to WHO, IRS is indicated only in those settings where it can be implemented effectively, which calls for a high and sustained level of political commitment (WHO, 2006). Transmission control operations based on IRS, or any other vector control intervention, have to be maintained at high coverage levels for extended periods of time, for as long as impact is needed. However, it is unclear whether the capacity to do this exists in Uganda. In the annex to the paper by Schapira it is noted that "in very low income countries it is not possible to routinely meet the logistical demands of ensuring that trained spray teams equipped with working spray pumps and sufficient insecticide arrive at each village in time to spray before the malaria season." The situation may be different in Uganda given the international interest in funding IRS activities (see Box 3). But it might be prudent to perform a risk assessment identifying factors likely to cause a break in spraying coverage - and how these can be mitigated - in order to minimize a resurgence of malaria in areas where spraying has begun.
Strategy for Vector Resistance Management

Recommendation 5: The Ministry of Health should develop a long term vector insecticide resistance management strategy that considers the different options for preventing the evolution and spread of vector insecticide resistance.

The available tools for vector control are limited and availability of new agents to which there is no resistance will be few in the near future (IRAC, 2006). Therefore once resistance to most key insecticides has developed, options become very limited. In order to minimize resistance, strict insecticide management practices should be instituted. These should include tracking of insecticides movements and use, judicious use, avoidance of illegal use or diversion of insecticides to agriculture, and assurance of quality application of the insecticides.

A number of strategies have been developed to help prevent or delay the evolution and spread of vector insecticide resistance that include the quality of insecticides used and the quality technique of the actual spraying. In addition to these “quality” issues, the Insecticide Resistance Action Committee (IRAC) has developed guidelines to assist in this area (see Box 4; IRAC, 2006).

<table>
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<th>Box 4</th>
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<td><strong>IRAC Guidelines to Prevent or Delay Vector Insecticide Resistance</strong></td>
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- Insecticides have to be applied at the concentrations recommended by WHO and by the manufacturers (label instructions), avoiding over-dosages which are costly and potentially hazardous, as well as under-dosages which are not effective enough and may accelerate the development of resistance.

- Insecticides of the same chemical group, acting on the same target site, should be considered as a single product as far as resistance is concerned.
- The use of one chemical class against both larval and adult life stages should be avoided.
- The combined use of unrelated insecticides (for example in rotation) should be preferred to the continuous use of a single insecticide for extensive periods of time.
- Soon after resistance is detected in a target vector population, another unrelated insecticide should be introduced, either alone or in combination.
- If necessary, an insecticide can still be used for some time when resistance is at a low frequency, especially when resistance is recessive and individuals are mostly heterozygous. However, such an insecticide should preferably be replaced by a non-related one when vector populations are seasonally expanding or at their peak density (for example at the beginning and during the rainy season for tropical mosquito species).

Source: IRAC, 2006

It should be noted that for a “rotation” or “mosaic” of insecticides to delay the evolution of resistance, the different insecticides used must not show cross resistance to different classes of insecticides.

**Community Engagement on IRS / Community Participation in the IRS Programme**

**Conclusion 6:** High community acceptance is essential for a successful IRS programme. It is vital that pervasive Information Education and Communication (IEC), be undertaken throughout the period of implementation and even afterwards. It is also good practice to use spray operators that are acceptable to the community.

**Recommendation 6:** The Ministry of Health should employ effective IEC to engage communities in the targeted districts well before IRS is carried out. The process of community dialogue should be dynamic and well contextualised in order to achieve high and continued acceptability of the intervention. It should employ more than
community awareness building (i.e. community sensitisation) and should involve monitoring of people’s perceptions and acceptability of IRS. The Health Promotion and Education department of the Ministry of Health should be strengthened to support and oversee the targeted districts to carry out continuous community dialogue on this and other malaria control interventions.

On August 14, 2007 Uganda’s New Vision newspaper reported that malaria control spray operators were met with resistance in Munyonyo, a suburb of Kampala, as they attempted to conduct an IRS program. The spraying program in Munyonyo was managed by a private contractor, Balton, and even though they used pyrethroids, the community assumed the insecticide being used was in fact DDT. Following improved outreach and communication efforts by Balton, residents were given the facts and felt reassured, and subsequently, allowed spray operators to enter their homes. This incident highlights the need for good information, education and communication (IEC) in malaria control and further elucidates the fact that community mobilisation and cooperation is essential in obtaining maximum cooperation from households in order to effectively carry out IRS campaigns (AFM, 2007) especially since repeated spraying of houses commonly generates fatigue and refusal by householders. Similar statements can be found in the WHO position statement on IRS (WHO, 2006c) and in Schapira’s concluding remarks (see Part II).

IMPLEMENTATION PHASE

Monitoring and Evaluation of IRS Programme

Conclusion 7: Implementation of an IRS programme will be dynamic and will require up to date information to inform and refine the IRS strategy for vector resistance management.

Recommendation 7: The Ministry of Health should have a strategy for monitoring the operational effectiveness and quality of IRS implementation, in order to document the success of the programme. This will involve collecting data on transmission intensity, including changes in clinical variables such as parasite prevalence, malaria case incidence and entomological variables such as species
distribution, vector density, Entomological Inoculation Rate (EIR$^5$), insecticide effectiveness on the walls and the duration of the residual effect of the insecticide. For purposes of evaluating the effectiveness and persistence of the insecticide on the walls, it is essential that fully susceptible mosquitoes are used in the WHO cone bioassays. To assess the quality of the spraying, the committee recommends an evaluation be conducted within a month of the spraying, whereas to assess the residual life of the insecticide, the committee recommends an evaluation be conducted in three month intervals. (Maintain a colony of susceptible mosquitoes or use wild types as long as they are known to be susceptible.)

To document success of an IRS programme, baseline data is needed to monitor levels of resistance over time (WHO, 1992). Such monitoring should be standardised to ensure comparability of data from different sources; hence a standardised test system is a prerequisite. In this context, definition of standards and procedures and assuring access to quality assured test materials and kits is essential. To confirm the validity of laboratory test results, laboratory workers should always conduct a second trial concomitantly with susceptible mosquitoes as a control (Brogdon and McAllister 1998a; Brogdon and McAllister 1998b).

The quality of an IRS programme can be evaluated if certain clinical and entomological variables are collected. Such data might include the following:

- Mosquito populations for susceptibility to insecticides, to detect selection for physiological and behavioral insecticide resistance associated with IRS use;
- Indoor *Anopheles* vector densities, to detect changes in IRS insecticidal efficacy and changes in man-vector contact rates; and
- The quality assurance of IRS treatment, to verify both the initial efficacy and longevity of IRS treatment.

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$^5$ The number of infective bites per person per night is known as the Entomological Inoculation Rate (EIR) and is calculated as: EIR = man-biting rate x sporozoite rate (%)/100 (WHO, 2003).
Program performance in terms of services delivered (houses missed, quality of spraying) is another component of quality assurance (Booman et al., 2003) which if carried out by an entity external to the IRS program, would avoid the risk of conflict of interest.

Despite the need for quality assurance monitoring, according to the President’s Malaria Initiative (PMI), the Monitoring and Evaluation (M&E) unit of the National Malaria Control Programme (NMCP) lacks critical human resource capacity, a malaria M&E plan, and equipment (computers and accessories, scanners, and photocopiers) to operate the unit. Currently there is no mechanism in place at the malaria control program to evaluate large-scale malaria interventions. PMI intends to strengthen national level analysis of malaria surveillance data with seconded staff to provide M&E support for NMCP as well as support for equipment and other supplies. This intervention is estimated to total 100,000USD (PMI, 2007b).

**POST IMPLEMENTATION PHASE**

*Routine Surveillance of Vector Susceptibility*

Recommendation 8: During the maintenance phase, the Ministry of Health and the District Health teams will need to monitor vector susceptibility. The level of vector susceptibility will guide frequency of testing. If there is full susceptibility (i.e. 98-100% mortality within 24 hours after 60 minutes exposure), testing can take place once a year. If the susceptibility is between 80-97%, then the resistance should be confirmed with repeated tests at the same site and more tests in additional sites. Once resistance is confirmed, it should be monitored more frequently at least every six months and the mechanism of resistance determined through molecular and biochemical techniques. If susceptibility is less than 80%, then resistance management strategies should be considered and the appropriate options implemented. At the level of less than 80%, susceptibility monitoring is required at least every six months.
Although extensive discussions have taken place regarding an appropriate susceptibility level cut-off before recommending a switch of insecticides, there are at present no clear guidelines available and it seems that each situation must be considered individually when implementing resistance management even in the absence of resistance. However, the following WHO criteria for vector susceptibility to insecticides was used as a guide for the frequency of testing needed:

- Mosquito tests with mortality of greater than 98% indicate susceptibility to resistance;
- Mortality between 80% and 98% suggests a need for verification of the results; and
- Mortality of less than 80% indicates that resistance is present. In this case, additional tests are also required and field observations should be intensified with parallel, periodical checking of resistance levels in order to confirm the results (Davidson and Zahar, 1973).

To compromise insecticide vector control, the level of resistance must be high enough to adversely affect disease transmission. In many cases, vector control may not be affected by the level of resistance (Brogdon and McAllister, 1998a). The experience in Western Kenya provides an operational example of the coexistence of resistance and disease control efforts. Soon after bed nets were introduced in the region, pyrethroid resistance emerged. However, after two years, the level of resistance had not changed significantly, possibly because of the continual massive introduction of susceptible genes (Vulule JM et al., 1996; Brogdon and McAllister, 1998a). Because results of susceptibility tests cannot represent the actual reaction of vector populations to an insecticide as applied in the field, these tests should not be taken as the sole basis for replacing an insecticide. But for an activity controlling less than 80% of the vector population, the control operations will need to be evaluated and improved or a different method will need to be used.

When the presence of physiological resistance has been confirmed and its geographical extent has been delineated, routine susceptibility tests should be conducted to determine the change in the levels of resistance. It is then the task of the entomologist and the malariologist, to project the results of these susceptibility tests in terms of field mortality in the vector population.
under field dosage and spraying rounds. In addition, they should determine the role played by the proportion of the population surviving to an epidemiologically significant age and the resulting parasitological consequences in the human population. This may vary from one vector to another depending on their vectorial efficiency (Davidson and Zahar, 1973).

**Networking**

**Recommendation 9:** The Malaria Control Programme manager should ensure that the programme has representation on WHO entomological networks and committees such as the African Network for Vector Resistance Monitoring where information on vector resistance can be shared and advice can be gained on how to manage resistance.

An IRS programme stands to gain from linking up with stakeholders beyond national borders and monitoring the development and spread of resistance at the country, regional, and international levels. This leads to better coordination and enables malaria control programmes to develop harmonized approaches to managing vector resistance within their borders (ANVR, 2006). Many studies do show that international networking and cooperation towards strengthening malaria control programmes across the region is of vital importance to the success of any such programme (Mabaso et al, 2004).

Initiatives such as the African Network for Vector Resistance (ANVR) are designed to create a platform for effective networking and capacity building, which has led to increasing partnerships between research institutions, national vector control programmes and the private sector. Because many national malaria control programmes lack the required capacity to monitor and manage vector resistance, due to lack of trained staff and absence of guidelines, the ANVR can play a role by contributing to the development of technical capacity at the national level and increasing knowledge on the status of malaria vectors at the regional level in regard to resistance. The network allows national programmes to come together to share information and offers technical support to promote the effective management of disease vectors (WHO, 2005).
**Monitoring Insecticide Concentration**

**Recommendation 10:** The Ministry of Health and other line ministries should support studies that monitor the concentration of insecticides in the environment and food chain, including toxicity studies in spray operators and others that handle insecticides.

The particular concern over DDT is that its breakdown product, DDE is an extremely stable compound with a long half-life of about 7-11 years (Rogan and Chen, 2005; Wolff et al., 2000); however, it is because of these properties - persistence in the environment so it does not have to be reapplied often; water insoluble so it does not wash off by rains - that DDT and other organochlorines also serve as powerful insecticides against susceptible malaria vectors (Oregon State University, 2007). Both DDT and DDE are highly soluble in lipid and can thus accumulate in fatty tissue. They can be transferred from the placenta and breast-milk to fetuses and infants. In areas where DDT has been used for vector control in the past 5-10 years, DDT concentrations in human tissues can be high (Waliszewski et al., 2001; Bouwman et al., 1990; Bouwman et al., 1991); and DDT spray operators in Mexico, were shown to have much higher concentrations of serum DDE than children and adults who lived in sprayed houses but were not otherwise exposed to DDT (Yanez et al., 2002).

Although toxic effects of DDT have been demonstrated in laboratory animals, exposure to DDT or its metabolite DDE have not been sufficiently shown to:

- Increase human cancer risk;
- Detrimentally affect reproductive health; or
- Affect infant and child survival or cause neuro-behavioural abnormalities in persons with higher serum DDE concentrations obtained through the food supply (Rogan and Chen, 2005; López-Cervantes et al., 2004; Maelé-Fabry et al., 2006; Dalvie et al, 2004a,b; Ribas-Fitó et al., 2007).
In countries where insecticides are used (whether for plant protection or for IRS), constant surveillance and monitoring of their use is of critical importance. This is necessary in order to monitor for inappropriate use and to detect pesticide residues of compounds that could have negative ramifications for the ecology and the economy. As noted above, DDT is of particular concern because of its persistence in the environment and because of the controversy surrounding its use. Although the European Union (EU) denies allegations that Ugandan products would be totally banned from the market upon using DDT for IRS (Apio, 2006), there is a maximum residual level for DDT and other pesticides in food products intended for human or animal consumption set by the EU that would need to be monitored. The level allowed by the EU for DDT usually ranges from five to ten times lower than equivalent levels for other countries like Japan and the US.

Confirmatory tests that assure Ugandan exports do not exceed the strict EU limits would be needed for monitoring. At the same time, farmers who typically store or dry their export products (like coffee beans) indoors, could be encouraged to always store export products in an outhouse which is not sprayed. This would avoid the risk that products might pick up minute traces of DDT if the farmer’s house had been previously sprayed.

**CLOSING REMARKS**

In summary, the recommendations contained in this report are designed to provide a roadmap for how the Ugandan Ministry of Health can minimize resistance to insecticides used for indoor residual spraying and thus maximize the effectiveness of insecticide spraying in Uganda. Although pilot programs have already begun in some districts, it is not too late to lay the foundation for expansion of the spray campaign by conducting baseline susceptibility tests in targeted areas. This is also the time to characterize the mosquito population, select the insecticide, initiate long-term strategic planning, and begin strengthening the capacity to implement IRS and vector insecticide resistance management at all levels. Similarly, outreach to IRS experts and local communities should start during the pre-implementation phase and continue through the actual implementation phase where monitoring and evaluation of the IRS programme is paramount. During the maintenance or post implementation phase, there is continued monitoring of vector susceptibility as well as environmental testing which ultimately guides the selection of insecticide for IRS in the target area.
REFERENCES


PART II
CURRENT STATE OF KNOWLEDGE AND POLICIES

Framing the Issues

OVERVIEW

This section addresses three important issues with regard to vector resistance namely: that resistance in African malaria vector mosquitoes is becoming an increasing problem for vector control programmes; that resistance monitoring can be used in planning malaria control programmes as part of an integrated vector management strategy; and that most of the data on resistance is from West Africa. There is very little published data on vector resistance in Uganda. Limited data from neighbouring countries of Kenya, Tanzania, and Sudan show varying levels of resistance to insecticides. Highlighted issues from the papers indicate that determining the extent of insecticide resistance is an urgent issue and early detection of resistance is the key to rational insecticide resistance management strategies. Expressed is the need to develop a decision making support system which incorporates a rational insecticide usage plan. Such an IRS plan might address acceptance of IRS by the population, preservation of spray on wall surfaces, and whether the plan is suitable for rapid population protection or for progressive introduction and incorporation into sustainable population habits. It might also consider challenges of implementing malaria vector control in the context of various eco-epidemiological types. It is important to base decisions on evidence through studies in affected areas to design malaria control programs that put into consideration proper vector...
resistance management strategies. One such example is at the Obuasi gold mine in Ghana. Results of investigations into malaria vector resistance to insecticides in this Ghanaian gold mining town had implications for malaria control which was part of a larger integrated vector management programme.

**Current State of Malaria Vector Resistance in East Africa and Uganda**

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**Introduction**

The *Anopheles* vectors of malaria can be controlled through Indoor Residual Spraying of insecticides (IRS) and distribution of insecticide-treated bed nets (ITNs) (Kleinschmidt et al., 2006; Sharp et al., 2007). Resistance to insecticides approved for IRS (DDT; pyrethroids; carbamates and organophosphates) and ITNs (Pyrethroids) now represent a major threat (Nauen et al., 2007). New insecticides for the public health sector are unlikely to be developed in the near future and improved monitoring and evaluation of the effectiveness of current insecticides is crucial to extend their lifespan. Knowledge of resistance is central to this strategy but little is known of the extent of resistance in field populations from East Africa.

Insecticide resistance stems primarily from mutations that alter insecticide target sites or from metabolic resistance via detoxification enzymes, and is now widespread in *Anopheles* malaria vectors, threatening disease control (Denholm et al., 2002; Coleman et al., 2006; Nauen et al., 2007). Identification of the genes responsible for insecticide resistance will permit the presence of key genetic variants (alleles) to be screened for directly in the field as part of monitoring programmes. This will allow early identification of resistance or prediction of future resistance in a population by detecting rare or recessive resistance alleles, complementing and extending the information provided by standard bioassays (WHO, 1998), and providing information critical for strategic insecticide deployment (Nauen et al., 2006).
Resistance Monitoring in Eastern Africa

Most monitoring work on natural populations screens for just a few mutations affecting insecticide target sites, especially the DDT and pyrethroid knockdown resistance mutation \textit{kdr}, (Martinez et al., 1998), which sometimes, but not always, correlates with resistance in the field (Awolola et al., 2003; Vulule et al., 1999; Yawson et al., 2004). Yet, resistance is likely to be more complex, and biochemical assays frequently indicate a role for metabolic resistance in \textit{Anopheles} populations (Awolola et al., 2003; Vulule et al., 1999; Etang et al., 2003; Chandre et al., 1999; Brogdon et al., 1999; Brogdon et al., 1999 (Journal of Economic Entomology); Casimiro et al., 2006; Corbel et al., 2007), although they cannot identify the loci responsible. By contrast, laboratory studies have identified a number of metabolic resistance-associated genes, but their importance in the field populations is unknown. Most reports on insecticide resistance in sub-Saharan Africa are confined to West Africa (Coleman et al., 2006) and only a few studies have been conducted in Uganda and neighboring countries. The following summary of studies will concentrate on resistance to pyrethroids and DDT. Much of the data available describes the mechanisms of resistance and this brief review reflects this fact.

\textit{Sudan}

In Sudan, beginning in the late 1980s DDT resistance was observed in both \textit{Anopheles gambiae} and \textit{A. arabiensis}. In both species, resistance was attributed to an over-expression of a detoxification enzyme belonging to the Glutathione-S transferase group with no evidence for the involvement of \textit{kdr} (Janet Hemingway \textit{pers comm}). More recently knockdown resistance mutations have been reported in \textit{A. arabiensis} from Sudan (Himeidan et al., 2007). Two resistance mutations were observed. The first, a leucine-phenylalanine substitution at position 1014 of the sodium channel gene, termed L1014F, is widely spread in \textit{A. gambiae} s.s. in Western Africa and was recently observed in East Africa. The second \textit{kdr} allele, a serine replacement (L1014S) at the same position, was initially identified in Eastern Africa and has been found in parts of Central Africa (Ranson et al., 2000; Pinto et al., 2006).
Tanzania

Very high levels of DDT resistance have been observed in *A. gambiae* populations from Zanzibar (Prapanthadara et al., 1995). Again resistance was attributed to increased GST activity. The expression levels of several of the up-regulated GSTs declined with age and therefore resistance frequency was underestimated when field collections of mosquitoes of indeterminate age were bioassayed. The L1014F *kdr* allele has also been observed at low frequency in *A. arabiensis* populations from Tanzania (Kulkarni et al., 2006). However, recent data show no evidence for phenotypically detectable levels of resistance in either *Anopheles gambiae* s.l. or *A. funestus* s.l. Both species groups were highly susceptible to permethrin (mortality 87-100%) and deltamethrin (mortality 100%) in WHO tests in 1999 and 2004 (Kulkarni et al., 2007).

Kenya

A relatively small foci of resistance has been documented in western Kenya in an area on the shores of Lake Victoria adjacent to Uganda. Multiple resistance mechanisms have been described in the *A. gambiae* s.s. populations. The mechanisms are L1014S *kdr* allele which was effectively DDT specific and produced very little obvious pyrethroid resistance, a GST and a P450 mechanism (Vulule et al., 1999; Vulule et al., 1994; David et al., 2005). The L1014S *kdr* allele was observed recently in *A. arabiensis* from Kenya (Stump) at an extremely low frequency (Stump et al., 2004).

Uganda

Only one publication was found in the peer-reviewed literature that discussed any aspect of resistance in Ugandan malaria vectors. This methodological paper reported detection of both *kdr* in *A. gambiae* s.s. and the L1014S allele in *A. arabiensis* (Verhaeghen et al., 2006). No collection site details were given.
Conclusion

At present there are insufficient data on the resistance status of vector populations in Uganda, and in eastern Africa as a whole, to make an informed decision on the use of DDT. There is an urgent need to determine the extent of insecticide resistance. This necessitates the establishment, or expansion, of a network of sentinel sites where insecticide resistance can be regularly monitored. Monitoring should not be solely reliant upon phenotypic bioassays. WHO bioassays are often viewed as the gold-standard for resistance detection. However, there is no simple relationship between designation of a vector population as resistant and control failure. The standard bioassay definition of resistance is if a certain percentage of the population survive exposure to a discriminant dose. The discriminant dose chosen and the percentage cut offs for population resistance characterisation are themselves highly arbitrary. Furthermore, bioassays have limited throughput, require live material and have limited power to detect resistance alleles when at low frequencies in sample populations. This latter point is particularly important as early detection of resistance is the key to rational insecticide resistance management strategies. There are increasing numbers of molecular analyses available or becoming available. These should be integrated into any monitoring and evaluation programme. Another key point to consider, which is essential to the long–term management of resistance, is the development of a decision support system which incorporates a rational insecticide usage plan.

REFERENCES


The Role of IRS in Contemporary Malaria Control

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Introduction

In this paper, the principles of Indoor Residual Spraying (IRS) for malaria control, the evidence on its effectiveness and the requirements for its implementation will be summarized. Recent data from a study comparing cost-effectiveness of IRS and Insecticide Treated Nets (ITNs) will be included. This will be followed by a summary of the recommendations regarding the role of IRS in malaria vector control, as defined through recent consultative meetings convened by WHO (WHO, 2006). Finally, the practical implications of these recommendations for malaria control and research in the coming years will be discussed on the background of growing international financial support for malaria control.
What is indoor residual spraying?

Indoor residual spraying refers to the spraying of all the stable surfaces inside human habitations using an insecticide with residual action. The expected result of indoor residual spraying is mainly to reduce the survival of vectors entering houses (WHO, 2000). It takes a minimum of 8–22 days (shorter at higher ambient temperatures) for malaria parasites in a blood-meal taken by an anopheline from an infected human to develop to a stage, when they render the mosquito infective. If the life-span of the vector mosquitoes is reduced below the length of this period, they become incapable of transmitting the disease. Therefore, methods targeting adult mosquitoes are in most circumstances far more effective than anti-larval measures, which only reduce mosquito density. IRS is a highly standardised intervention with specific requirements in terms of management, logistics and programme infrastructure. The financial costs of IRS are in most countries, around US$ 2–5 per person protected per spray-round, varying with insecticide, labour and transport costs. Twelve insecticides are currently recommended by WHO for IRS (see Annex 1). Recent trends in the use of IRS are illustrated in Annex 2.

History of malaria vector control, and malaria eradication and elimination

Following the demonstration of the role of anophelines in malaria transmission in 1898, malaria vector control efforts were generally focused on reduction of the breeding of anophelines based on identification of the larval habitats of local vectors. The main interventions were environmental management and chemical or biological larviciding. In tropical areas with intense transmission, it was found that anti-larval measures needed to be applied with a high degree of perfection to be effective. They were therefore mainly used in economic development projects, the Panama Canal being the most famous example. In the 1940s, trials in South Africa showed that DDT was highly effective for malaria control, when applied to indoor surfaces of human dwellings, thanks to a residual insecticidal effect lasting for several months. This led to the development of Indoor Residual Spraying (IRS) with DDT and other residual insecticides.
IRS revolutionized malaria control. It became possible in huge areas, where the classical methods of larval control had not worked or not even been envisaged. Based on the good results, especially in southern Europe, leading scientists in 1955 advised the World Health Organisation it would be possible to eradicate malaria from the world by a time-limited campaign. A short period, about 10 years, was recommended. It was considered unlikely that spraying operations could be maintained with necessary high coverage and quality for many years, because of concerns about the development of mosquito resistance to DDT and related compounds. The global malaria eradication campaign was adopted by the World Health Assembly in 1956 (WHO, 1957).

As a result of the campaign, malaria was eliminated or greatly reduced in most of the world outside tropical Africa. Southern Europe, most islands in the Caribbean, the former Soviet Union and many other areas in Asia and the Americas were freed of malaria. In most of the Indian sub-continent, South and Central America, the eradication campaign led to major reductions in the malaria burden, but not elimination. When it was called off in 1969, international support vanished. Malaria resurfaced in many areas where it had not been eliminated, sometimes temporarily becoming worse than before, because people had lost their immunity to malaria (Najera et al., 1998). Since then, most of these countries have succeeded in bringing their malaria problem under a degree of control, so that it now persists mainly in remote and/or conflict-ridden areas with poorly developed health services. In some forested areas in tropical Asia and South America, where anophelines often tend to rest outdoors (exophily) and/or people do not live in dwellings with sprayable walls, IRS was found to be practically useless. Some of these places remain as malaria “hotspots” to this day.

In southern Africa and the Horn of Africa, where transmission is generally of low intensity or epidemic, malaria was greatly reduced by IRS, and in some areas eliminated. Setbacks were observed as a result of factors such as inadequate funding, conflicts, changing climate, insecticide resistance and lack of collaboration of the populations. After 2000, there has been renewed progress in most of these countries (Mabaso et al., 2004).

When the global eradication campaign was launched, it was not known, whether it was feasible in areas of tropical Africa with intense year-long malaria transmission. To assess the possibilities, “pre-eradication
schemes" were initiated. In highland areas and some forested areas, it was possible to interrupt malaria transmission, but the difficulties proved much greater in the lowland savannah environment. Well documented trials in savannah areas with intense transmission, showed that IRS could substantially reduce transmission, morbidity and mortality, but not interrupt the transmission (Fontaine et al 1978; Molineaux et al., 1980). The marked exophily of some sub-species of *Anopheles gambiae* was one of the main explanations.

On this background, there was consensus during the 1980s, that in areas of intense transmission in sub-Saharan Africa, IRS should not be promoted as a general malaria control tool, because sustaining the results would require multiple spraying cycles per year. It was considered impossible to maintain these for many years (Gramiccia et al., 1988). It was also envisaged that insecticide resistance could lead to great operational difficulties and increased costs.

**The introduction of insecticide-treated nets**

Mosquito nets reduce the risk of malaria to the users. The insecticidal treatment of nets adds a chemical barrier to the physical barrier provided by the net and thus improves its effectiveness in personal protection. In addition, community-wide use of ITNs reduces the vector population and shortens the mean mosquito lifespan. Thus, ITNs, at high coverage levels, similarly to IRS, are a vector control measure of general applicability. Community-based randomized trials in Africa, showed that when applied with high levels of coverage, this intervention can substantially reduce all-cause mortality and malaria morbidity in children under the age of five years (Lengler, 2004).

Following the good results of trials in Africa, ITN was widely embraced as the prevention of choice in areas of intense malaria transmission. Once introduced, ITNs often became popular, more because they reduce mosquito nuisance than because they reduce malaria. They also became popular among some donor agencies; because it appeared that they could be promoted through marketing approaches rather than through public services. However, most social marketing has benefited urban populations more than rural ones, and has not been effective for maintaining high coverage of insecticide treatment (Lines et al., 2003).
On the background of widespread traditional use of mosquito nets and the failure of IRS in some forested areas, ITNs were vigorously promoted in WHO's Western Pacific Region, where they have now to a large extent replaced IRS (Schapira, 2002). High coverage has been reached as a result of distribution of free or highly subsidized nets, but the maintenance of regular re-treatment has been variable. In most of these countries, IRS has been kept as the intervention of choice for curbing epidemics, and in problem areas, where ITNs do not afford enough impact. In other parts of Asia and the Americas, ITNs are now being introduced cautiously to replace IRS. In India, for example, ITNs have in recent years become popular among populations and health care providers in the eastern part of the country. But in the north-western part of the country, with extremely high summer temperatures, ITNs have failed, and the malaria control programme has reverted to IRS and larval control (the latter being feasible in the very arid environment).

In Africa, the progress in ITN coverage has been slow (WHO et al., 2005). There is growing evidence and understanding, that high coverage rates can be attained rapidly through combination with immunization and antenatal care services, with the groups, which are most vulnerable to malaria (Grabowsky et.al.2005, Webster et al., 2006). Furthermore, long-lasting insecticidal nets (LLINs) are now making it much easier to rapidly scale up and maintain ITN coverage especially in hard-to-reach populations.

**Comparison of Cost-effectiveness of ITN and IRS**

The Swiss Tropical Institute has recently completed a comparative study of the cost-effectiveness of ITN and IRS in Africa (details, see Annex 4). The main conclusion is that the cost per person protected per year is USD 1.18-2.64 for LLINs, USD 1.43-6.05 for conventional ITNs and USD 3.27-3.90 for IRS. Thus, LLINs appear to be the “best buy”, but it needs to be cautioned that the long-term experience with LLINs as a large-scale public health intervention is limited. The authors of the comparative study rightly concluded that “all these vector control programmes are excellent public health investments.”
Conclusions

On the background of the availability of new evidence on interventions and new products, WHO convened a Study Group on Malaria Vector Control and Personal Protection in 2004 (Mabaso et al., 2004). The main conclusions and recommendations pertaining to the role of IRS in the report are summarized in Annex 3. For a more detailed discussion the reader is referred to the full report. The detailed technical conclusions and recommendations from the Study Group meeting related to IRS could be simplified as follows:

IRS requires the acceptance of the population of spraying once or twice a year and a reasonable preservation of sprayed surfaces without replastering. Thus IRS is suitable for the rapid protection of a population, but when IRS needs to be continued for many years, there may be an attrition of people’s acceptance. In contrast, ITNs are more suitable for progressive introduction and incorporation into sustainable population habits. Available evidence suggests that with high coverage and good implementation standards, the two interventions have similar epidemiological impact, although it is likely that in some situations, one will have an edge over the other (Curtis et al., 2000; Lengler and Sharp, 2004; Yadav, 2005).

In general terms, therefore, the choice between IRS and ITNs should be based primarily on consideration of operational factors and sustainability.

Countries with intense malaria transmission and no organized large scale IRS

ITNs are, especially with the availability of LLINs, easy to implement, and their scale-up to full coverage of vulnerable populations is the top priority.

Given the availability of increased funding, the opportunity should be taken to establish a capacity for selective application of IRS in ministries of health, because:
• Many countries have some areas, e.g. mountain-fringes, or situations, e.g. refugee camps, where epidemics may occur; an IRS rapid response can then be life-saving.

• In some areas, for example around economic development projects, the reduction of transmission afforded by ITNs may not be deemed sufficient. IRS could then be applied. Such situations could provide opportunities for assessing whether there is added benefit from combining IRS with ITNs.

• In some areas, it may be found that the population for one reason or another will not accept ITNs or use them correctly, or that the expected impact is not achieved. The causes of such problems should be ascertained, and if they cannot be remedied, IRS should be considered.

**Countries with a strong IRS tradition**

Countries with a strong IRS tradition, should review their malaria control strategy to assess whether the vector control interventions are well selected and well targeted. A transition from IRS to ITNs should be considered, but should not be automatic. There may be areas under IRS coverage, where transmission has become so low that insecticidal coverage can be withdrawn altogether and replaced with surveillance. Furthermore, ITNs may not always be better than IRS. Population fatigue, related to ITNs has not been observed in the same way as with IRS, but then the experience with the former is not as long.

Countries, which are approaching elimination on the basis of IRS, should normally continue to use IRS until elimination has been achieved, but may consider combining with other interventions.
Annex 1

The twelve insecticides recommended for IRS by WHO’s Pesticide Evaluation Scheme

The selection of insecticide for a given area is based on data on insecticide resistance, costs, safety, type of surface to spray and local experience. Different insecticides have different lengths of action. Of the ones currently in use, DDT has the longest action. On certain surfaces, especially after repeated applications, one spray cycle with DDT may cover for over a year. DDT remains the cheapest insecticide per kg and per m² sprayed, but because of its weight and bulk, it may be more expensive to use, where transport is costly. WHO has issued a position paper on DDT (WHO, 2004).

<table>
<thead>
<tr>
<th>Compound and formulation</th>
<th>Class</th>
<th>Dosage g/m²</th>
<th>Duration of effect (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-cypermethrin – WP; SC</td>
<td>P</td>
<td>0.02–0.03</td>
<td>4–6</td>
</tr>
<tr>
<td>Bendiocarb – WP</td>
<td>C</td>
<td>0.10–0.40</td>
<td>2–6</td>
</tr>
<tr>
<td>Bifenthrin – WP</td>
<td>P</td>
<td>0.025–0.050</td>
<td>3–6</td>
</tr>
<tr>
<td>Cyfluthrin – WP</td>
<td>P</td>
<td>0.02–0.05</td>
<td>3–6</td>
</tr>
<tr>
<td>DDT – WP</td>
<td>OC</td>
<td>1.0–2.0</td>
<td>3-18</td>
</tr>
<tr>
<td>Deltamethrin – WP</td>
<td>P</td>
<td>0.010–0.025</td>
<td>2–3</td>
</tr>
<tr>
<td>Etofenprox – WP</td>
<td>P</td>
<td>0.10–0.30</td>
<td>3–6</td>
</tr>
<tr>
<td>Fenitrothion – WP</td>
<td>OP</td>
<td>2.0</td>
<td>3–6</td>
</tr>
<tr>
<td>Lambda-cyhalothrin – WP</td>
<td>P</td>
<td>0.02–0.03</td>
<td>3–6</td>
</tr>
<tr>
<td>Malathion – WP</td>
<td>OP</td>
<td>2.0</td>
<td>2-3</td>
</tr>
<tr>
<td>Pirimiphos-methyl – WP; EC</td>
<td>OP</td>
<td>1.0–2.0</td>
<td>2–3</td>
</tr>
<tr>
<td>Propoxur – WP</td>
<td>C</td>
<td>1.0–2.0</td>
<td>3–6</td>
</tr>
</tbody>
</table>

WP = wettable powder, SC = suspension concentrate, EC = emulsifiable concentrate
C = carbamate, OC = organochlorine, OP = organophosphate, P = pyrethroid

Annex 2
A1: Current levels and trends in IRS use

Total number of households sprayed by country and region, 2000–2003

<table>
<thead>
<tr>
<th>Country</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFRICA</td>
<td>1897</td>
<td>2155</td>
<td>2451</td>
<td>2755</td>
</tr>
<tr>
<td>ASIA</td>
<td>2297</td>
<td>3167</td>
<td>2412</td>
<td>3052</td>
</tr>
<tr>
<td>AMERICAS</td>
<td>411</td>
<td>637</td>
<td>753</td>
<td>229</td>
</tr>
</tbody>
</table>

Only countries with complete reporting for 2000–2003 have been included.

Sources:
Africa and Asia: World Malaria Report, 2005
Americas: Unpublished data, PAHO/AMRO
The country that has the largest population covered by IRS has for many years been India:

**A2: Insecticide wise population targeted for spray during the past 5 years**

**Source:**
Background Material 2 from National Consultative Workshop on National Vector Borne Disease Control Programme, Delhi, 16\textsuperscript{th} March 2006
Annex 3

Summary conclusions and recommendations from the Report on the Study Group on Malaria Vector Control and Personal Protection convened by WHO in 2004

In areas where malaria vectors are fully susceptible to pyrethroids, side-by-side comparison of the same pyrethroid used for both methods against malaria transmitted by *An. gambiae* s.s. and *Anopheles funestus* showed very similar impact on the Entomological Inoculation Rate (EIR) of the vector population, incidence of malaria infection and malaria morbidity in children. A review of the remarkable results achieved in the 1950s, 1960s and 1970s with IRS, in highly endemic areas of Africa, shows that so far none of the recent ITN trials has done as well. However, IRS programmes were larger in scale than the relatively smaller ITN efficacy trials, so the comparison conflates two methods and scales of intervention.

Cost comparisons of IRS and ITNs yielded surprisingly variable results. The encouraging past results with IRS in tropical Africa did not lead to nationwide campaigns. It can be argued that this has been because in very low income countries, it is not possible to routinely meet the logistical demands of ensuring that trained spray teams equipped with working spray pumps and sufficient insecticide arrive at each village in time to spray before the malaria season. Furthermore, it can be argued, it is more feasible to supply ITNs in such circumstances because this does not impose similar logistics requirements.

Moreover, the experience of long-term use of IRS by organized anti-malaria campaigns in many parts of the world has frequently shown a progressive development of people’s fatigue and reluctance to allow intrusion into their homes. This phenomenon may be less likely to occur with the use of ITNs, which are far more under the control of households. In contrast, in rapid response to epidemics, there are good reasons to favour a trained and equipped IRS “fire brigade” capable of moving quickly to an area where there is a high likelihood of a malaria epidemic.

IRS and ITNs can be considered measures of almost general applicability, while other measures may be applicable in particular circumstances. The process of deciding about which mosquito control method is appropriate in
a given situation should be guided by an analysis of the level of malaria endemicity and vector bionomics, the eco-epidemiological setting, the health management system and an estimate of the programme sustainability.

The following recommendations should be taken into consideration in such selection:

• IRS should only be adopted if the necessary infrastructure exists or can be created to achieve and sustain high coverage and where local vectors are susceptible to the insecticides used.

• An ITN programme should aim for high coverage and use and should ensure that all mosquito nets are treated with insecticide, through regular free re-treatment or distribution of LLINs. Follow-up should be carried out to ensure continuous availability as well as regular and appropriate use of ITNs.

• Considering the magnitude of disease transmission, there is a need to combine vector control interventions in the context of integrated vector management. In specific circumstances larviciding, eventually associated with environmental management, might be a useful complement to IRS or ITNs.

Knowledge of both vector ecology and behaviour, will determine the choice of intervention to be used. This will include chemical control (use of adulticides and/or larvicides), a combination of methods (e.g. ITNs and IRS; ITNs and larviciding), mechanical control (house screening) and/or source reduction (e.g. drainage).
A3: Requirements for successful use of indoor residual spraying, insecticide-treated nets and larval control for malaria vector control

| Indoor residual spraying | • Indoor resting vectors (endophilic species)  
|                          | • Houses with walls and ceilings  
|                          | • Most malaria infections acquired indoors (endophagic species)  
|                          | • People not nomadic (permanent homesteads)  
|                          | • Willingness to accept spraying  
|                          | • Ability to organize the delivery of spraying on time to all malaria areas including information on number and location of houses to be sprayed |
| Insecticide-treated nets | • At least some of the vectors biting at hours when and where people are in bed  
|                          | • Willingness of people to use nets  
|                          | • An adequate delivery system for nets and insecticide including information on number and location of houses and sleeping units requiring nets  
|                          | • Ability to organize a net treatment programme free of charge or to switch to use long-lasting insecticidal nets |
| Larval control           | • Breeding in semi-permanent sites  
|                          | • Ability to locate and map out a very large proportion of the breeding sites within mosquito flight range of the community which it is required to protect  
|                          | • Proper selection of anti-larval measures (e.g. use of larvivorous fish, bacteria, oiling)  
|                          | • Community participation for mosquito breeding sites reduction and/or elimination |
### A4: Effectiveness and challenges of implementing malaria vector control and personal protection strategies in the context of various eco-epidemiological types

<table>
<thead>
<tr>
<th>Eco-epidemiological type</th>
<th>Recommendation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>African Savannah</td>
<td>ITNs are the preferred option, but the use of IRS in special situations is not excluded.</td>
<td>Urgent need for strengthening implementation to achieve high coverage of ITNs.</td>
</tr>
<tr>
<td>Plains and valleys outside Africa</td>
<td>IRS has worked well, but may meet operational difficulties; ITNs should be considered as replacement.</td>
<td></td>
</tr>
<tr>
<td>Forest and forest fringes in Asia and the Americas</td>
<td>ITNs are the preferred option.</td>
<td>ITNs are not universally effective, mainly because of human ecology. In some situations, novel tools such as hammock-nets should be tried.</td>
</tr>
<tr>
<td>Highland and desert fringes</td>
<td>IRS is usually best option to curb epidemics, but choice should be made according to local situation.</td>
<td>It is important not to waste resources on vector control, where transmission is minimal or absent most of the time.</td>
</tr>
<tr>
<td>Wetland and coastal areas</td>
<td>The basic method should be ITNs, but limited IRS with stable insecticides is justified in areas with special problems. Environmental management may play an important role.</td>
<td>Combined measures are often warranted.</td>
</tr>
<tr>
<td>Urban and peri-urban areas</td>
<td>IRS is often used, but in many cases it could be replaced by ITNs, as the mosquito nuisance motivates people.</td>
<td>Attention must be given to development of resistance by nuisance insects.</td>
</tr>
<tr>
<td>Agricultural development projects</td>
<td>IRS and ITNs may be used.</td>
<td>Environmental management should be explored.</td>
</tr>
<tr>
<td>Socio-political disturbances</td>
<td>IRS is often useful in the acute phase.</td>
<td>Novel approaches have shown promising results, particularly insecticide-treated tents, tarpaulins, blankets, top-sheets and insect repellents.</td>
</tr>
</tbody>
</table>
Annex 4


Excerpt from Executive Summary

Five Insecticide Treated Nets (ITN) programs and two Indoor Residual Spraying (IRS) programs operating at large or national scale in sub-Saharan Africa were assessed comparatively using a standardised methodology. The ITN programmes were chosen to represent the major distribution systems being currently used in sub-Saharan Africa. The two IRS programs were chosen based on scale and accessibility of cost data.

The ITN programs represented the following models:
- Free ITN delivery through routine services and at the community level (Eritrea)
- Free ITN delivery through integrated vaccination campaigns (Togo)
- Highly subsidized ITN delivery through routine services in the frame of a social marketing program (Malawi)
- Largely commercial sector promotion (Senegal)
- Commercial sector promotion through social marketing, combined with vouchers to reach high-risk groups (Tanzania).

The two IRS programs represented:
- A national program funded locally (KwaZulu-Natal, South Africa)
- An international intervention funded by donors and a public-private partnership (Lubombo Spatial Development Initiative, Southern Mozambique).

Costs were measured locally or derived from existing studies and focused on the provider perspective, supplemented by direct costs to users for net procurement. Effectiveness was measured by combining outputs (number
of nets distributed, person-years of protection) with standard impact indicators derived from the existing Cochrane review on ITNs. Effectiveness for IRS was based on the same data because of (1) lack of IRS-specific data, and because (2) past research showed the efficacy of the two interventions to be similar in African settings.

Conventional ITNs:
Average annual economic costs per net distributed varied by about a factor of 3 across sites, i.e. from USD 3.23 per net in Togo to USD 8.05 in Senegal. The cost per net-year of protection ranged from USD 1.43 in Eritrea to USD 6.05 in Senegal. When re-treatments were not included, to make results more comparable (re-treatment rates are highly variable between countries), the cost per DALY (Disability Adjusted Life Years) averted ranged from USD 37 to USD 89, and the cost per death averted from USD 1,174 to USD 2,926 (in Togo and Senegal, respectively). When re-treatments were included, the cost-effectiveness varied from USD 16 to USD 67 per DALY averted and from USD 521 to USD 2,199 per death averted.

Long Lasting Insecticidal Nets (LLIN) with three years protection and a USD 5 price: Average annual economic costs per net distributed were significantly higher than for conventional nets because of the higher purchasing price of the nets, ranging from USD 3.47 in Togo to USD 7.64 in Eritrea. But the cost per treated-net year of protection was generally significantly lower, ranging from USD 1.48 in Eritrea to USD 2.64 in Senegal. As a result, the cost per DALY averted ranged from USD 16 in Eritrea to USD 29 in Senegal and the cost per death averted ranged from USD 539 to USD 960.

Long Lasting Insecticidal Nets (LLIN) with five years protection and a USD 7 price: Average annual economic costs per net distributed were again higher than for conventional nets, ranging from USD 3.23 in Togo to USD 7.78 in Eritrea. The cost per treated-net year of protection was even lower than for a 3-year LLIN, ranging from USD 1.18 in Eritrea to USD 1.90 in Togo. As a result, the cost per DALY averted ranged from USD 13 in Eritrea to USD 21 in Togo and the cost per death averted ranged from USD 431 to USD 692.
IRS: The costs per person-year protection (for all ages) were USD 3.27 in KwaZulu Natal (KZN) and USD 3.90 in Mozambique. This is clearly higher than the 3-year and 5-year LLIN options and indicates that the implementation costs for IRS are in any case greater, even when the whole population is targeted. If only children under five years are included in the denominator (because the vast majority of expected benefits accrue due to prevented child mortality), the cost per person-year of protection becomes substantially higher for IRS: USD 23.96 (KZN) and USD 21.63 (Mozambique). As a result, the costs per DALY averted (USD 119-132) and per death averted (USD 3,933-4,357), were much higher for IRS compared to LLINs in the presence of targeting.

Sensitivity analysis for ITN programmes indicated that the parameters with the largest effects on overall cost and cost-effectiveness ratios were: the cost of the nets, the length of protection offered by ITNs (or re-treatment kits), and usage rates of nets by children. These results suggest the clear potential for improving the cost-effectiveness of programs, by switching to long-lasting insecticidal nets (LLINs) and/or stronger netting, targeting nets to highly vulnerable groups, and heeding the importance of behavioral factors associated with net programs. The cost of nets represented approximately 60% of the overall costs.

For IRS programs, sensitivity analysis revealed that the most important parameters were the compliance/acceptability of spraying, insecticide choice, and the number of spraying rounds required per annum. For IRS programs, insecticide and staff costs accounted for the largest share of overall economic costs.

Clearly, some strategies are more cost-effective than others, but each strategy also brings specific advantages, both in the short and long-term. It is the consideration of these many factors, based on the cost-effectiveness results presented in this report, which should guide national planners.

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Insecticide resistance in malaria vector mosquitoes in a gold mining town in Ghana and implications for malaria control.

M. Coetzee (1, 2), P. van Wyk (3), M. Booman (4, 5), L. L. Koekemoer (1, 2) & R. H. Hunt (1, 5)

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Summary: Malaria control programmes in Africa, for the most part, address only treatment of the disease and supply of insecticide treated bed nets. The impact of these restricted programmes has been limited and new approaches are being advocated, including integrated vector management strategies and partnerships with industry. Mosquito surveys were carried out for AngloGold/Ashanti in preparation for their implementation of an integrated malaria control programme at the Obuasi gold mine in Ghana. Malaria vectors that were collected inside houses were identified to species and molecular forms by PCR, and tested for insecticide resistance using standard WHO bioassays and molecular target site insensitivity (kdr) assays. Species were identified as An. funestus s.s. and An. gambiae S and M forms. The An. gambiae S form samples showed resistance to DDT, pyrethroids and carbamates while An. funestus was resistant to DDT and carbamates. The An. gambiae M form occurred in very low numbers and
could not be assessed reliably for resistance. The standard PCR assay for detection of the kdr mutation in An. gambiae S form showed little association with pyrethroid resistance. Subsequent sequencing of the IIS6 domain containing the kdr mutation from nine surviving mosquitoes showed that eight were homozygous resistant and one heterozygous. This correlated with the bioassay results and with previous studies on West African An. gambiae, but raised concerns about the reliability of the PCR assay for detection of the kdr mutation. As a result of these investigations AngloGold/Ashanti are implementing, in addition to treatment and case management, a vector control programme that includes insecticide resistance management by alternation of various classes of insecticides for house spraying, supply of ITNs, screening of houses and environmental management where appropriate, i.e. integrated vector management.

Introduction

Insecticide resistance in African malaria vector mosquitoes is becoming an increasing problem for vector control programmes. A recent malaria epidemic in South Africa was caused by the presence of insecticide resistant Anopheles funestus (Hargreaves et al., 2000; Coetzee, 2005). The efficacy of insecticide treated bed nets has been assessed in the face of widespread pyrethroid resistance in An. gambiae in West Africa with varying results (Chandre et al., 2000; N’guessan et al., 2001). It is therefore prudent for any new initiative to assess the resistance status of the local vector mosquitoes, before deciding on strategies for malaria vector control.

The Health Ministry of Ghana, rates malaria as the number one cause of death amongst children in the country. Approximately three million Ghanaians are affected by malaria annually. Malaria is not a notifiable disease but information regarding morbidity and mortality are collected at health facility level. The use of ITNs, early treatment, education and environmental sanitation are the main control measures against malaria in Ghana. In 2003 only 3.5 % of children under five and 2.2% of pregnant women slept under bed nets (Ministry of Health, Ghana, unpublished report, 2003).

Obuasi sub district is situated in south-west Ghana in the district of Adansi-West. The 2003 unpublished Ministry of Health annual report from this
district indicated that malaria was one of the major health concerns (figure 2.1).

2.1: Malaria incidence rate (clinical cases) by sub-district, Adansi-West, Ghana, 2001-2003

Obuasi has a population of 173,447 people who are mostly dependent on the AngloGold/Ashanti operation for their income. Other economic activities are farming and minor trading. The crops cultivated include cocoa and citrus. During 2003, only 1000 nets were sold in the Obuasi sub-district but according to the annual report, plans are underway to strengthen seven identified bed net sales points in the district. The majority of uncomplicated (83%) and complicated (89%) malaria cases were in the age group 5 years and older. Severe anaemia was predominantly (65%) in the older age group. Data for age specific incidence rates were not available. These disease profiles are not typical of tropical Africa and have a marked impact on the workforce of the mine and the local economy in general. The malaria cases and case fatality rate at the AngloGold/Ashanti hospital for 2003 were on average over 6000 and 17 per month respectively (unpublished data). This has considerable impact on the productivity of the mine personnel.

The aim of the Anglogold/Ashanti malaria control programme at Obuasi is to implement an integrated strategy to reduce transmission of *Plasmodium falciparum*. Specific aims of the vector control programme are to implement an Integrated Vector Management (IVM) programme. This includes Indoor Residual Spraying (IRS) with an appropriate insecticide, screening of houses, supply of Insecticide Treated bed Nets (ITNs), selective larviciding...
and environmental management where appropriate. In order to do this, base line surveys of the mosquito populations were carried out.

Insecticide resistance studies of malaria vector mosquitoes in Ghana have been limited in their scope. Kristan et al. (Kristan et al., 2003) carried out bioassay studies on populations of An. Gambiæ complex from south-western Ghana and showed resistance to both DDT and permethrin but did not correlate these results with presence or absence of the West African \textit{kdr} allele. Yawson et al. (Yawson et al., 2004) on the other hand, at 11 localities from coastal to far northern Ghana reported on the occurrence and distribution of the \textit{kdr} mutation in \textit{An. gambiae} complex mosquitoes but did not carry out insecticide bioassays.

The results of baseline surveys of mosquito populations at Obuasi, Ghana, for species composition, infectivity, insecticide susceptibility and \textit{kdr} assays are presented here.

\textbf{Material and Methods}

\textit{Study Site}

The Obuasi area (06°15′N, 01°36′W) is characterized by its equatorial climate and hilly terrain greatly modified by mining activity and urban development. Mine personnel are to some extent housed in accommodation provided by the mine in housing clusters scattered within Obuasi town, but a fair proportion live amongst the general town and peri-urban population. Drainage in the town to remove liquid waste is via surface drains on the side of the roads. Some of the better maintained sections have well constructed concrete drains, in some instances covered with concrete slabs. In many areas the drains are damaged, creating swampy areas.

\textit{Collections}

The survey was conducted during the dry season in April 2004 and again when the rains started in June 2004. Houses were searched and resting mosquitoes were collected by hand. Samples of wild females were exposed to insecticides in the field, while sub-samples were returned to the laboratory in Johannesburg for rearing of F-1 progeny and further analysis.
Insecticide Susceptibility Tests

The standard WHO susceptibility tests were conducted. Adults from the April collections were exposed for one hour to 4% DDT, 0.05% deltamethrin, 0.1% bendiocarb and 5% malathion. The June collections were exposed for one hour to 0.05% lambda-cyhalothrin, 0.15% cyfluthrin, 0.15% etofenprox, 0.1% propoxur, and for two hours to 1% fenitrothion. Dead and alive mosquitoes were stored separately on silica gel for molecular analysis and sporozoite infectivity tests.

Species Identification

The *Anopheles gambiae* complex was identified initially by morphology (Gillies et al., 1987), to species level by PCR assay (Scott et al., 1993) and to M and S molecular forms by the PCR method of Favia et al. (Favia et al., 1997). The *An. funestus* group was identified using the method of Koekemoer et al. (Koekemoer et al., 2002).

Molecular assay for knockdown resistance (kdr)

The mutation responsible for knockdown resistance to pyrethroids in West Africa was described by Martinez-Torres et al. (Martinez-Torres et al., 1998) and this methodology was modified here for assaying members of the *An. gambiae* complex. Two independent PCR runs were set up for each sample: the first contained the primers Agd2 + Agd4 to identify the susceptible allele and the second contained primers Agd1 + Agd3 identifying the resistant allele.

Sequence analysis of the IIS6 domain

The 293 bp fragment of the IIS6 domain containing the *kdr* mutation was amplified from 9 mosquitoes using primers Agd1 and Agd2 (Martinez-Torres et al., 1998). This fragment was sent to Inqaba Biotechnical industries, South Africa, for sequencing.
ELISA for sporozoite assay for *P. falciparum* infectivity

The infectivity rates of female mosquitoes were tested using the enzyme-linked immunosorbent assay (ELISA) (Wirtz et al., 1987).

**Results**

Mosquito surveys carried out in April and June 2004, showed the presence of three anopheline species resting inside houses: *An. gambiae* (“S” form predominated with very few “M” form identified), *An. funestus* and a small number of *An. pharoensis*. Both the former species were found infected with *P. falciparum* but not the latter, based on ELISA (table 2.1).

Resistance, as determined by the standard WHO susceptibility tests, to three classes of insecticides (pyrethroids – 46.4% mortality, organochlorines – 30.8% and carbamates – 45.3%) was demonstrated in *An. gambiae* (table 2.2), with 100% susceptibility shown only to organophosphates.

In *An. Funestus*, resistance to two classes (organochlorines – 60.9% mortality and carbamates – 71.4%) was shown, with full susceptibility to pyrethroids and organophosphates (table 2.3).

**Table 2.1: Species composition and *Plasmodium falciparum* infectivity rates in malaria vector species at Obuasi.**

*Composition des espèces et taux d'infestation du Plasmodium falciparum chez les espèces de vecteur du paludisme à Obuasi.*

<table>
<thead>
<tr>
<th>Date</th>
<th>species</th>
<th>Number identified</th>
<th>Number tested for P. Falciparum (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2004</td>
<td><em>An. gambiae</em> ‘S’ Form</td>
<td>111</td>
<td>92 (4.35)</td>
</tr>
<tr>
<td></td>
<td><em>An. funestus</em></td>
<td>152</td>
<td>221 (1.81)</td>
</tr>
<tr>
<td></td>
<td><em>An. pharoensis</em></td>
<td>12</td>
<td>12 (0)</td>
</tr>
<tr>
<td>June 2004</td>
<td><em>An. gambiae</em> ‘S’ form</td>
<td>175</td>
<td>175 (5.5)</td>
</tr>
<tr>
<td></td>
<td><em>An. funestus</em></td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>An. pharoensis</em></td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2.2: Insecticide susceptibility tests of *An. gambiae* S form from Obuasi.

*Tests de sensibilité aux insecticides d’*An. *gambiae* *forme S à Obuasi*  

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>number tested</th>
<th>% 24-hr mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deltamethrin</td>
<td>54</td>
<td>75.9</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>27</td>
<td>12.5</td>
</tr>
<tr>
<td>Etofenprox</td>
<td>21</td>
<td>57.1</td>
</tr>
<tr>
<td>DDT</td>
<td>26</td>
<td>30.8</td>
</tr>
<tr>
<td>Bendiocarb</td>
<td>39</td>
<td>56.4</td>
</tr>
<tr>
<td>Propoxur</td>
<td>38</td>
<td>34.2</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>89</td>
<td>100</td>
</tr>
<tr>
<td>Malathion</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2.3: Insecticide susceptibility tests of *An. funestus* from Obuasi, Ghana.


<table>
<thead>
<tr>
<th>Insecticide</th>
<th>number tested</th>
<th>% 24-hr mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deltamethrin</td>
<td>53</td>
<td>100</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>DDT</td>
<td>23</td>
<td>60.9</td>
</tr>
<tr>
<td>Bendiocarb</td>
<td>56</td>
<td>71.4</td>
</tr>
<tr>
<td>Malathion</td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2.4: Kdr gene involved in insecticide resistance in *An. gambiae* S form from Obuasi.

*Gène Kdr impliqué dans la résistance aux insecticides chez An. gambiae forme S à Obuasi.*  

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Kdr</th>
<th>% 24-hr mortality (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deltamethrin</td>
<td>S.S</td>
<td>69.2 (13)</td>
</tr>
<tr>
<td></td>
<td>RS</td>
<td>90.7 (43)</td>
</tr>
<tr>
<td></td>
<td>RR</td>
<td>66.7 (6)</td>
</tr>
<tr>
<td>DDT</td>
<td>S.S</td>
<td>75.0 (8)</td>
</tr>
<tr>
<td></td>
<td>RS</td>
<td>50.0 (4)</td>
</tr>
</tbody>
</table>
Table 2.5: Kdr mutation in Deltamethrin survivors determined by sequencing and the Martinez-Torres PCR assay (Martinez-Torres et al., 1998).

Mutation du Kdr chez les survivants au deltamethrin déterminé par le séquençage et les dosages de la PCR Martinez-Torres (Martinez-Torres et al., 1998).

<table>
<thead>
<tr>
<th>Kdr Martinez-Torres</th>
<th>mortality/survival (n)</th>
<th>Kdr Sequencing (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.S</td>
<td>survived (2)</td>
<td>RR (2)</td>
</tr>
<tr>
<td>RS</td>
<td>survived (4)</td>
<td>RR (4)</td>
</tr>
<tr>
<td>RR</td>
<td>survived (2)</td>
<td>RR (1) RS (1)</td>
</tr>
<tr>
<td>RR</td>
<td>died (1)</td>
<td>RR (1)</td>
</tr>
</tbody>
</table>

Molecular assays for the knockdown resistance mutation (kdr), showed no correlation with the bioassay data (table 2.4) with both survivors and susceptible being found positive for kdr in each of the three classes RR, SS and RS. However, sequencing of the kdr fragment showed good correlation with the bioassay data (table 2.5).

Discussion

The difference in species composition between the two collections in April and June 2004 was marked. The dry season vector populations were largely excluded from the town, probably due to the highly polluted state of the surface water. However, large populations, particularly of An. funestus, could be found just outside the urban area. The predominance of this species in the dry season is because it is dependent on permanent breeding sites such as swamps and ponds. Anopheles gambiae, dependent on temporary sunlit pools of rain water, was understandably in the minority. In June the picture was very different with An. gambiae predominating and breeding in large numbers in rain puddles, wheel ruts, etc. Such breeding sites were common in many parts of the town and mine housing complexes, resulting in generalized mosquito populations particularly in the low lying parts of the town.

In the two communities, that were sampled to obtain mosquitoes for the susceptibility tests, the use of mosquito coils was much more common during the rains than in the dry season. This was because there were many
more mosquitoes, making the expense worthwhile. In fact, this reduced the populations to a level that made it difficult to obtain meaningful samples in June, which was not the case in the dry season in April.

The bioassay mortality results for *An. gambiae* S form are markedly lower than those reported in Kristan *et al.* (Kristan *et al.*, 2003). Twenty-four hour mortality for deltamethrin at Obuasi was 75.9% while it was >97% in the Kristan study and on DDT was 30.8% at Obuasi compared with >94% in the Kristan study. Resistance levels in mosquito populations may vary with locality and usage of insecticides in agriculture and the home. But, it is probable that these levels also vary with season, as mosquito populations vary in numbers with the increasing or diminishing availability of larval breeding habitats.

The marked lack of *An. funestus* in the rainy season was unexpected considering its utilization of permanent breeding sites, but could be explained by the swamps being filled to overflowing by rainfall and thus washing out mosquito larvae from their preferred habitat. We have no data to support this speculation, but cannot otherwise explain the decrease in numbers of *An. funestus* in the rainy season.

Most studies to date show strong correlation between the *kdr* mutation and bioassay insecticide resistance in *An. Gambiae* in West Africa (Chandre *et al.*, 2000; Chandre, Darriet *et al.*, 1999; Chandre, Manguin *et al.*, 1999). Chandre *et al.* (Chandre *et al.*, 2000) in Cote-d'Ivoire showed not only positive correlation of the *kdr* mutation with the resistance phenotype, but also demonstrated that the trait was incompletely recessive. They suggested that the continued efficacy of insecticide treated bed nets against resistant mosquitoes was due to either the continued killing effect of the nets because of prolonged contact with pyrethroids during blood seeking, and/or relatively few *kdr* females able to take blood meals after prolonged contact with the nets. These studies have led to the assumption that all *An. Gambiae* populations in West Africa will display similar correlations of resistance phenotype to the *kdr* allele. Yawson *et al.* (Yawson *et al.*, 2004), carrying out *kdr* analysis on *An. gambiae* from Ghana, unfortunately did not correlate their results with bioassay data but only with the molecular M and S forms.
We carried out the Martinez-Torres PCR assay (Martinez-Torres et al., 1998) in two steps in order to maximize accuracy of the assay. Results showed that *An. gambiae* S form was both resistant to pyrethroids/DDT and had the *kdr* mutation, but that these two conditions were not correlated (table IV). Some mosquitoes that survived on either deltamethrin or DDT were found to be homozygous susceptible for the *kdr* allele, while some individuals homozygous for resistant *kdr* were found to be susceptible to deltamethrin. Heterozygotes on deltamethrin gave a high mortality supporting incomplete recessiveness (Chandre et al., 2000), while the DDT exposures did not. Sequencing of the *kdr* fragment of the IIS6 domain from nine individuals that survived exposure to pyrethroids showed close correlation between the mutation and resistance phenotype, contrary to data from the Martinez-Torres PCR assay (table V). These results highlight a problem with the standard PCR assay and either this assay must be modified to produce more reliable results or a new technique is needed.

The infection rates with *P. falciparum* of both *An. Gambiae* and *An. funestus* are typical of those found in West Africa (Gillies et al., 1968) with *An. gambiae* being a better vector than *An. funestus*.

**Conclusion**

The results presented here have been used in planning the malaria control programme for the Anglogold/Ashanti mine in Obuasi. An initial indoor residual spray round with an organophosphate is being implemented in early 2006. Integrated Vector Management (IVM) through supply of ITNs, screening of houses, larviciding and environmental management, forms the basis of the vector control programme. Monitoring and surveillance of the mosquito populations will be carried out and decisions on rotation of insecticides and further IVM interventions will be made based on future results.

**Acknowledgements**

Personnel at the Obuasi mine, who assisted with logistical issues and mosquito collections, are thanked for their help. Dr R. Wirtz, CDC Atlanta, USA, is thanked for the supply of the ELISA reagents for detection of *P. falciparum.*
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Monitoring Resistance and Maximising Insecticide Effectiveness

OVERVIEW

This section presents the principles, regulations and issues that the National Environment Management Authority (NEMA) considered before approving the use of DDT and other pesticides in Uganda; and it looks in detail at the factors that drive pesticide effectiveness with a focus on DDT. DDT was singled out because it is among the 12 Persistent Organic Pollutants (POPS) identified in the Stockholm Convention and because of the controversy surrounding its use. Although the author acknowledges the risks involved with using POP compounds, he still advocates the use of DDT in IRS to control malaria given the magnitude of the problem in Uganda. But introduction of POPS necessitates a strong environmental monitoring system. The environmental management tools used by NEMA are based on the precautionary principle (international legal instruments, national legislation and standards, and Environmental Impact Assessment). These are tools intended for prevention versus the polluter pays principle (environmental inspection, Environmental Audits, and technical infrastructure for environmental monitoring), which utilizes tools for detection of a problem before taking corrective action. Therefore, national monitoring of DDT is based on monitoring compliance with international obligations, national laws and conditions for approval of the insecticide. The second paper focuses on how to maximize the effectiveness of DDT and other insecticides. The drivers of effectiveness are categorised into intrinsic and external drivers. Intrinsic drivers are those that relate to the internal properties of the pesticides that kill the vector (quality of pesticide, length of residual action in relation to length of disease transmission). External drivers relate to the overall effectiveness of the pesticide (probability of vector coming in contact with the pesticide, vector resistance
to the pesticide, timeliness of spraying in relation to timing and intensity of transmission, compliance to best practices by spray operators and adherence among the targeted household). These drivers can be enhanced to ensure effectiveness. The author also emphasises the importance of an effective pesticide management system to facilitate judicious use and continued utility of insecticides.

Monitoring DDT in the Environment

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National Environment Management Authority, Kampala, Uganda

Introduction
DDT is among the 12 Persistent Organic Pollutants (POPs) that were identified as requiring immediate control action under the Stockholm Convention. POPs are organic compounds that, to a varying degree, resist photolytic, biological and chemical degradation. POPs are often halogenated and characterized by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues. POPs are also semi-volatile thus being able to move long distances in the atmosphere before disposition occurs. Highly stable POP compounds can last for many years or decades in the environment before breaking down.

Proposed re-introduction of DDT

Uganda experiences malaria all year round although the intensity of the disease is varied from region to region. The cases have continued to increase since launching Roll Back Malaria (RBM) in 1998 from 5.5 to 12 million in 2005. This has been mainly due to insufficient action to break the transmission cycle. A multi-faceted approach utilizing all available strategies and interventions is therefore required to control malaria in Uganda, including IRS using DDT.

Environmental Monitoring

According to the National Environment Act Cap 153, environmental monitoring means the continuous determination of actual and potential
effects of any activity or phenomena on the environment, whether short term or long term.

**Environmental Monitoring Tools**

Environmental monitoring tools include, tools that are intended for prevention (based on the Precautionary Principle) and those that are intended to detect the problem when it occurs and then take corrective action (based on Polluter Pays Principle).

**Precautionary Principle**

**International Legal Instruments Relevant to DDT**

*The Stockholm Convention*

Under the Stockholm Convention, production and/or use of DDT is restricted for disease vector control, in accordance with the World Health Organisation recommendations and guidelines on the use of DDT. This is when locally safe, effective and affordable alternatives are not available.

In the controls on DDT, Parties are obliged to among others:

- Restrict production and/or use of DDT for disease vector control in accordance with the World Health Organisation recommendations and guidelines on the use of DDT.
- Notify the Secretariat of the Stockholm Convention and the World Health Organisation (WHO) if they decide to use DDT for disease vector control to have their names added to the DDT Register.
- Provide the Secretariat of the Stockholm Convention and the World Health Organisation with information on the amount used, the conditions of such use and its relevance to that Party’s disease management strategy.

The production and/or use of DDT for disease vector control is further controlled by requiring Parties, with the goal of reducing and ultimately eliminating the use of DDT, to;
• Develop and implement an action plan on DDT as part of the National Implementation Plan (NIP) including:
  o A regulatory and other mechanisms to ensure that DDT use is restricted to disease vector control;
  o Implementation of the use of suitable alternative products, methods and strategies, including resistance management strategies to ensure continuing effectiveness of these alternatives; and
  o Measures to strengthen health care and to reduce the incidence of the disease.

• To promote research and development of safe alternative chemical and non-chemical products, methods and strategies for Parties using DDT, relevant to the conditions of those countries and with the goal of decreasing the human and economic burden of disease.

The Conference of the Parties is mandated, in consultation with the World Health Organisation, to evaluate the continued need for DDT for disease vector control on the basis of available scientific, technical, environmental and economic information every three years, and pay special attention to:

• The production and use of DDT;
• The availability, suitability and implementation of the alternatives to DDT; and
• Progress in strengthening the capacity of countries to transfer safely to reliance on alternatives.

Article 6 of the Convention, obliges Parties to ensure that stockpiles and waste consisting of POPs, are managed in a manner protective of human health and the environment by developing appropriate strategies for managing stockpiles in a safe, efficient and environmentally sound manner. Under the enabling activities for the Stockholm Convention coordinated by NEMA, Uganda is finalizing undertaking an inventory on stockpiles and contaminated sites of POPs and pesticides in general to develop actions for the management of stockpiles and remediation of contaminated sites during the National Implementation Plan (NIP) process.

*Rotterdam Convention on the Prior Informed Consent Procedure for certain Hazardous Chemicals and Pesticides in International Trade*
The Rotterdam Convention seeks to encourage environmentally sound management of certain hazardous chemicals and pesticides in International Trade, through provision and sharing of accurate information on their characteristics, potential dangers and safe handling and use. This applies to DDT, because its production and use is severely restricted in a number of Party States.

Prior Informed Consent (PIC) is a procedure aimed especially at helping developing countries, many of which find difficulties to monitor and control imports. Under this Convention, when a State Party decides not to import a particular chemical or pesticide covered by the PIC procedure, other State parties agree not to export this product to that country. If a country decides to limit the importation of a chemical to certain uses, exporting countries agree to respect those limits. Uganda is at the final stages of ratifying this Convention.

The Basel Convention

The Basel Convention seeks to regulate the trans-boundary movement of hazardous wastes, by providing obligation to its parties to ensure that such wastes are disposed of in an environmentally sound manner. One of the main principles of the Convention is that the hazardous waste should be treated and disposed of as close as possible to their source of generation. In addition, the Basel Convention urges that the generation and movement of hazardous waste should be minimized. The Convention is relevant to handling of DDT waste when disposal facilities, in an environmentally sound manner are not available, within the Party State.

National Legislation and Standards

The National Environment Act (Cap 153)

Section 52 of the National Environment Act, imposes a duty to every person to manage any waste generated by their activities or of those persons working under their direction, in such a manner that does not cause ill health to persons or damage to the environment.
Following Uganda’s ratification of the Basel Convention in 1999, her obligations were transformed into national requirements through the formulation and operationalisation of the National Environment (Waste Management) Regulations, 1999.

The Regulations prohibit import of Hazardous waste into the country and provide for a licensing mechanism for persons involved in the storage, transportation and disposal of hazardous waste generated locally.

**Environmental Impact Assessment and Post EIA Monitoring**

*Environmental Impact Assessment (EIA)*

The National Environmental Act Cap 153 (Section 19) provides that projects listed in the third Schedule to the Act undergo the Environment Impact Assessment (EIA) process before implementation. The Environmental Impact Statement (EIS) documents commitments from the developers (in this case Ministry of Health) of a project for the mitigation of negative impacts of the project to the Environment. Subsequently, the tool is important for post EIA environmental monitoring, based on the Environmental Monitoring and Management Plan. The Environmental Impact Assessment for DDT re-introduction for Indoor Residual Spraying (IRS) was conditionally approved by NEMA.

**Polluter Pays Principle**

*Environmental Inspections* 

Section 2 of the National Environment Act Cap 153, provides that NEMA ensures the principles of environment management are observed. Section 2, subsection 2, states the principles of environmental management to include among others:

- Providing adequate environmental protection standards and monitoring changes in environmental quality
- Requiring prior environmental assessments of the proposed projects, which may significantly affect the environment or use of natural resources
- Ensuring that the true and total costs of environmental pollution are borne by the polluter

The Act also states that regular environmental inspections will apply to Internal Residual Spraying (IRS) of DDT.

**Environmental Audits**

The purpose of environmental audits is to establish the environmental performance of an existing project. If any weaknesses are identified they are addressed. The Regulations for Environmental Audits are in place. Environmental Audit in IRS for DDT, can apply to evaluate compliance with WHO Guidelines and the implementation of guidelines on Indoor Residual Insecticides Spraying (IRS), for national and district level managers. Further, audits can be undertaken, to evaluate compliance with the EIA mitigation measures and conditions of EIA approval.

**Technical infrastructure for Environmental Monitoring**

Presently, studies for determination of occurrence, concentrations and trends of DDT in humans, foodstuffs and the environment are not many. Some studies are being undertaken to obtain data from background and potential hot-spot contamination sites, for a very preliminary risk assessments under the Enabling Activities for the Stockholm Convention. Existing analytical laboratories (in the field of chemistry) are being used to conduct residue analysis and monitor possible adverse effects. Clinical studies have also been undertaken in western Uganda. [UNAS 2007]

**Conclusion**

Monitoring of DDT requires monitoring of compliance with international obligations, national laws, commitments and conditions of EIA approval as well as environment management systems for indoor residual insecticide spraying (IRS) and DDT residue in the components of the environment.
REFERENCES

The National Environment Act (Cap 153).
The National Environment (Standards for Discharge of Effluent into Water or on Land) Regulations, 1999.

Maximizing the effectiveness of DDT and other insecticides

Jacob Williams
Research Triangle Institute

Purpose of IRS

The purpose of IRS is to kill the female Anopheles mosquito, before the part of the life cycle of Plasmodium in the vector is completed. Specifically, before the mosquito become infective. By targeting the mosquito vector, IRS is aimed to reduce vector-man contact and therefore reduce transmission and ultimately to lower the malaria burden.

Drivers of pesticide effectiveness

The effectiveness of a pesticide is driven by a number of factors, which may be broadly categorized as either intrinsic or external.

Intrinsic drivers relate to the internal properties of the pesticide that elicit the desired goal of killing the targeted mosquito:

- *The quality (specification) of the pesticide*. An incorrect specification and concentration of the active ingredient may result in an under-dosage of the pesticide that is deposited on the wall, and may lead to intervention failure.

- *The length of the elicited residual action of the pesticide, in relation to the length of disease transmission*. The period of residual action
is a function of the half-life of the active ingredient, the formulation of the pesticide, as well as the behaviour of the active ingredients on different wall substrates.

**External drivers** that impact on the overall effectiveness of a pesticide used for IRS include:

- *The probability of the vector coming into contact with the pesticide.* The mosquito vectors that are targeted should predominantly exhibit endophagic (indoor feeding) and endophilic (indoor resting) behaviour.
- *Vector resistance to the pesticide.* Resistance is usually due to the development of physiological changes in the vector. A lesser well known phenomenon is behavioural resistance. This comes about as a result of changes in mosquito behaviour to avoid contact with the pesticide. *An. arabiensis* has been demonstrated to show significant tendency for exophagy (outdoor feeding), postprandial exophilic behaviour (tendency to exit and rest outdoors following an indoor blood meal) and an avoidance of DDT-sprayed surfaces after blood meals (Ameneshwa et al., 1996). Exophilic tendencies may result from long-term use of excito-repellant insecticides such as DDT and pyrethroids.
- *The timeliness of spraying in relation to the timing and intensity of transmission.* Usually, spray operations coincide with the onset of seasonal transmission or the seasonal peaking in malaria, which is linked to the upsurge in mosquito vector populations with rainfall. A delay in the timing of IRS may undermine the ability of the pesticide to disrupt the transmission of malaria. The higher the transmission intensity, the more critical the timing of the IRS.
- *Compliance of the spray operator with best practices* such as ensuring the use of the right amount and concentration of pesticide and employing appropriate spraying techniques.
- *Adherence among the targeted households,* such as avoiding the re-plastering or washing of walls. IRS requires good public education and information.
Maximizing the drivers of pesticide effectiveness

The intrinsic and external factors can be manipulated to enhance the overall effectiveness of the pesticides.

Internal drivers

- The residual activity of a pesticide may be increased by improving its formulation and application technologies. For example, the wettable powder formulation of lambda-cyhalothrin (ICON WP) exhibits a residual activity of between 3-6 months, while the newer suspension concentrate formulation (ICON CS) has residual activity of at least 6 months (WHO, 2007). Since the length of residual action varies with the type of wall substrate, the actual length of new pesticide formulations has to be validated within the local setting. Furthermore, to provide the desired protection, the residual activity of the pesticide should occur throughout the whole period of malaria transmission. It may therefore be necessary to repeat the spray operation, in situations where the length of the residual action is shorter, than the period of malaria transmission.
- Sound pesticide registration procedures (including vendor licensing), and procurement procedures that stipulate purchasing from legitimate and certified sources would help to assure pesticide quality. Procured pesticides must meet World Health Organisation Pesticide Evaluation Scheme (WHOPES) specifications. Ideally, routine sampling should be established to verify the specifications of an imported pesticide. However, most malaria endemic countries do not have the capacity, in terms of financial and other resources, to carry out routine pesticide sampling. In such circumstances, a verifiable and secure supply chain is of utmost importance.

External Drivers

- The development of credible vector control functions in entomology and eco-epidemiological evaluation is fundamental to assuring pesticide effectiveness in IRS. These capacities are often neglected because most programs are constrained financially. They are however needed for good resistance management, for
example, to ensure timely response to changing physiological and behavioral resistance in vector populations, as well as foster inter-sectoral collaboration (e.g. with Ministry of Agriculture on integrated pesticide/ vector management and NEMA on environmental management). Entomological evaluations are needed for sound selection and judicious use of insecticide to promote effectiveness and safeguard the ongoing utility of the available insecticides.

- The timeliness of spraying operations may be enhanced by developing adequate capacities for forecasting (e.g. the onset of epidemics), assuring adequate recurrent budget, strengthening logistics capacity, supply chain of commodities and strengthening program management.
- There are a number of opportunities for promoting best practices among spray operators. It includes enhancing training (linked to each spraying season), effective field supervision and auditing, by paying special attention to compliance with dosage and wall layering.
- Household adherence (avoiding re-plastering, wall washing and non participation), could be facilitated through vibrant IEC campaigns, as well as enhancing direct spray-operator communication.

Conclusion

The effectiveness of insecticides used for IRS, is linked to the overall efficiency of a vector control program. A careful appraisal of policy perspectives is needed to enhance IRS effectiveness in Uganda. A national vector control strategy that targets the development of a responsive, cost effective and sustainable infrastructure for IRS deployment is needed. It should address needs for effective pesticide management to facilitate judicious use and continued utility of insecticides. There is need for a clear perspective on the appropriate role of IRS, in an integrated vector management plan to maximize the contributory impact of IRS.

REFERENCES


A number of important elements guide malaria control programs on the selection of insecticides to be used for IRS. Two of these elements are addressed by the authors in this chapter that include: (1) detecting and monitoring vector resistance and (2) the residual life of an insecticide. In the paper by Kolaczinski et al., the authors presented results of their study that looked at the residual life of the insecticide lambdacyhalothrin (ICON) 10 CS when applied to various wall surfaces – mud, painted and plain plaster. Results showed that ICON 10 CS appears to last well over a 12 month period on all 3 surfaces tested achieving over 80% mortality 12 months after spraying took place while standard formulations of pyrethroid insecticides have a residual life of between 3-7 months. The authors point out that spraying only once a year would provide considerable cost savings over insecticides that require more frequent application. In their paper, Birungi and Mukwaya looked to establish a baseline for resistance monitoring in two mosquito populations in Uganda using the Centers for Disease Control (CDC) bottle assays. Susceptibility testing for resistance to DDT and Lambdacyhalothrin was conducted on a sample of mosquitoes from the Bugala and Nsadizi islands on Lake Victoria. These populations were selected because they constitute areas assumed not to have experienced insecticidal pressures. However, preliminary results detected some resistance to DDT in both island populations. These results reinforce the need for testing resistance prior to initiating IRS or changing insecticides. Likewise, the author Peter Mohloai addresses the issue of resistance in his paper by monitoring the development and spread of insecticide resistance in southern African mosquito vectors and how this might impact policy for insecticide choice and use in this region. His results showed strong linkages between agricultural pesticide usage and vector resistance which have serious implications for sustainability of vector control programs.
control using insecticides. Given that agriculture contributes to the viability of rural communities in Africa, he organized a multidisciplinary forum that encouraged community participation in order to improve local understanding of the linkages between and ramifications of agricultural use of pesticides and malaria vector resistance; and to develop community-based solutions to the malaria problem in the southern African region.

Monitoring Insecticide Resistance in Malaria Vectors Using the CDC Bottle Assay

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Introduction

Malaria is one of the major public health problems in the world today. Almost 90% of malaria occurs in sub-Saharan Africa. Patterns of transmission, environmental and cultural factors greatly vary throughout Africa. Even within individual countries, differences in malaria prevalence and incidence occur even between neighboring villages. The magnitude of the disease is further compounded by extreme poverty in sub-Saharan Africa. In Uganda, the malaria vector control strategies rely heavily on synthetic insecticides for use in insecticide treated bed nets (ITNs) and indoor residual spraying (IRS). Insecticide resistance has however been observed within the major Afro-tropical malaria vector Anopheles gambiae in several West African countries and parts of East Africa, including Uganda (Elissa et al., 1993; Vulule et al., 1994; Chandre et al., 1999; Verhaeghen et al., 2006). The emergence and presence of such resistance among vector populations may have implications for the long term effectiveness and sustainability of insecticide-based malaria control (Curtis et al., 1998). Therefore, there is a need for knowledge of insecticide resistance, coupled with surveillance for other efficacy parameters (e.g., parasite infection rates) during ITN use and IRS malaria control in Uganda.

The extent and distribution of insecticide resistance, from either biological evidence (bioassays) or molecular (kdr allele) detection in Uganda is limited. Verhaeghen et al., 2006 using molecular (kdr allele, the mutation associated with pyrethroid and DDT resistance [Soderland & Knipple,
2003]), reported a resistance in a few sites in Uganda. However, the use of molecular techniques is relatively expensive and would require a laboratory with the appropriate equipment. The use of bioassays offers a cheaper means to monitor resistance in population. Commonly, the WHO standard test kit has been used. However, it has limitations in that the procurement process in the Ugandan setting is difficult and the kit has a short shelf life. Here, we provide preliminary data on the use of the CDC bottle assay (Brogdon and McAllister, 1998) to monitor insecticide resistance in natural populations. The technique is simple, rapid, economical and different dosages of insecticides can be evaluated.

**Methods**

a) *Sample collection*

Indoor resting adult female *Anopheles gambiae* mosquitoes were collected in June 2006. Mosquitoes were collected by aspiration in houses from island populations in Uganda. Lake Victoria island populations were selected as they constitute areas that have not experienced insecticidal pressures. The mosquitoes were taken to the laboratory and either the wild caught females or their progenies were tested. Either non-blood-fed females or females fed with 10% sugar solution were tested for susceptibility to insecticides.

B) *Susceptibility testing*

CDC bottles were coated with technical grade DDT or LambdaClothaldrin (ICON) as described by Brogdon and McAllister, (1998). Since susceptible populations could not be established in populations collected, a diagnostic dose (the optimal dose for detecting resistance) of 25ug/bottle for ICON and 100ug/bottle for DDT estimated for *Anopheles* from the Amazon was used. For each population, 25 mosquitoes were gently aspirated into each coated bottle. Four replicates, for each test bottle and one bottle coated with acetone, as control, were used. The number of dead mosquitoes was recorded after every 15 minutes. After testing, all dead mosquitoes were preserved in 80% alcohol.
Results

In this report, results for susceptibility testing from two Lake Victoria island populations of Bugala and Nsadzi are provided (Fig.2.2).

![Comparison of Resistance to DDT and LambdaCyhalothrin (ICON) in Nsadzi and Bugala islands.](image)

The vertical line represents the resistance threshold, which is the upper range limit for survival of a susceptible population. Both populations were found to be susceptible to ICON, as all were dead within 30 minutes. On the other hand, a few mosquitoes in both populations were resistant to DDT. No mosquitoes died in the control bottles.

Discussion

Bugala and Nsadzi islands were among the populations that were assumed to be naïve to insecticide. These populations were selected in order to establish a baseline for resistance threshold in Ugandan populations. However, resistance to DDT was detected in both populations. These results highlight the importance of detecting and subsequently monitoring resistance to insecticides in populations prior to applying ITN or IRS. Such tests would guide malaria control programs on the appropriate insecticide for use in a particular population. Additional tests including molecular
detection using the kdr allele complemented with monitoring of sporozoite/infection rates will be useful in establishing the extent and distribution of insecticide resistance and evaluating the effectiveness of the control measures implemented in Uganda.

Acknowledgment

Acknowledgement is made of support from the Presidential Malaria Initiative (PMI), Dr. Raymond Beach from Centers for Disease Control (CDC), Atlanta for introducing and training on the use of the CDC bottle assay. Acknowledgement is also extended to His Excellency, the president of Uganda for the funds that enabled the collection of mosquitoes and the Uganda Malaria Research Center for the funds to continue this work which is currently ongoing. The Uganda Virus Research Institute (UVRI) Entomology division staff that made the collections and assisted in the testing of various populations are also acknowledged.

REFERENCES

Evaluation of the residual efficacy of Lambda-cyhalothrin 10 CS (25mg a.i/m² and 50 mg a.i/m²) in field use of Indoor Residual Spraying

SUMMARY OF A PRESENTATION

Kate Kolaczinski, PhD - Malaria Consortium; James Kirunda - Vector Control Division, MoH; Juma Mpima - Wakiso District

Introduction

Indoor Residual Spraying (IRS) is known to be an effective tool for prevention of malaria and is widely used in Southern Africa. Its use in Eastern Africa is increasing, where it is particularly appropriate in urban and peri-urban settings, epidemic prone areas or refugee camps. Its use in large scale rural highly endemic areas may also increase considerably in the future if donor support allows. One of the disadvantages of IRS is the need to reapply insecticide on a repeated basis. Standard formulations of pyrethroid insecticides have a residual life of between 3-7 months. This means that at least two spray rounds per year would be the goal in an area of perennial transmission with two annual rainy seasons. IRS is currently most often used where transmission has seasonal peaks. Here the persistence of the insecticide must be long enough to last from application, perhaps 1-2 months prior to the transmission season, throughout the transmission season itself. ICON® wettable powder (WP) is known to have a residual life of 4 months (Le Suere et al., 1993). Icon 10 CS may have a considerably longer insecticidal life. This study was carried out to assess the residual life of ICON® 10 CS, a lambda-cyhalothrin based insecticide formulation developed by Syngenta (Basel. Switzerland), applied on various wall surfaces. ICON 10 CS at 25mg a.i/m² has previously been demonstrated to achieve optimal performance in contact bioassays up to 9 months after application on mud and thatch walls in a field setting (Curtis et al., 1998). The formulation has also been tested in the laboratory for residual efficacy on various substrates over a 12 month period (Marharah et al., 2005). These laboratory tests showed the CS formulation continued to result in optimal mosquito mortality on all substrates up to 10 months, with some decline in the persistence of the insecticide on some substrates seen in the 10 - 12 months post application period (e.g. on the mud surface),

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6 >80% mortality of mosquitoes 24 hours after a 30 minute exposure
though on reed and cement surfaces the insecticide continued to result in >80% mortality up to 12 months after application. The promising results in the laboratory tests and the field results up to 9 months prompted Syngenta to commission a field trial of this new formulation of insecticide in a household setting over a 12 month period. WHO guidelines for testing mosquito adulticides for indoor residual spraying (WHO, 2003) describe three phases of testing. Phase I, is laboratory studies. Phase II, is small scale field trials and Phase III, is large scale field trials. Each phase of study requires various components with different aims. The current study was designed to examine the persistence of ICON 10 CS in a small scale field trial and therefore falls under the phase II component. The study objectives did not examine efficacy, vector behaviour or acceptability.

Outcomes

The primary outcomes examined were:

- Mean % mosquito knockdown 1h after 30 minute exposure in contact bioassays at various intervals post application (0, 4, 5, 6, 7, 11, 12 months), with statistical comparison at the 12 month point
- Mean % mosquito mortality 24h after 30 minute exposure bioassays at various intervals after application (0, 4, 5, 6, 7, 11, 12 months) with statistical comparisons made at the 12 month point

Statistical comparisons at the 12 month point were made between:

- The two doses (25 mg ai/m² and 50 mg ai/m²)
- The three different substrate types (mud, painted plaster and plain plaster walls)

Methods

Insecticide

Lambdacyhalothrin 10 CS (ICON® 10 CS) (Syngenta, Basel, Switzerland) was tested with untreated surfaces tested as controls. Two different doses were tested: 25 mg ai/m² and 50 mg ai/m². The insecticide was supplied in 1 litre bottles by Syngenta.
**Substrates**

The insecticide was applied and tested in houses with walls of 3 different substrates: mud, unpainted plaster and painted plaster. Mud walls were of the common East African type, with a fairly smooth mud surface with no additional finishing. Plain plaster walls were plastered, unfinished walls sometimes with rough surfaces. Painted plaster walls were plastered walls, smooth surfaced and painted with oil based gloss paint, which is commonly used inside houses in the peri-urban and urban East African setting. The intention had been to also include unfinished brick walled houses, but these were insufficient in the study area, to allow inclusion.

**Location**

The study took place in a peri-urban area around 20km from Kampala, at Kajansi trading centre, in Wakiso district. An initial survey of the district was carried out to identify an area where houses of different substrates were found (e.g. cement, brick, painted plaster, plain plaster, mud). The selected area was a residential area of Kajansi trading centre known as Kajansi B.

**Recruitment and insecticide application**

House to house visits were made, explaining the study and requesting informed consent to take part. A standard information checklist and questionnaire, was administered through observation and conversation with the household representative to record detailed information about the house construction and history of spraying (private companies offer IRS services in this area). Recruited houses were indicated with a sticker on the front door, on which a study number was written in permanent marker pen. Twenty-six houses of each wall type were recruited to the study and allocated a study number. Following recruitment, the complete list of household numbers and types was used to randomly allocate houses of each type to receive either 25 mg ai/m$^2$ or 50 mg ai/m$^2$ (using random number allocation in Microsoft Excel).

The number of houses recruited and sprayed, was based on 10 houses per treatment per dose, to remain compliant through the 12 month period to allow a range of houses for bioassay tests. An average 30% drop out rate
where consent was withdrawn at some point during the study period, was estimated requiring 13 houses to be recruited for each house type / dose.

Spraying took place with 5 teams of two spray operators over three days. They were all experienced spray operators, who were given refresher training using the WHO manual for application of residual sprays for vector control (WHO, 2003). This was 3 days prior to the spraying, to ensure consistency between sprayers and accuracy of application. Hudson Xpert™ sprayers were used and calibrated prior to commencement of spraying.

**Bioassay tests**

To examine the effect of the insecticide on knockdown and mortality, over a 12 month period, 30 minute exposure contact bioassays were carried out. Bioassays were carried out using wild caught non-blood-fed female anopheline mosquitoes. Over 95% of the anopheline population in the mosquito collection site is *An. gambiae sensu lact*, with the majority being *An. gambiae sensu stricto* and *An. funestus*. The two nights prior to the bioassay testing night, landing catches were performed in an area of high mosquito densities, to collect wild host seeking mosquitoes. The anopheline population of the area was sampled and demonstrated to be susceptible to Lambdacyhalothrin. Female anophelines were selected out of these catches and held in paper cups with access to sugar solution until needed for bioassay. Bioassay testing took place over a 2-3 day period. The testing framework, ensured houses of different substrates and different doses were tested on each day, to avoid any variation in test results due to variations in the mosquitoes.

Thirty minute exposure bioassays in WHO plastic bioassay cones were performed with knockdown of mosquitoes recorded 1 hour after exposure and mortality recorded 24 hours after exposure. Mosquitoes were exposed in batches of 8-10. To provide control data, mosquitoes were exposed using the same methodology to untreated walls. Table 2.6 shows the number of mosquitoes exposed to each dose and each house type at each monthly testing interval. Variations were based on numbers of wild caught mosquitoes available, at each monthly test point, but were all above a cut off of 50 mosquitoes per dose per house type, as per WHO guidelines for testing persistence of insecticide.
Bioassays were carried out at four places on the wall of the main room in each house. The bioassays were carried out on the same area of wall each month. One bioassay replicate was carried out in each test cone, resulting in a total of four replicates per house. At least 100 mosquitoes were tested for each house type/dose combination at each test interval. Variations, about 100, depended on the number of mosquitoes caught in the night landing catches. The number of houses tested during each test interval, depended on the number of mosquitoes available for bioassays at each point. The order of houses to be included was the same each month.

Households were asked whether mud had been replastered or walls washed between testing. If this had occurred the household was dropped from the study.

Note: Light trap catches, were planned to assess differences in mosquito densities, within houses. This component of the study was withdrawn as a result of low wild mosquito numbers in this area, which would have not given sufficient data to see an impact.

**Statistical analysis**

Proportional data (knockdown and mortality), from the contact bioassays were analysed using blocked logistic regression (STATA 6 software). Comparisons between treatments were made by successively dropping treatments from the overall comparison. This process ensures mosquito data is pooled for analysis and allows each treatment to be compared with every other. Blocked logistic regression uses log-transformed data in the analysis. Means and confidence limits of the estimate of the co-efficient of each treatment were therefore back-transformed to give percentage values for presentation. Back-transformation was carried out as follows:

\[
x' = \frac{1}{1 + (1/ \exp(x))}
\]

Where: \(x'\) = back-transformed value
\(x\) = the value from the logistic regression

**Timeframe**

Spraying was carried out in June 2006 with the 12 months post-spray tests completed in June 2007.
Results and discussion

Results of the bioassay tests are shown in the four figures below. Some variation in the numbers of mosquitoes knocked down and killed is seen over the 12 month period. This is a result often seen when tests are carried out in field settings, where the test conditions (temperature and humidity) cannot be regulated. In this study, wild caught mosquitoes were used. These have the additional issue of inter batch variability and unknown ages. Mortality in mosquitoes unexposed to insecticides at each testing interval, is shown and variation in this, is likely to have some impact on variability in the knockdown and mortality data.

On all substrates, and for both the 25 and 50 mg ai/m$^2$ doses, mosquito mortality remained above or around the 80% mark over the 12 month period (Figures 2.3 and 2.4). Despite some variability, confidence intervals span the 80% mark at every test point. At 12 months, mosquito mortality on the 25 mg dose was above 80% for all 3 house types (Painted plaster: 81.8%, 95%CIs: 76.9-85.9; Plain plaster: 84.2%, 95%CIs: 79.8-87.8; Mud: 81.6%, 95%CIs: 76.7-85.7) (Figure 2.3). For the 50 mg dose, mean mosquito mortalities are just below 80%, at the 12 months test point with the confidence intervals extending over the 80% mark (Painted plaster: 88.9%, 95%CIs: 83.4-92.7; Plain plaster: 76.1%, 95%CIs: 69.3-81.8; Mud: 77.7%, 95%CIs: 71.6-82.7). There is no clear pattern of decline over the 12 month period.

The number of mosquitoes knocked down, also show some variation over the 12 months period, though percentages knocked down remain above 80% at all test points as well as for all substrate types except for the mud and plain plaster walls at 12 months. There is no clear pattern of decline over the 12 month period.

Neither the mortality, nor the knockdown results or any of the 3 house types show a clear difference between the dosages tested. There does not appear to be any benefit in using the high dose in terms of insecticidal persistence.

In neither the mortality, nor the knockdown results and for neither of the 2 doses, is there a clear difference between the house types tested. The
insecticide formulation appears to last similarly well on each of the wall types.

The number of mosquitoes exposed at each test point is shown below in Table 2.6. The variation in numbers exposed was a result of a reliance on wild mosquito landing catches for the test batches. A cut off point of 50 mosquitoes, to be exposed per house type, per dose for each monthly test interval was imposed, based on WHO testing guidelines for examining insecticide persistence. Testing was planned for month 7, for example, but mosquito numbers were too low, to carry out the tests. Months where mosquito numbers were at the lower end, still demonstrated fairly tight confidence intervals, allowing clear conclusions to be formed from the data.

**Table 2.6: Number of mosquitoes tested at each test interval**

<table>
<thead>
<tr>
<th>House type</th>
<th>Dose</th>
<th>Months since spraying</th>
<th></th>
<th></th>
<th></th>
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<th></th>
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<td></td>
<td></td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>11</td>
<td>12</td>
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<tr>
<td>Mud</td>
<td>25</td>
<td>61</td>
<td>92</td>
<td>220</td>
<td>200</td>
<td>179</td>
<td>288</td>
</tr>
<tr>
<td>Painted plaster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain plaster</td>
<td>76</td>
<td>51</td>
<td>216</td>
<td>398</td>
<td>72</td>
<td>323</td>
<td></td>
</tr>
<tr>
<td>Mud</td>
<td>50</td>
<td>95</td>
<td>97</td>
<td>156</td>
<td>231</td>
<td>72</td>
<td>215</td>
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<tr>
<td>Painted plaster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain plaster</td>
<td>82</td>
<td>53</td>
<td>158</td>
<td>239</td>
<td>72</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Untreated walls (negative control)</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td>142</td>
<td>238</td>
<td>148</td>
<td>81</td>
<td>144</td>
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</tbody>
</table>
Figure 2.3: Mosquito mortality 24h after 30 minute exposure in cone bioassays on walls of different substrates sprayed with lambda-cyhalothrin CS at an application rate of 25mg ai/m²

Figure 2.4: Mosquito mortality 24h after 30 minute exposure in cone bioassays on walls of different substrates sprayed with lambda-cyhalothrin CS at an application rate of 50mg ai/m²
Figure 2.5: Mosquito knockdown 1h after 30 minute exposure in cone bioassays on walls of different substrates sprayed with lambda-cyhalothrin CS at an application rate of 25mg ai/m²

Figure 2.6: Mosquito knockdown 1h after 30 minute exposure in cone bioassays on walls of different substrates sprayed with lambda-cyhalothrin CS at an application rate of 25mg ai/m²
Conclusions and recommendations

ICON 10 CS, appears to last well over a 12 month period on all the 3 house types tested. It continues to achieve over 80% mortality, 12 months after spraying has taken place.

This insecticide formulation may be one that can be applied once a year only. This would provide considerable cost savings over insecticides that require more frequent application, especially in settings of high year round transmission where IRS is now being considered. The intervention will be most effective, if year round protection is provided, rather than spanning only a short transmission season. Given this finding, it would be useful to examine the cost effectiveness of this formulation, compared to other available insecticides and insecticide formulations for use in indoor residual spraying.

This study follows WHO test procedures and could be used as a part of a set of data from other studies, for a phase II evaluation of these insecticide formulation. Whilst the formulation has received recommendation from the WHO Pesticide Evaluation Scheme (WHOPES) (WHO, 2006) for use in indoor residual spraying, it is highly recommended that the data results from this and other field trials are submitted to WHOPES, to inform further assessment of the estimated persistence of this formulation.

There appears to be no benefit of using a higher dose of ICON 10 CS, for indoor residual spraying, with similar results attained for the 25 and 50 mg ai/m² dosages tested.

Additional studies that would be useful for ICON 10 CS, as well as other insecticides for indoor residual spraying, would look at mosquito behaviour and its impact on transmission reduction. There is currently discussion of the relative roles of repellency, deterrence and killing effect in reducing malaria transmission through IRS. It would be useful to know these properties for several of the insecticide options for IRS. It would also be useful to examine the impact of these different properties, on test results in contact bioassays. Given that the higher dose does not appear to persist longer or result in significantly greater knockdown and mortality than the lower dose in these tests, it is likely that there is a complex combination of issues at play in the test methodology and results. For example, higher irritability may result in mosquitoes possibly coming into contact with the
insecticide less at higher doses. Differences in knockdown time may result in differences in mortality, as rapid knockdown may result in less exposure to the insecticide in the cone tests.

**REFERENCES**


An Ecosystem Approach towards developing policies appropriate to sustainable pesticide use in agriculture and malaria control.

Peter Mohloai

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**Abstract**

**Background**

The Southern African region relies heavily on residual insecticide house spraying, for vector control and increasingly on ITN’s. Prior to this project, there was little or no expertise in the region, to assess insecticide
resistance in natural populations of malaria vector mosquitoes. The objectives of the study were to determine the relative importance of anti-malarial and agricultural pesticide usage, in the selection of insecticide resistance. The study set about to monitor the development and spread of insecticide resistance, in southern African mosquito vectors and establish a rational policy, for insecticide choice and use for southern Africa. This also included the development of a sustainable network that could be expanded to other African countries, for resistance monitoring, including a national capacity in various southern African states.

**Methods**

All mosquito collections were made over three years, from 1999 to 2001 in KwaZulu/Natal, Botswana, Swaziland, Mozambique, Zambia and Gabon. Permanent breeding sites were mapped and agricultural pesticide usage, documented in all study areas. Biochemical assays were performed on individual 1-3 day old adult F1 progeny, for altered acetylcholinesterase (AChE), glutathione S-transferases (GSTs), esterase and monooxygenase-based resistance mechanisms. PCR method of Scott et al. (Scott et al., 1993) for the *An. gambiae* complex and Koekemoer et al. (2002) for the *An. Funestus* was used. The diagnostic PCR developed by (Martinez-Torres et al., 1998) to distinguish between ‘resistant’ and ‘susceptible’ kdr alleles was employed.

**Results**

WHO discriminating dose assay, showed pyrethroid resistance in Mozambique, cross-resistance between DDT/permethrin in KwaZulu/Natal and cross-resistance between DDT/deltamethrin at Fiwale, Ndola, in Zambia. Altered AChE conferring resistance to OPs and carbamates, was found in all the three major malaria vectors in southern Africa. The presence of non-specific esterase-based pyrethroid and OP resistance mechanism was widespread in this region. An elevated monooxygenase-based mechanism was also present, in all the three vectors. High levels of GST activity were detected in all study areas. Kdr-type based resistance mechanism conferring cross-resistance to DDT/pyrethroids, was only detected in *An. gambiae* s.s at Fiwale, Ndola, Zambia (Mohloai, 2006). An ecosystem (holistic) approach, to study the linkages between pesticide usage, resistance and malaria incidence was successfully implemented.
**Conclusion**

Strong linkages between agricultural pesticide usage and vector resistance, were observed during this study. Due to the complexity of the factors involved in agricultural activities, an ecosystem approach was deemed necessary in South Africa, to evaluate the impact of human activities on linkages between total agricultural and public health pesticide usage, vector resistance and malaria transmission. This approach requires local know-how, assisting researchers to guide communities, towards solutions to problems that are perceived by them as priorities (Forget and Lebel 2001).

**Keywords:** Ecosystem approach, insecticide resistance, malaria transmission.

**Introduction**

WHO susceptibility tests showed pyrethroid resistance in Mozambique, cross-resistance between DDT/permethrin in KwaZulu-Natal (KZN) and cross-resistance between DDT/deltamethrin at Fiwale, Ndola, in Zambia (Mohloai, 2006). Altered AChE conferring resistance to organophosphate and carbamate insecticides, was found in all the three major malaria vectors (*An. gambiae* s.s, *An. arabiensis* and *An. funestus*) in southern Africa (Mohloai, 2006). The presence of non-specific esterase-based pyrethroid and OP resistance mechanism was widespread in this region (Mohloai, 2006). An elevated monooxygenase-based mechanism was also present in all vector species. High levels of GST activity were detected in all study areas. Kdr-type based resistance mechanism, conferring cross-resistance to DDT/pyrethroids, was detected only in *An. gambiae* s.s at Fiwale, Ndola, Zambia (Mohloai, 2006). The development of insecticide resistance in malaria vector mosquitoes has been associated with pesticide usage in agriculture (Martinez-Torres et al., 1998). This has serious implications for sustainability of vector control, while high levels of disease affect agricultural development (Abamu et al., 2003). Agriculture contributes to the viability of rural areas, by generating employment and helps to maintain the rural infrastructure, a point emphasised in the World Summit on Sustainable Development, the Johannesburg Declaration, 2002 (www.rdfs.net/ linked-docs/SARDmESP_1XII103.pdf). Malaria occurs in areas of semi to high agricultural development, where agricultural activities
are the major means of poverty alleviation. Development is a human problem, aimed at changing the human, economic, social and ecological environment leading to improved health (in Mohloai, 2006). Forget and Lebel (2001), state how public health thinking has evolved over the last quarter century towards a more global, ecological approach, which basically recognizes that the health problems of the world, including malaria, have had serious impact on human development. It is clear, that these problems cannot be solved by a single scientific discipline working alone. This approach in turn has led to natural resource management thinking, including consideration of environmental and social factors together with economic parameters (Forget and Lebel 2001). This has stimulated an integrated approach to management of health and environment (Forget and Lebel 2001). Human activities in the form of agriculture for example, have had a serious impact on people’s health in the form of malaria in this case (SIMA 2005). Due to the complexity of the factors involved in agricultural activities, an ecosystem approach was deemed necessary in South Africa, to evaluate the impact of human activities on linkages between total agricultural and public health pesticide usage, vector resistance and malaria transmission. This approach requires local know-how, assisting researchers to guide communities towards solutions, to problems that are perceived by them as priorities (Forget and Lebel 2001).

Aims

The aim of the multi-sectoral forum was to provide a discussion opportunity, in which all relevant stakeholders could participate, aiming to develop a protocol for integrated pest and pesticide management as well as usage. A second aim was to make recommendations for policy decision making, in both the agricultural and health sector, through which appropriate interventions could be developed and maintained. This approach should enable the different stakeholders, to advocate and agree on strategies for improving agricultural production, including measures to ensure appropriate pesticide use.

When documenting pesticide usage in agricultural and health sectors, during this PhD (Mohloai, 2006), it became clear that a more inclusive approach was needed in order to obtain data and influence the behaviour of the different sectors. An ecosystem approach was introduced, in order to promote interactions between communities and the environmental, health
and agricultural sectors, which should lead to increased awareness, exchange of information and joint action in anti-malaria and agricultural activities.

It is envisaged that the Ecosystem Approach, to address problems associated with pesticide usage and effects on vector control, will incorporate the many systems upon which these factors impact. The participatory process will ensure that the opinions of all stakeholders are expressed, and taken into consideration, in the formulation of policy recommendations.

**Materials and Methods**

As stated earlier, two of the main objectives during this PhD study, were to determine the relative importance of anti-malarial and agricultural pesticide usage in the selection of insecticide resistance. It was also to help establish a rational policy for insecticide choice and use for southern Africa. Two workshops were held in October 2003 at the Department of Health, Jozini, KZN. The first workshop was a result of visits to the institutes, groups and organisations by the author to discuss the ecosystem approach, aimed at solving the problems of insecticide resistance and the perceived association of vector insecticide resistance with agricultural pesticide usage and malaria transmission. During the identification of stakeholders, it was ensured that both men and women were included in the participatory process. Women formed the bulk of the stakeholders, due to the migration of men to the cities for better jobs. The second workshop was held in November 2003, at the Makhathini Agricultural Research Station, Mamfene, Jozini. A Participatory Rural Appraisal (PRA) tool was employed during this second workshop, to assess the ecosystem structures, in order to develop community-based solutions to the malaria problem in the southern African region. This also provided an opportunity to determine and understand the gender-specific impacts of ecosystem health risks. In addition to holding the workshops, communication to all the different stakeholders was carried out, and a steering committee was chosen, which will foresee the implementation of the project and its management.

The PRA method applied in this study was a mapping exercise that is modeling of the problem (Mohloai, 2006). This exercise, conducted in Zulu, the language spoken in KwaZulu/Natal, was a starting point for recognizing
and discussing differences in agricultural practices in the form of pesticide usage in the community. The PRA tool was also used, to explore local perceptions about wealth, and therefore helping to design and develop the sequence of interventions to address socio-economic issues.

The stakeholders’ forum, identified from the different institutional and community sectors, was divided into two groups. These were the collaborating partners and partners based on the different capacities of the sectors. The steering committee, with a duty to oversee the implementation of the project, considered individuals from all the collaborating partners. Efforts were made to reduce barriers or obstacles that could preclude or debilitate equitable participation within the stakeholder forum.

To undertake this linkage analysis, four villages Kwa-Jobe, Makhathini, Ndumo and Mzinyeni, being ecologically characteristic of the target communities in the region, were identified, based on the initial insecticide resistance analysis (Mohloai, 2006). These sites had the highest levels of insecticide resistance encountered during the study period. Women were invited to participate in the forum as they form the bulk of farmers and farm labourers in almost all the rural areas of South Africa. Improved understanding and monitoring of pesticide usage should help to reduce pesticide accumulation, in breeding sites and decrease insecticide selection pressure on malaria vectors, resulting in more efficient malaria vector control programmes.

**Results**

The participatory process was successfully implemented in October 2003, at the Makhathini Research Station in Jozini. The group made recommendations for policy decision making, for both the agricultural and health sectors, and established a community vehicle through which appropriate interventions could be developed and maintained. All participants in this forum were given the opportunity to voice their knowledge about malaria, insecticide resistance and agricultural practices in the form of pesticide usage. The forum, will promote interactions between communities and the environmental, health and agricultural sectors, which will lead to increased awareness, exchange of information and joint action in anti-malaria and agriculture activities. It will also lead to stakeholder capacity development, for interdisciplinary participatory research. This will
therefore lead to promotion of holistic approaches to malaria reduction, based on improved management and utilization of natural resources. It will subsequently lead to implementation of ecosystem-based and environmentally-sound best practices, for reductions in malaria risk at study sites, with known malaria and social-ecological characteristics. This work will help to assess the impact of interventions, aimed to control malaria and agricultural productivity, as well as document their impact on the sustainability of insecticide usage in both programs.

**Discussion**

The success of the stakeholders’ forum, suggests that it should now form the basis for recommendations to commence similar initiatives in the remaining malarious provinces of South Africa, as well as the entire southern African region, where pesticides are used in agricultural production. The forum process, advocates for the judicious use of pesticides, in both the agricultural and health sectors.

Improved understanding and monitoring of pesticide usage would help the women to reduce its usage in farming, and hence avoid pesticide accumulation in breeding sites. This will consecutively reduce the insecticide selection pressure, on the malaria vectors, resulting in more efficient malaria vector control programs. In addition, a well coordinated judicious use of pesticides in agriculture should result in increased agricultural productivity, which will improve the women’s economic status and therefore enable them make decisions regarding their health status and of their offspring.

A project team and management structure, including a steering committee, was established (Mohloai, 2006). Based on the discussions during the workshops, it became clear that the development of insecticide resistance in insects of both health and agricultural importance had serious implications, for all the stakeholders for sustainability of vector control as well as agricultural developments.

The established stakeholders’ forum, became aware of how health problems, especially malaria in this case, cannot be solved by a single
scientific discipline and therefore a holistic approach to address health issues was urgently needed in their community. To address these issues, the study looked at activities that introduced a cross-disciplinary and participatory approach to develop an integrated pest control program, pesticide management and insecticide usage program with the objective of sustainable and efficient vector control, by insecticides.

The major accomplishment of this pilot study has so far been both conceptual and methodological. The concepts and methods to be employed have been successfully debated and understood by the different stakeholders during the two participatory workshops (Mohloai, 2006). No empirical data exists at the moment, to supply a comprehensive numerical estimate of the linkages between pesticide usage, vector resistance and malaria transmission and therefore the health status of the given ecosystem. But the forum should allow these linkages to be fully investigated. The stakeholders were successfully included in both the definition of the system and its description (Mohloai, 2006).

The stakeholders have formed a part of the research and are involved in developing the solutions to insecticide resistance selection, which will lead to a higher chance of sustainability of the system. Their involvement at this stage of the research was in problem identification, planning and implementation of the pesticide usage, resistance management and monitoring. They will consequently therefore take part in the analysis of the data obtained through the system. They now own the problems, the solutions and the means to solve them.

**Summary and Conclusions**

The multidisciplinary forum used Participatory Rural Appraisal (PRA) research, to identify how communities perceive linkages between pesticide usage, vector resistance and malaria. The above agreements, for future research, have now established the framework in which the stakeholders’ forum will operate in the future. This along with the resistance baselines established (Mohloai, 2006), will lay the groundwork for the next decade of insecticide based malaria control activities in South Africa.
The ecosystem approach process envisaged, will analyze the complex situation in the developing agricultural sector, of the malaria endemic areas of KZN in regard to insecticide resistance, pesticide use, environmental, demographic, social and health data, in respect to the study community. It will create awareness amongst farmers, concerning inappropriate agricultural usage practices and enforce the need for development of best management practices.

Long term benefits from the planned work, will be that community participation in the project will enable community inputs in policy making, thereby making it oriented towards the needs of the community.

Sustainable malaria control through insecticide use, as well as technological improvement (pesticide choice), will benefit the local communities’ health and their economic status. The improved pesticide use practices in agriculture and malaria control should result in increased agricultural productivity and better health, leading to enhanced economic status within the community. This will lead to an improvement of the environment, through integrated pest control and pesticide management, thus increasing the longevity of the natural ecosystem, providing sustainable tourism opportunities and economic growth. Based on a better understanding of the situation, policy decisions will be formulated with regards to pesticide use in the area by all sectors.

Experience from this work in KZN, will form the basis for recommendations, to inform similar initiatives in the remaining malarious provinces of the Southern Africa region, where pesticides are used to increase agricultural production. This will enable different stakeholders, to advocate and agree on strategies for improving agricultural production, including measures to ensure appropriate pesticide use. The information dissemination strategy will be targeted at local, provincial, national and international communities and institutions through a variety of media on appropriate pesticide use, agricultural practices and health related issues. This will not be targeted at malaria and agricultural communities only, but also at a variety of disciplines affected by this process. For example:

- Communities in malaria prone areas – workshops, community radio stations, community information booklets, posters and drama (all in the local language)
• Policy makers – meetings, workshops, reports on policy recommendations
• Researchers – peer-review publications, local and international conferences on research findings, recommendations, new methodologies and tools
• Developers – internet access, newspaper articles on research findings and implications of development on malaria incidence
• Agricultural extension officers – workshops, reading material and reports
• Media – press releases and interviews

Monitoring will be carried out by providing financial evaluation of suitable technologies and farming practices in the form of pesticides. It will also involve follow-up risk perception and exposure investigation, as well as environmental contamination measures and farmer knowledge regarding pesticide use. The participatory process will continually be evaluated.

Acknowledgements

This work was made possible through the contribution of Multilateral Initiative on Malaria/Tropical Diseases Research (MIM/TDR), Systemwide Initiative on Malaria and Agriculture (SIMA), International Development Research Centre (IDRC), FRD/British Council, SAMRC, Hemingway Group & Liverpool School of Tropical Medicine, and the Oversees Research Council.

REFERENCES


GLOSSARY OF TERMS

An endophagic mosquito: A mosquito that feeds indoors

An endophilic mosquito: A mosquito that tends to inhabit/rest indoors. Endophilism facilitates the blocking of malaria transmission through application of residual insecticides to walls.

An exophilic mosquito: A mosquito that tends to inhabit/rest outdoors. Residual insecticides in buildings are less effective at controlling exophilic mosquitoes.

Entomological Inoculation Rate (EIR): The number of infective bites per person per night:

\[ EIR = \text{man-biting rate} \times \text{sporozoite rate} \times 100 \]

Full vector susceptibility: 98-100% mortality within 24 hours after 60 minutes exposure

Knock down resistance: Knockdown resistance (kdr) is a well-characterized mechanism of resistance to pyrethroid insecticides in many insect species and is caused by point mutations of the pyrethroid target site the para-type sodium channel.

Insecticide Effectiveness: Is measured as impact under programme, rather than trial conditions

Insecticide efficacy: is measured as impact of an insecticide under controlled trial, i.e. almost ideal conditions

IRS spray round: the administration of insecticide to walls or other surfaces in a defined area; the number of spray rounds that take place in a given year in the defined area is dependent upon factors such as the effective residual life of the insecticide, resistance to the sprayed insecticide, the length of the transmission season, and the number of rainy seasons.

Pyrethroid: A class of insecticides derived from the natural pyrethrins

Susceptibility tests: A measure of vector susceptibility to a given insecticide based on standard bioassays.

Vector susceptibility: Can also be called vector sensitivity. It is the ability of the vector to succumb or survive when exposed to an insecticide.
REFERENCES


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<tr>
<th>Acronym</th>
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<tr>
<td>ACTs</td>
<td>Artemisinin-based Combination Therapy</td>
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<td>AIDS</td>
<td>Acquired Immunodeficiency Virus</td>
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<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<td>DALY</td>
<td>Disability Adjusted Life Years</td>
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<td>DDHS</td>
<td>District Directorate for Health Services</td>
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<td>DDT</td>
<td>Dichlorodiphenyltrichloroethylene</td>
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<td>EIA</td>
<td>Environment Impact Assessment</td>
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<td>EIR</td>
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<td>EU</td>
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<td>Glutathione-S Transferases</td>
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<td>Home Based Management of Fever</td>
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<td>Health Management Information System</td>
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<td>Lubombo Spatial Development Initiative</td>
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<td>OPs</td>
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<td>PPE</td>
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<td>World Health Organisation</td>
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<td>WHOPES</td>
<td>World Health Organisation Pesticide Evaluation Scheme</td>
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Appendix A

The insecticide resistant status of the main malaria vectors in Uganda
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Abstract
A rational choice on insecticides in the framework of malaria control programmes can only be made if information on the insecticide resistance status of the vectors is available. Therefore WHO bioassay were done in seven sentinel districts throughout Uganda to assess the insecticide resistance status of the main malaria vectors An. gambiae s.s. and An. funestus. Tests were done with diagnostic concentrations of permethrin 0.75% and DDT 4%. Additional tests were done with deltemethrin 0.05% in one study area.

DDT resistance in An. gambiae s.s. seemed to be widespread in Uganda, mainly in the central end eastern parts, whereas permethrin resistance was mainly found in the densely populated eastern part of the country (around Tororo) and in Apac where cotton was grown in the past. In this study site, An. funestus showed possible resistance to DDT and permethrin. No resistance has been detected so far in the north western part of the country.

This first study on insecticide resistance in Uganda showed that resistance in the main vectors is present and that tolerant populations can be found throughout the country. This information has to be taken into account when planning vector control interventions in Uganda.
Introduction

The socioeconomic burden associated with malaria is a serious impediment to development in many tropical countries (Gallup & Sachs 2001). 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. In the high transmission areas malaria incidence can only be reduced if a comprehensive control strategy, including early diagnosis, correct treatment and very effective vector-control interventions, is implemented. In Africa important efforts are being made to scaling up insecticide treated bed nets (ITNs) and insecticide treated materials (ITMs) which are expected to reduce the malaria burden significantly (WHO 2000; Roll Back Malaria 2005). However, ITNs remain highly dependent on a single class of insecticide namely synthetic pyrethroids. Recently a renewed interest in indoor residual spraying (IRS) has been seen. This intervention gives the possibility to change insecticides according to operational factors, residual effects, cost and presence of insecticide resistance.

Knowledge of vector resistance and changing trends of resistance in target species are basic requirements to guide insecticide use in malaria control programmes (WHO 2006). In Uganda no data are available on insecticide resistance in the main malaria vectors therefore WHO bioassays were done in seven sentinel districts in order to assess the level of DDT and pyrethroid resistance.

Material and Methods

Mosquito sampling, identification and bioassays: seven different districts were involved in the monitoring of insecticide resistance, namely Apac, Arua, Jinja, Kanungu, Kyenjojo, Mubende and Tororo. The districts are sentinel sites for the monitoring of anti-malarial drug efficacy and were selected by the National Malaria Control Program in the framework of the East African Network for monitoring Anti-malarial Treatment (EANMAT). In each district at least two villages were selected for the insecticide resistance monitoring (Fig1). Adult Anopheles mosquitoes were collected during the night and put in paper cups with access to 10% sugar solution. The following morning mosquitoes were morphologically identified using a simplified illustrated key adapted from Gillies & Coetzee (1987). Subsequently, bioassays were done on An. gambiae s.l. and An. funestus. They were performed according to the WHO standard protocol (WHO 1998)
using the discriminative concentrations of DDT 4%, and Permethrin 0.75%. Deltamethin 0.05% was tested in one district namely Tororo. The impregnated and control papers were supplied by the Vector Control Research Unit, Malaysia and were not used more than 5 times. Mortality was observed after 24 hours, after which mosquitoes were stored individually in small tubes and put over siligacel.

**Mosquito processing:** The morphological identification of the mosquitoes was verified using the technique of Scott *et al.* (1993) for *An. gambiae* s.l. and the PCR-RFLP for *An. funestus* (Garros *et al.* 2004).

**Detection of Kdr frequency:** The *An. gambiae* s.s. and *An. arabiensis* samples were analysed on the presence of knockdown resistance by using a slightly adapted version of the FRET/MCA developed by Verhaeghen *et al.* (2006). The secondary PCR assay on the iCycler (Biorad, Hercules, USA) was performed in a 20 µl reaction, containing 1 x iQ supermix (Biorad, Hercules, USA), 1 mM MgCl₂, 500 nM of AgdF-ROX, 100 nM of AgdR and 1 µl of a 10-fold dilution of the primary PCR product resulting from amplification with Agd1 and Agd2 (Verhaeghen *et al.* 2006).

**Biochemical assays:** biochemical assays were performed on the *An. gambiae* s.s. populations of eastern Uganda (NAA and NAB) in order to assess whether metabolic resistance mechanisms confer DDT or pyrethroid resistance these *An. gambiae* s.s. populations. The abdomen of individual mosquitoes was removed and the head-thorax portion was homogenized in 200 µl distilled water. Non-specific esterase (PNPA), monooxygenase and GST (CDNB) levels were measured as described by Penilla *et al.* (1998). After the biochemical assays, the remaining mosquito homogenate was spotted on a filter paper and subsequently used for DNA extraction. Because no fully susceptible reference strain was available, the *An. gambiae* s.s. population collected in 2005 in southwestern Uganda (KHA) was selected as reference. This population showed a 24h mortality of 100% against permethrin and 97% against DDT. The two-sample Kolmogorov-Smirnov Z test (SPSS 15) was used to compare the enzyme levels of the different *An. gambiae* s.s. populations.

**Results**

**Molecular identification:** In total 1529 morphologically identified *An. gambiae* s.l. were identified by use of the multiplex PCR. Identifications
were well done in all sites except Jinja and Arua. In Jinja (JIA, JIB) only 48% belonged to the species complex, in Arua this was 50%. Bioassays tests on \textit{An. funestus} were only performed in Apac district and identifications were well done.

\textbf{Bioassays:} the bioassay results of Jinja were excluded from the analysis because of the unreliable morphological identification. In Arua no resistance was observed, neither in \textit{An. gambiae} s.s. nor in the misidentified mosquitoes. \textit{Anopheles gambiae} s.s. from Tororo and Apac showed high levels of DDT and Permethrin resistance (Table 1, Fig 2). In 2004, an other village was tested in Tororo (NAB), in this village possible resistance was found against both DDT and Permethrin (data not shown in table 1). In other districts Kanungu, Kyenjojo and Mubende, susceptible and possible resistant populations of \textit{An. gambiae} s.s. were found. In Mubende (MUA) repeated measures of resistance showed very different results (Table 1, Fig 2). \textit{Anopheles funestus} was possible resistant against permethrin in two study villages in Apac district. DDT tolerance in \textit{An. funestus} was only found in one village (Table 1).

\textbf{Metabolic resistance:} In the pyrethroid resistant \textit{An. gambiae} s.s. populations of NAA and NAB the esterase activity was significantly increased compared to the activity measured in the KHA \textit{An. gambiae} s.s. population. The monooxygenase level and GST activities measured in the \textit{An. gambiae} s.s. populations of NAA and NAB did not differ from the levels measured in the KHA \textit{An. gambiae} s.s. population.

\textbf{Kdr frequency:} In total, 744 \textit{An. gambiae} s.s. and 24 \textit{An. arabiensis} mosquitoes from 8 sites throughout Uganda were analysed on the presence of knockdown resistance by use of the FRET/MCA assay. This revealed the presence of the L1014S \textit{kdr} allele in all Ugandan \textit{An. gambiae} s.s. populations with a frequency varying from 14% to 50%.

\textbf{Discussion}

A rational choice on insecticides in the framework of malaria control programmes can only be made if information on the resistance status of the vectors is available. This is of even greater importance given the recent initiatives to roll back malaria in which vector control has a prominent place (Roll Back Malaria 2005; USAID 2007). Therefore WHO bioassay was used
in seven sentinel sites throughout Uganda to assess the insecticide resistance status of the main malaria vectors *An. gambiae* s.s. and *An. funestus* (Okello *et al.* 2006). Three insecticides, Permethrin, DDT and Deltamethrin, were tested using the discriminating dosage as defined by WHO (1998). One of the problems encountered during the study was the morphological identification of the vector species resulting in a loss of information. This problem could be avoided for the biochemical tests and the detection of *kdr*, since all analyses were based on molecularly confirmed *An. gambiae* s.s.

DDT resistance or at least tolerance in *An. gambiae* s.s. seemed to be widespread in Uganda and was found mainly in the central and eastern parts of the country, whereas permethrin resistance was mainly observed in the densely populated eastern part of Uganda (around Tororo) and in Apac where cotton was grown in the past. Moreover, *An. gambiae* s.s. from Tororo showed possible resistance against deltamethrin. In Apac, *An. funestus* populations were found that showed possible resistance to DDT and permethrin. No resistance has been detected so far in the north western part of the country. The resistant status of *An. gambiae* s.s. in Mubende still need some clarification since the bioassays done at different occasions showed conflicting results. Knockdown resistance was present in all study populations and metabolic resistance could only be tested in populations from Tororo and Kanungu. The results suggest that in Uganda the L1014S *kdr* frequency at genomic DNA level is not a good predictor of phenotypic resistance as measured by the WHO bioassay and that probably other resistance mechanisms, such as increased esterase levels, are involved in the observed insecticide resistance.

The presence of resistance in a bioassay does not automatically means that the malaria vector programmes are becoming ineffective. Trials in West Africa showed that insecticide treated nets (ITNs) remain effective against resistant *An. gambiae* s.s. mosquitoes having the L1014F *kdr* mutation. (Chandre *et al.* 2000). The impact of metabolic resistance mechanism coupled with the *kdr* mutation, as found in eastern Uganda, on the efficacy of ITNs is not understood. The impact of insecticide resistance on IRS insecticide resistance is expected to be dramatic as shown by Hargreaves *et al.* (2000) in South Africa, where the presence of resistant *An. funestus* resulted in a malaria upsurge in the region. Consequently, the fact that resistance is present should be taken into account when planning vector control interventions in Uganda. Moreover assessing the susceptibility status of the main vectors to organophosphate and carbamate
insecticides will provide essential information on possibility of insecticide rotation or mosaic treatment using compounds with different mode of action.

Acknowledgements
We thank the Ministry of Health of Uganda for facilitating this research. We are grateful to the School of Medical Entomology in Kampala, Uganda, for the excellent entomological work. Financial support: this research was financed by the Belgian Directorate-General for Development Co-operation through the framework agreement Institute of Tropical Medicine - East African Network for Monitoring Anti-malarial Treatment (EANMAT).

References


Table 1. Overview of the percentage mortality recorded in the WHO bioassays done in the years 2005 and 2006. Number of tested mosquitoes are between brackets.

<table>
<thead>
<tr>
<th>Species</th>
<th>District</th>
<th>Village</th>
<th>2005</th>
<th>2006</th>
<th>Summary(^1)</th>
<th>2005</th>
<th>2006</th>
<th>Summary(^1)</th>
<th>2006</th>
<th>Summary(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>funestus</td>
<td>Apac</td>
<td>ADA</td>
<td>99 (101)</td>
<td>100 (80)</td>
<td>S</td>
<td>99 (97)</td>
<td>93 (80)</td>
<td>S/T</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADB</td>
<td>97 (97)</td>
<td>81 (80)</td>
<td>T</td>
<td>98 (138)</td>
<td>92 (75)</td>
<td>S/T</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gambiae</td>
<td>Apac</td>
<td>ADA</td>
<td>-</td>
<td>76 (80)</td>
<td>R</td>
<td>-</td>
<td>81 (80)</td>
<td>T</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADB</td>
<td>-</td>
<td>63 (80)</td>
<td>R</td>
<td>-</td>
<td>80 (80)</td>
<td>T</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aria</td>
<td>AUA</td>
<td>99 (200)</td>
<td>-</td>
<td>S</td>
<td>100 (193)</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUB</td>
<td>100 (202)</td>
<td>-</td>
<td>S</td>
<td>100 (199)</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanungu</td>
<td>KHA</td>
<td>97 (197)</td>
<td>-</td>
<td>T</td>
<td>98 (197)</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KHB</td>
<td>99 (199)</td>
<td>99 (78)</td>
<td>S</td>
<td>88 (191)</td>
<td>95 (80)</td>
<td>T</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyenjojo</td>
<td>KYA</td>
<td>92 (101)</td>
<td>81 (85)</td>
<td>T</td>
<td>95 (80)</td>
<td>61 (85)</td>
<td>T/R</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KYB</td>
<td>98 (80)</td>
<td>-</td>
<td>S</td>
<td>100 (75)</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mubende</td>
<td>MUA</td>
<td>92 (83)</td>
<td>72 (85)</td>
<td>T/R</td>
<td>99 (81)</td>
<td>54 (85)</td>
<td>S/R</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MUB</td>
<td>97 (79)</td>
<td>T</td>
<td></td>
<td>100 (99)</td>
<td>S</td>
<td></td>
<td>-</td>
<td></td>
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</tr>
</tbody>
</table>
Table 2. Mean and standard error (SE) obtained for the esterase assay, mono-oxygenase and GST assay on An. gambiae s.s. populations of Uganda. The esterase activity was measured with para-nitrophenyl acetate (PNPA), whereas the GST activity was measured with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

<table>
<thead>
<tr>
<th>Study site and year</th>
<th>WHO bioassay</th>
<th>Biochemical assays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DDT</td>
<td>Per-methrin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(µmol/min/mg protein)</td>
</tr>
<tr>
<td>KHA 2005 PR S ND</td>
<td>30</td>
<td>0.0403</td>
</tr>
<tr>
<td>NAB 2004 PR T PR PR</td>
<td>59</td>
<td>0.0846</td>
</tr>
<tr>
<td>NAA 2004 PR R PR PR</td>
<td>59</td>
<td>0.0905</td>
</tr>
</tbody>
</table>

1 S: mortality between 100-98%, T: mortality between 97-80%, R: mortality <80%. One class is indicated when repeated bioassays resulted in the same mortality class or when only one bioassay result is available. Combined classes indicate that repeated bioassays resulted in different mortality classes.

2 No results are available for 2005. Bioassay results of 2004 are recorded instead. In 2006 two bioassays were done, one in the month of February-March the second in November.

Table 2.
Mortality categories:

- **S** Susceptible (mortality between 98 and 100%)
- **T** Possibility of resistance (mortality between 80 and 97%)
- **R** Resistant (mortality <80%)
- **ND** not done

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2 p-values (two-sample Kolmogorov-Smirnov Z, two-tailed) are given for all populations. P values which indicate a significant increased level of esterase activity, GST activity or an elevated level of P450 compared to the KHA An. gambiae s.s. population are given in bold.

† Mean rank of population is increased compared to the mean rank of the KHA An. gambiae s.s. population.
Table 3. Frequencies of the L1014S kdr allele in the different *An. gambiae* s.s. and *An. arabiensis* populations of Uganda as determined by the FRET/MCA.

<table>
<thead>
<tr>
<th>Species (molecular identified)</th>
<th>N</th>
<th>L1014S allelic frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>An. gambiae s.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JIA 2005</td>
<td>62</td>
<td>33.06</td>
</tr>
<tr>
<td>KHA 2005</td>
<td>28</td>
<td>14.29</td>
</tr>
<tr>
<td>KYA 2005</td>
<td>80</td>
<td>33.75</td>
</tr>
<tr>
<td>KYB 2005</td>
<td>71</td>
<td>30.28</td>
</tr>
<tr>
<td>MUA 2005</td>
<td>52</td>
<td>50.00</td>
</tr>
<tr>
<td>MUB 2005</td>
<td>48</td>
<td>47.92</td>
</tr>
<tr>
<td>NAA 2004</td>
<td>265</td>
<td>48.87</td>
</tr>
<tr>
<td>NAB 2004</td>
<td>120</td>
<td>42.50</td>
</tr>
<tr>
<td>An. arabiensis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JIA 2005</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>NAA 2004</td>
<td>16</td>
<td>0.00</td>
</tr>
<tr>
<td>NAB 2004</td>
<td>7</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Figure 1. The study sites for the monitoring of insecticide resistance in Uganda.
Figure 2. Overview of insecticide resistance in *Anopheles gambiae* s.s. from Uganda

- **Susceptible**: mortality > 98%
- **Suspected resistance**: mortality between 97—80%
- **Resistant**: mortality < 80%

Bioassays done at different occasions showed different resistance classes namely: Suspected resistance and resistant
- **Resistant**: mortality < 80%
- **Susceptible and resistant**: Bioassays done at different occasions showed different resistance classes namely: Susceptible and resistant
- **Study sites**
Appendix B

Open Meeting Agenda

CONSENSUS STUDY COMMITTEE OF THE UGANDA NATIONAL ACADEMY OF SCIENCES (UNAS)

Assessing Malaria Vector Resistance to Insecticides Used for Indoor Residual Spraying in Uganda

Draft Agenda
Hotel Africana in Kampala, Uganda
Room F

Meeting Objective:
To provide background information to the UNAS consensus committee that will assist them in reviewing and assessing the current state of knowledge and policies related to monitoring malaria vector resistance; and to enable the committee to propose “best practices” with respect to maximizing the effectiveness of DDT and other insecticides that will help foster a successful implementation strategy for indoor residual spraying.

MONDAY, JULY 23, 2007

9:00 – 10:15  Closed session for committee members only
10:00 – 10:30  Registration of Participants
10:30 – 10:45  Welcome and Opening Remarks
  - Prof. Paul E. Mugambi, President, Uganda National Academy of Sciences
  - Dr. James Tibenderana, Malaria Research Centre, Kampala; Committee Chair
10:45 – 11:00  Self-Introductions: Committee Members; Forum and Executive Council Members

11:00 – 11:15  Keynote Presentation
   Dr. Sam Zaramba, Director of Health Services, Uganda (INVITED)

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**Session I: Framing the issues (11:15am-1:00pm)**

Moderator: James Tibenderana, Committee Chair

11:15 – 11:35  Current state of malaria vector resistance in East Africa and Uganda
   Martin James Donnelly, Vector Group, Liverpool School of Tropical Medicine

11:35 – 11:50  Discussion – 15 min

11:50 – 12:10  The role of IRS in contemporary malaria control
   Allan Schapira, Swiss Tropical Institute in Basle, Switzerland

12:10 – 12:25  Discussion – 15 min

12:25 – 12:45  Best practices for implementation of IRS that minimizes malaria vector resistance
   Maureen Coetzee, University of the Witwatersrand, South Africa

12:45 – 1:00  Discussion – 15 min

1:00 - 2:00  LUNCH

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**Session II: Monitoring resistance and maximizing insecticide effectiveness (2:00pm-4:00pm)**

Moderator: Nelson Musoba, Committee Vice-chair

2:00 – 2:20  Overview of vector control interventions in Uganda and their past performances
   John B. Rwakimari, National Malaria Control Programme, Ministry of Health, Uganda
2:20 – 2:35  Discussion – 15 min

2:35 – 2:55  Monitoring DDT in the environment
Waiswa Ayazika, National Environment Management Authority, Uganda

2:55 – 3:10  Discussion – 15 min

3:10 – 3:30  Maximizing the effectiveness of DDT and other insecticides
Jacob Williams, Research Triangle International

3:30 – 3:45  Discussion – 15 min

3:45 – 4:00  BREAK

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Session III: Issues to consider (4:00pm-6:30pm)

Moderator: James Tibenderana, Committee Chair

4:00 – 4:20  Molecular Techniques to Measure Insecticide Resistance
Louis Mukwaya, Uganda Virus Research Institute

4:20– 4:35  Discussion – 15 min

4:35 – 4:55  Trial of Icon CS: examination of persistence on walls over 12 months
Kate Kolaczinski, Malaria Consortium, Uganda

4:55– 5:10  Discussion – 15 min

5:10 – 5:30  Malaria and agriculture: the links and the challenges
Peter Mohloai, Malaria Programme, Medical Research Council, South Africa

5:30 – 5:45  Discussion – 15 min

5:45 – 6:00  Summary & Assessment
Maureen Coetzee, University of the Witwatersrand, South Africa

6:00 – 6:30  Open Discussion

6:30  ADJOURN
Appendix C

Biographies

COMMITTEE MEMBERS

DR. JAMES TIBENDERANA (PhD) is a medical doctor who trained as an epidemiologist at the London School of Hygiene and Tropical Medicine. His Ph.D was on the "Neurological Sequelae of cerebral malaria in Ugandan children and adults". He now works for the Malaria Consortium Africa Office, in Kampala Uganda, in several areas of work that include: Supporting the Ministry of Health with the change of antimalarial treatment policy from chloroquine + SP to Arthemether/Lumefantrine as first-line; Implementing interventions that focus on the health systems that aim to reduce the mortality of severe malaria in four districts in Uganda; Establishing a research centre for malaria in Uganda that aims to provide the evidence needed for effective implementation of malaria control interventions in the country; and helping the private sector to provide affordable and accessible Artemisinin-based combination therapy (ACTs). In addition to these activities, he is involved in conducting research on communicable diseases.

DR. NELSON MUSOBA is a Senior Health Planner and the Focal Point Person for the Public Private Partnerships in Health (PPPH) at Uganda’s Ministry of Health. In this capacity, Dr. Musoba, coordinates and supervises ground breaking health research and policy formulation across Uganda. He has also worked at the district and sub-district levels both as manager and operational level worker. He revitalized Rushere Hospital theatre for emergency obstetric care in 2000, after the private not-for-profit hospital spent over a year without functioning. He turned Lyantonde Health Center IV, into a model health center with a functioning theatre and blood transfusion unit. In 1996, he revitalized Kambuga Hospital theatre, after a-two year dormancy. In addition to this work, Dr. Musoba, specializes in HIV/AIDS management and advocacy. He currently serves as Executive
Director of the Action Group for Health, Human Rights and HIV/AIDS (AGHA) and as Chair of AIDS Information Center (AIC) Advisory Committee, Western region. He formerly served as Rakai district HIV/AIDS coordinator. Dr. Musoba, received his MB.Ch.B from Mbarara University of Science and Technology, and earned a Postgraduate Diploma in Anesthesiology and a Masters Degree in Public Health, both from Makerere University. In 2000, Dr. Musoba was awarded Best Health Worker of the Year in Uganda, by the Uganda Medical Association and the Ministry of Health.

PROF. MAUREEN COETZEE is the Head, Vector Control Reference Unit, at the National Institute for Communicable Diseases, in South Africa. She holds a PhD on Morphological study of malaria mosquitoes of the An. gambiae complex and an MSc from University of the Witwatersrand on Morphological and genetical study of the An. coustani group of mosquitoes. Before her current position, she was the acting Head, Department of Medical Entomology, South African Institute for Medical Research (SAIMR) and was a Medical Scientist with the Department of Medical Entomology, SAIMR, with joint appointments in the Department of Tropical Diseases, School of Pathology of the University of the Witwatersrand and Department of Zoology, University of the Witwatersrand for 10 years. She has had a number of academic appointments, the most recent being Research Professor of Medical Entomology, School of Pathology of the National Health Laboratory Service (NHLS) and the University of the Witwatersrand, Johannesburg, Honorary Professor, School of Animal, Plant & Environmental Sciences, University of the Witwatersrand, Johannesburg, and Professor and Reader of Medical Entomology in the Department of Clinical Microbiology & Infectious Diseases, School of Pathology of the National Health Laboratory Service (NHLS) [previously the South African Institute for Medical Research (SAIMR)] and the University of the Witwatersrand. Professor Coetzee, has had various scientific achievements and honours, and has been involved in many review panels and committees. She is an elected fellow of the Royal Entomological Society, and the Royal Society of Tropical Medicine and Hygiene among others. Her research interests include: mosquito insecticide resistance, mosquito systematics, and mosquito genetics.

PROF. BENSON B.A. ESTAMBALE is a clinician and tropical medicine specialist having obtained his professional qualifications in both the University of Nairobi, Kenya and Liverpool School of Tropical Medicine in
the UK. He is the current Director of the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID), having been appointed as its first director in 2004. He has been instrumental in the setting up of the Institute, including the establishment of Level-3 Bio-safety containment laboratory, to handle haemorrhagic fever and other emerging pathogens. Prior to his appointment, Prof. Estambale served as an academic faculty member of the Department of Medical Microbiology, in the School of Medicine at the University of Nairobi for over 20 years. Prof. Estambale is a member of the Kenya National Academy of Sciences (KNAS), a member of the British Society of Parasitology, American Society of Tropical Medicine and Hygiene and International AIDS society. He has been instrumental in the development of the KNAS 2006-2011 Strategic Plan which was launched in May 2007. He has sat on various task forces including the Health Research Capacity Strengthening and the Malaria vaccine decision making framework. Prof. Benson B.A Estambale is the director of the Nairobi Institute of Tropical and Infectious Diseases. He holds a PhD in Medical Microbiology and is widely published on Malaria in Kenya. He is a member of the Kenyan, British, and Danish society of parasitologists, as well as a member of the Kenya National Academies. He has held various positions, including coordinator of the Kenyan-Danish health research project on vector borne diseases, Principle Investigator of the Intermittent Preventive Treatment in schools in western Kenya, a project between the Nairobi Institute of Tropical and Infectious Diseases and London School of Hygiene and Tropical Medicine.

**DR. MARY HAMEL**, Malaria Branch Chief at the KEMRI/CDC Research Station, is a medical epidemiologist from the U.S. Centers for Disease Control and Prevention. In 1995, after completing her medical training at the University of Vermont School of Medicine and Residency in Internal Medicine at the University of Colorado Health Sciences Center, she joined the CDC as a Medical Epidemiologist in the Child Survival Activity. She subsequently was board certified in Infectious Diseases and has a Diploma in Tropical Medicine and International Health. Dr. Hamel has expertise in program evaluation, and has worked extensively on the evaluation of child survival and malaria-related guidelines, including the Integrated Management of Childhood Illnesses. She was the point person managing the multi-agency effort: the Bungoma Integrated Malaria Initiative, designed to implement malaria prevention interventions and reduce malaria related morbidity and mortality in a single district in western Kenya. She has
served as a consultant to WHO, and several African Ministries of Health, and currently is a member of several technical working groups for the Kenya Division of Malaria Control. She has led and supervised numerous malaria-related studies and clinical trials focusing on malaria and HIV interaction, intermittent preventive treatment for malaria in infants and pregnant women, drug efficacy studies, community-based evaluation of parasitemia and anaemia, and is currently a co-PI on a malaria vaccine trial, among others.

DR. JESCA NSUNGWA SABIITI is the Principal Medical Officer at Child Health-Ministry of Health. Her current work involves developing and monitoring child health policies at national level, setting standards and supporting districts to plan and implement these standards. Her work also includes district implementation of newborn and child health interventions and research and evaluation of large scale child health related programs, including health systems and community aspects of the programs. As a policy maker and a pediatrician involved in primary health care, she has worked on several aspects of child health including the integrated management of childhood illnesses, family care practices, home based management of fever, nutrition counseling and communication using trials of improved feeding practices, engaging informal drug sellers in management of malaria, pneumonia and diarrhea, and scaling pediatric ART using a decentralized primary health care approach. She has spearheaded the inclusion of newborn health into the national strategic plan for Uganda 2006-2011 and was involved in drafting the plan. Jesca has evaluated several programs in the country and worked as a consultant for WHO, UNICEF, USAID and the World Bank. She is currently involved in research on the overlap of malaria and pneumonia, using ACTs for home management of malaria and adherence to ARVs. Dr. Nsungwa holds a Bachelors degree in Medicine, Masters degree in Pediatrics and is currently enrolled for a doctorate with a thesis on Health Systems, Evaluation of home management of fever intervention in Uganda.

DR. CHARLOTTE MUHEKI ZIKUSOOKA is an Economist/Health Economist, with strong quantitative skills and nine-year experience in project/program coordination and management, research, training and consulting in the Health Economics field. Over the years, Charlotte’s research activities have mainly covered the areas of Health expenditure reviews and National Health Accounts, Health sector financing, assessment
of hospital efficiency, hospital organisational audits, evaluation of drug supply systems, economic evaluation of health programs/interventions, cost-effectiveness studies, and costing studies. She has also worked on key diseases, mainly Malaria and HIV/AIDS. Some of her special research skills include costing; cost-effectiveness analyses; econometric and decision analytic modeling; designing, conducting and analyzing household surveys, and good writing skills. She has excellent knowledge of computer programs (Word, Excel and PowerPoint) and some basic knowledge of relevant statistical and modeling software. She has substantive knowledge in Project/Program Management and Coordination and in Monitoring and Evaluation. Dr. Charlotte Muheki Zikusooka was specifically employed as the Monitoring and Evaluation Specialist with the Uganda Global Fund Project (Ministry of Health), where she was involved in the process of engaging and monitoring sub-grantees of grants implementation activities, performance and reporting, and other responsibilities. She has been involved in coordinating, monitoring and evaluating 2 long-term big research projects while employed at the Health Economics Unit (University of Cape Town). She is also involved in training/teaching, mainly on postgraduate programs (Masters Level), special courses for Health sector managers, research internship programs and special workshops organized by international institutions. Her teaching experience includes actual teaching (through up-to-date teaching styles and techniques), designing and convening teaching post-graduate modules, setting and marking assignments and exams, and providing supervisory support to students writing theses (through editing and providing guidance on their written work). She has provided services to organisations like UNAIDS, World Health Organisation and DANIDA, Action Aid International, as well as other private sector organisations such as Norvatis (a Pharmaceutical company).

**DR. JOHN W BAHANA** is current Uganda Director of Indoor Residual Spraying Project and Chief of Party of RTI International, the implementing partner of USAID/President’s Malaria Initiative (PMI) support to Uganda’s Ministry of Health National Malaria Control Programme. Dr Bahana holds a Ph D in entomology. He was previously Chief Scientist at the Zambia based International Red Locust Control Organisation for Central and Southern Africa where he spearheaded research and control of migratory pests in the region for more than 15 years. He has also worked at the International Centre of Insect Physiology and Ecology (ICIPE) with headquarters in Kenya as a scientist researching into the biological control of cereal stem
borers. Dr Bahana was one time (2001-2003) President of the ARPPIS Scholars’ Association an affiliate of ICIPE. ARPPIS (African Regional Postgraduate Programme in Insect Science) is a continent wide collaborative programme between ICIPE and a number of African Universities in which insect scientists are trained on the continent as an effort to contribute to stemming the tide of brain drain in Africa. He also served as Chief Editor of the African Association of Insect Scientist where he oversaw the Associations information exchange and publications. Dr Bahana has a number of publications both in international journals and local publications. He is chairman of a number of voluntary associations.

SPEAKERS’ BIO DATA

PROF. MAUREEN COETZEE (See Committee members)

DR. MARTIN JAMES DONNELLY is a vector biologist trained at Cambridge University and the Liverpool School of Tropical Medicine. He previously worked with the US Centers for Disease Control and Prevention (CDC) in Atlanta, USA (1999-2001). He joined the Liverpool School of Tropical Medicine as lecturer in 2001 and heads a research laboratory within the Vector Group. He is involved in an ongoing programme investigating the population genetics, phylogenetics and ecology of members of the *Anopheles gambiae* complex in Africa. As part of the Gates IVCC (www.ivcc.com) he is involved in developing improved diagnostics for insecticide resistance. This is a wide ranging programme that involves field collections, association mapping and microarray studies together with developing technologies that are appropriate to disease endemic country settings. Dr. Donnelly, together with a group of scientists, performs applied field research and recently completed a study on malaria transmission in urban settings of West Africa. This study was conducted in association with the International Water Management Institute (www.iwmi.org) and focused on the potential of urban agriculture to increase malaria transmission in cities.

DR. KATE KOLACZINSKI heads the Vector Control Section of the Malaria Consortium's Africa Office. As the Malaria Consortium's focal person for these subject areas, she represents the organisation on the East Africa Roll Back Malaria Network and the RBM Technical Support Network for
Complex Emergencies. As well as directly managing projects, she provides technical input into the Malaria Consortium’s projects in the African region as well as providing technical advice to partners in the region. From 2002 until 2004 she worked as the research advisor for an international NGO in Afghanistan and Pakistan on a malaria and leishmaniasis control programme which ran a comprehensive malaria control programme encompassing IRS, ITNs and larviciding alongside case management. Here she managed operational research projects to inform programming, particularly on the use of insecticide treated materials and new vector control tools and the use of disease modeling to geographically target malaria control interventions. Dr. Kolaczinski obtained both her MSc, on the biology and control of disease vectors, and her PhD, on malaria control in complex emergency settings, from the London School of Hygiene and Tropical Medicine.

**DR. PETER MOLAMU MOHLOAI** is a Senior Scientist at the Malaria Research Lead Programme, Medical Research Council, having recently finished a PhD on “Implementation of Biochemical and molecular tools for resistance detection, monitoring and management of malaria vectors in Southern Africa.” The project was a MIM/TDR initiative. He has substantial experience in developing and implementing resistance management strategies. Dr. Mohloai has established a stakeholder’s forum to implement an ecosystem approach in South Africa to study linkages between agriculture in the form of pesticide usage, vector resistance and malaria transmission, an approach that emphasizes natural resource management thinking, including consideration of environmental and social factors together with economic parameters. Dr. Mohloai holds a Bachelor of Science degree in Human Genetics. He has work experience that ranges from Medical Scientist to consultant on various programmes, some of which were on insecticide resistance, vector borne diseases, and molecular techniques. He has skills in resistance management strategies, biochemical and molecular tools for insecticide resistance detection in malaria vectors, techniques in molecular biology particularly associated with Polymerase Cahin Reaction (PCR), scientific field work associated with Palaeo-anthropology, and theoretical population genetics. Among others, he has also published on Implementation of biochemical and molecular tools for resistance detection, monitoring and management of malaria vectors in southern Africa, integrating a malaria control programme into the
general health services in Kwazulu/Natal, and Insecticide resistance in *An. funestus* (Diptera:Culicidae) from Mozambique.

**DR. JOSEPHINE BIRUNGI** is a Principal Research Officer at the Uganda Virus Research Institute (UVRI), Department of Entomology. She received her B.Sc. (Hons), (Zoology and Psychology), M.Sc. (Zoology/Entomology) at Makerere University, and a Ph.D. in Molecular Genetics at Makerere University/University of Copenhagen, Denmark. She then joined Yale University School of Medicine, Department of Epidemiology and Public Health as a Postdoctoral Associate in Vector genetics working with Dr. Leonard E. Munstermann. As a post doc, using population genetic markers, she worked on the invasion biology of the dengue fever vector, *Aedes albopictus* from the US, Brazil and Asia and population genetics of the sandfly species, *Lutzomyia longipalpis* and *Lu. cruzi* from Brazil and Bolivia. Subsequently, she joined UVRI and also serves as external examiner to graduate students at Makerere University. Although a lot of work has previously been done in the lab on the ecology, behavior and genetic differentiation of the yellow fever vector *Aedes simpsoni*, and recently on the genetic differentiation of *An. gambiae* complex, either older molecular approaches were utilized or molecular analyses were performed in external laboratories. Josephine would like to build a modern molecular genetics laboratory and strengthen the research capability in the field of vector genetics at the institute. Her research interests include applying morphological and molecular genetic methods in order to distinguish the human-biting from non-human biting vector populations and the complex forms of the yellow fever vector, *Aedes africanus*, in Uganda (and neighbouring countries). Specifically; (a) Using as many morphological and molecular markers of *Ae. africanus* from as many samples as possible and search for clusters of individuals or populations, which may be ecologically or genetically meaningful. (b) Studying the human-biting behaviour of mosquitoes from different populations in relation to morphological or genetic differentiation. (c) Determining the geographical and ecological (habitat) distribution of the different groups within *Ae. africanus*, with particular emphasis on the distribution of human-biting and non-biting forms, which are relevant for vector control. (d) Comparing molecular data of *Ae. africanus* with published data on other species, and attempt to reconstruct their phylogenetic relationships.
DR. ALLAN SCHAPIRA is 58 years old and was born in Copenhagen, Denmark. He finished his medical studies at the University of Copenhagen in 1975, after having spent 9 months as a volunteer in a primary health care project in Botswana. He holds a diploma in Tropical Medicine from Liverpool, UK, and a doctorate in medical science from the University of Copenhagen. In 1987 to 1988 he was a fellow in international health at the Harvard School of Public Health, Boston, US. After working as an intern in hospitals in Denmark, he worked as a district medical officer in Angoche, Mozambique from 1977 to 1979. Since 1982, his professional life has mainly been oriented towards malaria, starting with research on antimalarial drug resistance at Statens Serum Institut, Copenhagen, followed by field work in Mozambique. From 1990 to 2007, he was a staff member of the World Health Organisation. From 1999 to 2002, he was WHO Regional Adviser on Malaria in the Western Pacific Region and from 2002 to 2005 he was Coordinator of the Strategy and Policy team, Roll Back Malaria Department, WHO Headquarters, Geneva. From 2005 to 2006 he was coordinator of the Vector Control and Prevention team, Global Malaria Programme. He currently works as part of a team modeling the impact and cost-effectiveness of malaria control interventions in the Department of Public Health and Epidemiology of the Swiss Tropical Institute in Basle. He has lived in Denmark, Botswana, UK, Mozambique, USA, Switzerland, Vietnam and the Philippines. He has written a number of scientific articles about malaria, its treatment and control.

DR. JACOB E. WILLIAMS recently left his post as the point person on DDT related issues in the Roll Back Malaria Cabinet Project of the World Health Organisation (WHO). His primary role there was to coordinate Roll Back Malaria efforts on the sustainable reduction of DDT use for Malaria Vector control among Member states, and the effective management of current use. Previous assignments with the World Health Organisation include the Programme of the WHO/FAO/UNEP Joint Panel of Experts on Environmental Management for Vector Control (PEEM) and WHO Regional Centre for Environmental Health Activities. Dr. Williams also held the positions of Research Fellow/Lecturer with the Department of Zoology at the University of Ghana; Executive Director with the Organisation for Children In Need in Ghana; and Consultant to the World Bank and the United Nations Population Fund.