Republic of Zambia
National Malaria Control Centre

Monitoring the Insecticide Resistance Profiles of Major Malaria Vectors in Zambia

USAID
FROM THE AMERICAN PEOPLE

ZISSP
Increasing the use of High Impact Health Services

Co-Authors

Mulenga Musapa, Zambia Integrated Systems Strengthening Program, Lusaka, Zambia

Javan Chanda, Malaria Transmission Consortium, Lusaka, Zambia

Alister Kandyata, National Malaria Control Centre, Lusaka, Zambia

Idan Chabu, National Malaria Control Centre, Lusaka, Zambia

Osbert Namafente, Tropical Disease Research Centre, Ndola, Zambia

Lucy Muzia, Zambia Integrated Systems Strengthening Program, Lusaka, Zambia

Patrick Chewe, Zambia Integrated Systems Strengthening Program, Lusaka, Zambia

Peter Mumba, Zambia Integrated Systems Strengthening Program, Lusaka, Zambia

Elijah Sinyinza, Zambia Integrated Systems Strengthening Program, Lusaka, Zambia

Kathleen Poer, Zambia Integrated Systems Strengthening Program, Lusaka, Zambia

Emmanuel Chanda, Ministry of Health, National Malaria Control Centre, Lusaka, Zambia

Table of Contents ***Page***

Monitoring Insecticide Resistance Profiles of Major Malaria Vectors in Zambia 2011

1 Background

The recent massive expansion of the malaria vector control activities has employed a fairly uniform set of interventions and delivery systems: either Long Lasting Insecticidal Nets (LLINs) delivered through large-scale LLIN campaigns, or Indoor Residual Spraying (IRS) using DDT and pyrethroids in most cases. Moreover, in Zambia, the choice between these two interventions has often been made on the basis of program capacity and malaria endemicity, rather than on entomological criteria. However, vector control needs to become more diverse and more contingent on local entomological data. It is therefore, essential that Zambia develop entomological capacity to monitor, adapt and innovate.

Zambia has shown significant progress in malaria control, with a 53% drop in malaria parasite prevalence among children between the years of 2002 and 2008 (Chizema-Kawesha et al, 2010). However, between 2008 and 2010, Zambia experienced a resurgence of malaria and severe anaemia (Zambia Malaria Indicator Survey, 2010). The increase may have had several contributing factors and among these is the evidence from insecticide resistance monitoring data collected by the National Malaria Control Center (NMCC) and other partners in Zambia which has confirmed the selection of resistance in both *An. funestus* s.l and *An. gambiae* s.l to DDT and pyrethroids in some parts of the country. The problem of insecticide resistance in Zambia is a threat to malaria control efforts and can severely undermine the National Malaria Strategic Plan for the period 2011 to 2015, which has an overall objective of reducing malaria by 75 % by year 2015. Responding to insecticide resistant is not easy or cheap, largely because the approved insecticides used to control adult mosquitoes are so limited. Only four insecticide classes acting on two different target sites are currently registered. Of these, only pyrethroids remain uncontroversial with regard to human and environmental toxicity besides being relatively inexpensive. All four classes of insecticide currently recommended for IRS activities are neurotoxins. Organochlorines such as DDT and pyrethroids attach to the sodium channel of the insect's nerve cell membrane, keeping it open and not allowing the nerve impulse to recharge. The two other classes of insecticide, the carbamates and organophosphates, inhibit acetylcholinesterase (AChE), the enzyme that degrades neurotransmitter acetylcholine at nerve synapses. The activity of AChE terminates synaptic transmission. Blocking the function of AChE causes excessive acetylcholine to accumulate at the synaptic cleft, resulting in neuromuscular paralysis throughout the entire body and eventual death. This deficiency of classes of insecticide acting on different target site austerely limits options for management when resistance does arise.

These recent findings of insecticide resistance in some parts of the country present a serious challenge to malaria control in Zambia. This challenge also presents an opportunity for partner collaboration highlighted in the earlier publication (Chanda 2008). In an effort to improve the entomological monitoring and evaluation and a re-emphasis on the ideals of Integrated Vector Management (IVM) to strengthen the evidence-based implementation of interventions, the Ministry of Health (MoH) through the NMCC and in collaboration with the Zambia Integrated Systems Strengthening Program (ZISSP) has embarked on entomological monitoring including insecticide resistance surveillance. This will facilitate the introduction of a rational insecticide resistance management strategy. In pursuit of this partner collaboration, the NMCC has outlined short and long term strategies.

In the short term, there are three steps which the NMCC wishes to take to address the resistance issue:

1. Establish a network of entomological partners and resources in Zambia, so that the resistance status and mechanisms in different parts of the country can be examined systematically.

2. Form and lead a technical advisory committee that will interpret data collected by the network and make recommendations to the MoH. This committee should also include representatives from other agencies and ministries associated with pesticide use in Zambia, including the Ministry of Agriculture and Livestock and the Ministry Information, Broadcasting and Tourism

3. Develop a short-term response plan which may include recommendations on rotation and/or mosaic spraying of other classes of insecticides with different modes of action from those used previously.

In the long term, the NMCC will need to lead the development and implementation of a strategic plan for the management of insecticide resistance in Zambia. Key elements of this plan would likely include:

1. Determining epidemiological profiles in order to target IRS more effectively. IRS will become much more expensive if carbamates and organophosphates are introduced. It is more important than ever that IRS be used judiciously and effectively, not just as a malaria control tool in itself, but also, by using non-pyrethroids as a resistance management tool, especially when it is co-deployed with LLINs.

2. Assess the options for deploying new formulations of organophosphate insecticides (e.g., primiphos methyl CS) and new active ingredients (e.g., chlorfenapyr). The NMCC and collaborating partners will need to prepare the way for possible use of new formulations in the program.

2 Objectives

The overall objective is to assess the insecticide resistance status of the main malaria vectors: *Anopheles gambiae s.s*, *An. arabiensis* and *An. funestus s.s* in operational settings and characterize possible present resistance mechanisms. The generated data will act as a scaffold on which deliberations of the Insecticide Resistance Technical Advisory Committee will be based on in order to improve the vector control efficacy and inform policy as well as guiding the implementation of future tools against malaria.

3 Specific objective

- Identify and characterize all major malaria vectors in operational settings;
- Determine the specific resistance levels and potentially provide first alerts on the existence or emergence of resistance.

4 Materials and Methods

Mosquitoes were collected between December 2010 and June 2011 from various districts in the Copperbelt, Eastern, Northern, Luapula and Northwestern provinces. The two assay methods, World Health Organization (WHO) tube assay and Center for Disease Control (CDC) bottle bioassay that are commonly used to monitor resistance in mosquitoes, were employed. Mosquitoes were exposed to a wide range of different classes of insecticide to determine the knockdown rates.

Mosquito sampling and handling: By prior arrangement with the residents, live indoor resting adult female mosquitoes were collected from randomly selected houses using the modified CDC backpack aspirator. The collection occurred between December 2010 and June 2011 between 05:00 am and 09:00 am. Approximately 40 houses were sampled in each site. Mosquitoes were transported to the insectary at NMCC in Lusaka in BugDorm insect cages wrapped in a wet cloth. Upon arrival at the insectary, the mosquitoes were either left to rest for about four hours to recover from the stress before conducting the CDC bottle assay or left to lay batches of eggs to obtain F1 for use in the WHO tube assay. Only mosquitoes morphologically identified as *An. gambiae* s.l or *An. funestus* s.l were tested against the selected insecticides. Molecular identification subsequently performed on a subset of the mosquito used in the bioassay confirmed them as either *An. gambiae* s.s or *An. funestus* s.s species. Identification is based upon polymerase chain reaction (PCR) (Scott et al 1993), for *An. gambiae* complex and *An. funestus* differential PCR (Koekemoer et al 2002) and DNA extraction using the simple chelex method (Musapa et al).

5 Resistance Tests

CDC Bottle Bioassay: The resistance tests used the CDC bottle bioassay and followed the guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay (Brogdon and Chan). This bioassay employed 250 ml Wheaton bottles with screw lids, internally coated with the determined diagnostic dose of technical grade insecticide for the anopheline mosquito. For the bioassay of each insecticide in each site, five bottles were used, four for replicates and one for control. Using an aspirator, 10-16 mosquitoes were introduced into the control bottle. For each replicate bottle, 5-25 mosquitoes were introduced depending on the sample size available. All bottles were examined at time 0 minutes and the number of dead mosquitoes was recorded, if any. Dead and live mosquitoes were counted at 15 minute intervals until all mosquitoes were dead, or up to two hours. Mortality in the control bottle was examined at two hours.

WHO Tube Assay: WHO insecticide susceptibility test-kits and standard procedures (WHO 1998) were used to monitor the susceptibility of the wild *An. gambiae* and *An. funestus* populations to technical material of the four chemical groups of insecticides commonly used in IRS programs. Batches of 20 unfed females two to five days old were exposed to filter papers impregnated with 4% DDT (organochlorines), 0.05% deltamethrin, 0.75 % permethrin and 0.05% lambdacyhalothrin (pyrethroids), 0.1% bendiocarb (carbamate) and 5% malathion (organophosphate). Four to six replicates of 20 to 25 mosquitoes were exposed to each test paper. Indoor temperature and relative humidity were maintained at 27 + 2˚C and 70 – 80% respectively throughout the one hour exposure to insecticide impregnated papers. After one hour exposure, the mosquitoes were placed under observation with 10% sucrose solution, and mortality was scored 24 hours post exposure. Control samples were exposed to untreated papers. All exposed mosquitoes together with control samples were morphologically identified and subsequently identified to species level.

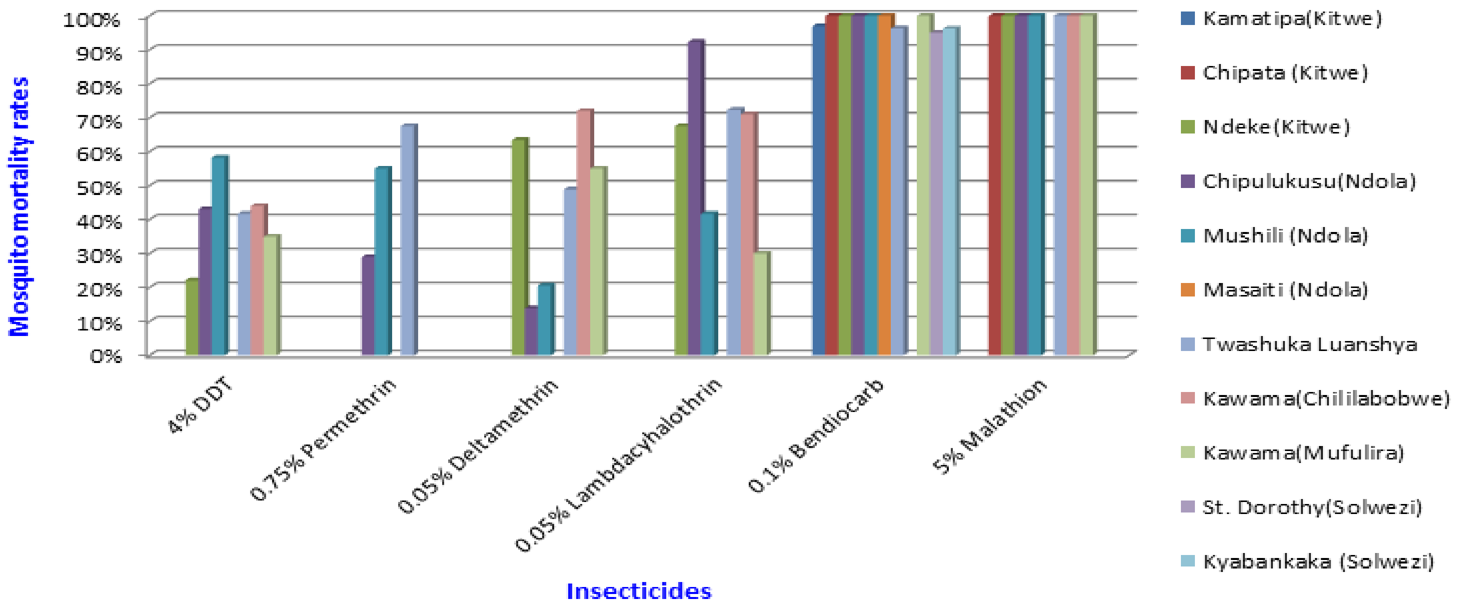
6 Results

Copperbelt Province

The selection of resistance in *An. gambiae* s.l to the two classes of insecticide, organochlorines particularly DDT, and pyrethroids, is still remarkably high and wide spread in the Copperbelt. The results obtained from the two bioassays indicated high levels of resistance to DDT with mortality between 0% and 58%. The mortality with deltamethrin and permethrin was also low between 13% and 72%, and 29% and 67.5 % respectively. The other two classes of insecticides, organophospahtes and carbamates, malation and bendiocarb, respectively have shown to be highly potent in all the mosquito populations tested, achieving 100% mortality rate in many areas.

One interesting observation is the identificarion of *An. funestus* s.l in the Copperbelt particularly in Kamatipa suburb of Kitwe. The sample size which was collected was not sufficient enough to provide for other insecticide testing and therefore, only bendiocarb was tested. The result confirmed susceptibility of this species to bendiocarb which achieved 99 % mortality (Annex 3).

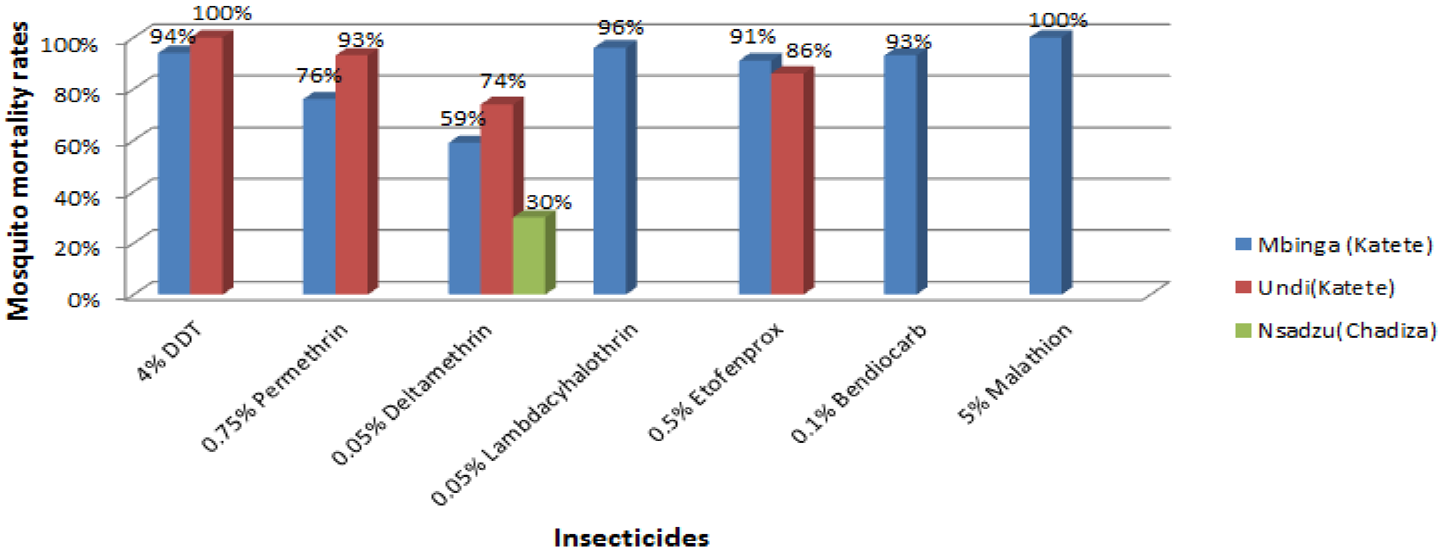
Fig 1: Percentage mortality in field (F1) samples of *An. gambiae* s.l using WHO test tubes.



Eastern Province

Eastern Province is inhabited by *An. funestus* as a major vector with apparent absence of *An. gambiae* s.l. Unlike the Copperbelt, DDT is still potent according to the WHO assay result where 100 % mortality was achieved with mosquitoes from Undi in Katete. But within Katete, Mbinga area, the population of funestus is being selected for DDT resistance as evidenced by mortality between 74% and 93% while selection against permethrin and deltamethrin was highest with mortally rates of 76% and 59% respectively. While it was noted that bendiocarb was potent, the Mbinga funestus population was somewhat not very susceptible. The mortality was 93% with WHO assay and therefore falls within the range of beginning to suspect resistance. Nonetheless, Malathion had achieved 100% demise. Deltamethrin resistance in Nsadzu, Chadiza is very high. Although the sample size was very small, the mortality rate of 30% is relatively low and pointing to a serious problem.

Fig 2: **Percent mortality in field (F1) samples of *Anopheles funestus* exposed to an array of insecticides using WHO assay**



Solwezi District

Solwezi district is unique with regards to vector inhabitants. The two vectors, *An. gambiae* and *An. funestus* exist sympatrically in St Dorothy and Kyabankaka areas. Using the CDC bottle assay, we found that in Kyabankaka the *An. gambiae* had developed high level resistance to DDT while the *An. funestus* was still susceptible. The resistant *An. gambiae* remained active throughout the test period and *An. funestus* all died at diagnostic time. All the results from St. Dorothy are based on the WHO assay and showed notable variation in terms of susceptibility to bendiocarb. *An. gambiae* showed reduced mortality (95%) in comparison to *An. funestus* which showed 100% mortality.

Northern and Luapula Provinces

The tests conducted in Northern and Luapula Provinces were performed between December 2010 and January 2011 and employed the CDC bottle assay. The different mosquito populations in the sampled areas showed 100% mortality to all the insecticides tested as shown in Table 2 above.

7 Discussion

There has been an overwhelming drive to control malaria in Zambia by National Malaria Control Program (NMCP) with support from various partners notably President's Malaria Initiative (PMI), Global Fund and the World Bank. However, the long-term sustainability of this effort depends on demonstrating a beneficial impact on the burden of malaria. Zambia has recorded significant progress in malaria control which has largely been accomplished through a massive increase in vector control through LLINs delivered through large-scale LLIN campaigns and IRS using DDT and pyrethroids. However, these compounding revelations of the evolution of resistance to DDT and pyrethroids in both *An. funestus* and *An. gambiae* in some parts of Zambia threaten to undermine malaria control efforts.

Copperbelt Province: There is now substantial evidence that resistance to DDT and pyrethroids – permethrin and deltamethrin - has evolved in the major vector *An. gambiae* in most parts of the Copperbelt Province. The high level of resistance to these insecticides was confirmed by the mortality results shown in Fig 1. Mortality with DDT was lowest between 22% and 58% in all the five sites sampled. For permethrin, between 29% and 67.5% was recorded whereas for deltamethrin, the range was 13% and 72%. The selection of resistance to deltamethrin and permethrin is of great detriment.

Table 1: Susceptibility status of mosquito vectors in Copperbelt Province- WHO Assay

Location	***An. gambiae s.l***						***An. funestus s.l***					
	% mortality delt. (n)	**% mortality DDT (n)**	**% mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyhalo. (n)**	**% mortality delt. (n)**	**% mortality DDT (n)**	**%.mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyhalo. (n)**
Chipulukusu-**Ndola**	**R**	**R**	**R**	**S**	**S**	**RS**						
Mushili-**Ndola**	**R**	**R**	**R**	**S**	**S**							
Twashuka-**Luanshya**	**R**	**R**	**R**	**RS**	**S**	**R**						
Chipata Cpd-**Kitwe**				**S**	**S**							
Ndeke-**Kitwe**	**R**	**R**		**S**	**S**	**R**						
Kamatipa-**Kitwe**				**RS**						**S**		
Kawama-**Chililabombwe**	**R**	**R**			**S**	**R**						
Kasapa-**Chililabombwe**												
Muchinshi-**Chingola**				**S**	**S**							

Location	*An. gambiae s.l*						*An. funestus s.l*					
	% mortality delt. (n)	**% mortality DDT (n)**	**% mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyhalo. (n)**	**% mortality delt. (n)**	**% mortality DDT (n)**	**%.mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyhalo. (n)**
Kapisha-**Chingola**												
Kawama-**Mufulira**	**R**	**R**		**S**	**S**	**?**						
Chembe East-**Kalulushi**				**S**	**S**							
Chishibambwe-**Masaiti**				**S**								

.

Key: **R**= Resistant, **RS**= Resistance Suspected and **S**= Susceptible

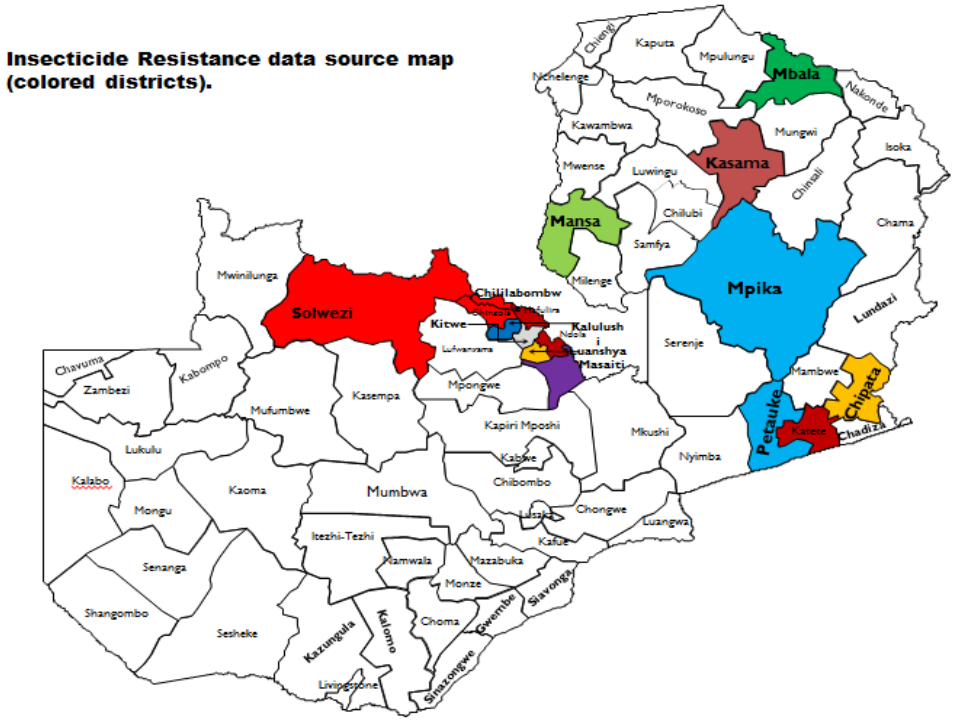
Eastern Province: The susceptibility status of the mosquito in Eastern Province is by and large pointing to targeted or mosaic spraying as a way of managing the problem of resistance to deltamethrin and permethrin (Table 2). In Undi, Katete, DDT remains potent with 100% mortality recorded. Although the tests were conducted on samples collected from a relatively small geographic area and with fewer insecticides tested, these indicators of resistance to pyrethroid must not be ignored. Moreover, NMCC had earlier documented the evolution of resistance in *An. funestus* in Eastern Province.

Table 2: Susceptibility status of mosquito vectors in Eastern Province - WHO Assay

Location	*An. funestus s.l*				
	% mortality delt. (n)	% mortality DDT (n)	% mortality perm. (n)	% mortality bend.(n)	% mortality malath.(n)
Mbinga-**Katete**	R	RS	R	RS	S
Undi –**Katete**	RS	S	RS		
Nsadzu-**Chadiza**	R				

Northern and Luapula Provinces: The issue of insecticide resistance in Mpika, Kasama and Mansa was not observed during the single monitoring activities that employed the CDC bottle assay. DDT and four pyrethroids, deltamethrin, permethrin, alpha-cypermethrin and lambdacyhalothrin, were tested. The results collected confirmed that the vectors, *An. gambiae* and *An. funestus* were susceptible to all four insecticides tested. However, there were observed indications that resistance may be developing to deltamethrin and permethrin as the mosquitoes during the tests were knocked down in the second half of the time period for insecticide exposure (Annex 3).

Solwezi District: There are apparently two major vectors in Solwezi, *An. gambiae* and *An. funestus*, existing sympatrically with each other (but differ in seasonality abundance based on empirical observation). The variation in selection of resistance to DDT observed in Kyabankaka by the two vectors presents a great challenge. According to the results obtained using the CDC bottle assay, DDT still remains potent against *An. funestus* and not efficacious against *An. gambiae*. While both species are known to be competent vectors, it is important to establish the relative contribution of each species to malaria transmission before consideration of the choice of insecticide to be used in vector intervention is made. The data on pyrethroid are not yet generated, however, the WHO assay results indicated that both vector species from both St. Dorothy and Kyabankaka areas are somewhat susceptible to bendiocarb but this needs further verification with a larger sample size.



In summary, given the central place of DDT and pyrethroid insecticides in malaria control today and the emerging threat of insecticide resistance, these two insecticides must be used judiciously. Only pyrethroid insecticides are licensed for use in ITNs. To the contrary, IRS has the advantage of being based on the broader group of licensed insecticides. However, no single intervention, IRS or ITNs, surpasses the other. Both have shown to be effective and are usually deployed in combination to achieve maximum impact. Although there are alternative insecticides to replace DDT and pyrethroids for IRS, there is currently none to replace pyrethroids used in ITNs, and the alternative insecticides for IRS will be relatively expensive and more complicated to use. It is therefore, essential that resistance management strategies be put in place to obviate the loss of ITN efficacy due to growing pyrethroid resistance. This requires that the spatiotemporal insecticide resistance profiles and present underlying resistance mechanisms be determined for the whole country so that an evidence-based decision making process is enhanced.

Reference

Chizema-Kawesha E, Miller JM, Steketee RW, Mukonka VM, Mukuka C, Mohamed AD, Miti SK, Campbell CC. 2010. Scaling up malaria control in Zambia: progress and impact 2005-2008. Am J Trop Med Hyg. 83(3):480-8.

Ministry of Health, Zambia National Malaria Indicator Survey 2008. Available at http://nmcc.org.zm/

Ministry of Health, Zambia National Malaria Indicator Survey 2006. Available at http://nmcc.org.zm/

Musapa M, Mkulama M, Chishimba S, Rouse P, Sikalima J, Norris DE, Thuma PE: Comparison of a simple chelex procedure against standard salt protocol for DNA extraction from wild Anopheles spp. American Society of Tropical Medicine and Hygiene, 56th Annual Meeting. Philadelphia, USA; 2008.

Scott, J. A., W. G. Brogdon and F. H Collins. 1993. Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg. 49(4):520-529.

Annexes

Annex 1: Approved Insecticides

Approved insecticides

Currently, the WHO has approved twelve different insecticides for IRS.

Insecticide	Class	Recommended dosage of active ingredient (g/m^2)[5]	Duration of effective action (months)	Estimated cost per house per 6 months (US$)[6]	WHO toxicity rating[7]
DDT	Organochlorine	1–2	>6	1.60	II
Fenitrothion	Organophosphate	2	3–6	14.80	II
Malathion	Organophosphate	2	2–3	8.20	III
Pirimiphos-methyl	Organophosphate	1–2	2–3		III
Propoxur	Carbamate	1–2	3–6	18.80	II
Bendiocarb	Carbamate	0.1–0.4	2–6	13.80	II
Alpha-cypermethrin	Pyrethroid	0.02–0.03	4–6		II
Cyfluthrin	Pyrethroid	0.02–0.05	3–6		II
Deltamethrin	Pyrethroid	0.02–0.025	3–6	1.60	II
Etofenprox	Pyrethroid	0.1–0.3	3–6		U
Lambda-cyhalothrin	Pyrethroid	0.02–0.03	3–6	8.60	III
Bifenthrin	Pyrethroid	0.025–0.05	3–6		II

Annex 2: Percentage mortality in field samples (F1) of *Anopheles gambiae* s.l. and *An. funestus* s.l 24 hours after a 1-hour exposure to insecticide impregnated papers in WHO test tubes

Location	***An. gambiae s.l***						***An. funestus s.l***					
	% mortality delt. (n)	**% mortality DDT (n)**	**% mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyhalo. (n)**	**% mortality delt. (n)**	**% mortality DDT (n)**	**%.mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyhalo. (n)**
Chipulukusu-**Ndola**	13.8 (96)	43(428)	29.3 (41)	100 (52)	100(27)	92.3 (26)						
Mushili-**Ndola**	20.6 (180)	58.2(244)	55 (31)	100 (102)	100 (47)							
Twashuka-**Luanshya**	48.9 (85)	41.7 (60)	67.5 (40)	96.3 (80)	100 (27)	72.3 (105)						
Chipata Cpd-**Kitwe**				100 (100)	100 (172)							
Ndeke-**Kitwe**	63.4 (82)	22.1 (68)		100 (138)	100 (152)	67.5 (40)						
Kamatipa-**Kitwe**				97 (209)						99 (100)		
Kawama-**Chililabombwe**	72 (65)	44 (45)			100 (38)	71.1 (65)						
Kasapa-**Chililabombwe**												
Muchinshi-**Chingola**				99 (324)	100 (66)							

Location	***An. gambiae s.l***						***An. funestus s.l***					
	% mortality delt. (n)	**% mortality DDT (n)**	**% mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyhalo. (n)**	**% mortality delt. (n)**	**% mortality DDT (n)**	**%.mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyhalo. (n)**
Kapisha- **Chingola**												
Kawama- **Mufulira**	55 (20)	30 (40)		100 (196)	100 (136)	30 (20)						
Chembe East- **Kalulushi**				100 (80)	100 (40)							
Chishibambwe- **Masaiti**				100 (60)								
Kyabankaka- **Solwezi**				96 (80)								
St. Dorothy- **Solwezi**				95 (40)						100 (40)		
Mbinga – **Katete**							59.4 (143)	93.9 (131)	76 (67)	93 (67)	100 (138)	96 (148)
Undi- **Katete**							93.8 (150)	100 (133)	92 (164)			
Nsadzu- **Chadiza**							30 (37)					

Annex 3: Percentage mortality in field samples of *Anopheles gambiae* s.l. and *An. funestus* s.l at diagnostic time using CDC bottle assay

Location	*An. gambiae s.l*						*An. funestus s.l*					
	% mortality delt. (n)	**% mortality DDT (n)**	**% mortality perm. (n)**	**% mortality α-Cyper**	**% mortality malath.(n)**	**% mortality λ-cyh. (n)**	**% mortality delt. (n)**	**% mortality DDT (n)**	**% mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyh. (n)**
Chipulukusu-**Ndola**	87 (107)	17 (82)	71 (112)	98 (54)		100 (97)						
Mushili-**Ndola**	49 (41)	0 (35)	5 (19)	88 (25)		96 (28)						
Twashuka-**Luanshya**		0 (69)		100 (65)								
Ndeke-**Kitwe**	98 (43)	0 (52)		100 (36)		100 (32)						
Kawama-**Chililabombwe**		0 (46)										
Mikambo - **Chililabombwe**		0 (48)										
Kawama-**Mufulira**	88 (51)	1 (82)	69 (70)	100 (84)		100 (98)						
Chembe East-**Kalulushi**	99 (81)	4 (67)	17 (72)	100 (79)		100 (73)						
Mbinga-**Katete**							39 (57)	74 (74)				84 (104)

Location	*An. gambiae s.l*						*An. funestus s.l*					
	% mortality delt. (n)	**% mortality DDT (n)**	**% mortality perm. (n)**	**% mortality α-Cyper**	**% mortality malath.(n)**	**% mortality λ-cyh. (n)**	**% mortality delt. (n)**	**% mortality DDT (n)**	**% mortality perm. (n)**	**% mortality bend.(n)**	**% mortality malath.(n)**	**% mortality λ-cyh. (n)**
Undi- **Katete**							25 (8)				78.6 (14)	55 (20)
Teleka-**Chipata**											88.9 (27)	
Chipungo- **Chipata**								54.6 (11)				
Nyane- **Chipata**												96.7 (30)
Zundo- **Chipata**								55.6 (11)				
Chikakala- **Mpika**							100 (59)		100 (23)		100 (55)	
Tibi- **Kasama**						100 (48)						
Kapapa- **Kasama**											100 (40)	
Mabumba- **Mansa**	100 (34)		100 (11)		100 (20)		100 (64)		100 (25)		100 (79)	
Chembe- **Mansa**	100 (56)		100 (42)		100 (84)		100 (24)		100 (51)		100 (82)	