REPUBLIC OF ZAMBIA MINISTRY OF HEALTH

INSECTICIDE RESISTANCE MANAGEMENT AND MONITORING PLAN IN ZAMBIA

2019

NATIONAL MALARIA ELIMINATION PROGRAMME WWW.NMEC.ORG.ZM

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Table of Contents

ABREVIATIONS

Ace-1R	Acethyl 1 Resistance
AIRS	Africa Indoor Residue Spraying
ANC	Antenatal Care
CDC	Centre for Disease Control
CS	Capsule Suspension
DDMS	Disease Data Management System
DDT	Dichlorodiphenyltrichloroethane
E8	Elimination 8
EC	Emulsifiable Concentrate
EHO	Environmental Health Officer
EHT	Environmental Health Technologist
EIR	Entomological Inoculation Rate
FTE	Field Technical Entomologist
GPIRM	Global Plan for Insecticide Resistance Management
GR	Granule
HLC	High Liquid Chromatography
HMIS	Health Management Information System
ACE	Acetylcholinesterase
ICEMR	International Centre of Excellency for Malaria Research
IRMM	Insecticide Resistance Management and Monitoring
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Net
kdr	Knock Down Resistance
LLINs	Long-lasting Insecticide-treated Mosquito Nets
LSM	Larval Source Management
M&E	Monitoring and Evaluation
MACEPA	Malaria Control and Evaluation Partnership in Africa
MIS	Malaria Indicator Survey
MOH	Ministry of Health
MPC	Ministerial Procurement Committee
NMEC	National Malaria Elimination Centre
NMEP	National Malaria Elimination Plan
OPD	Outpatient Department
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PM-CS	Pirimiphos-Methyl Capsule Suspension
PMI	Presidential Malaria Initiative
PPF	Pyriproxifen
PSC	Pyrethrum Spray Catch
PVC	Polyvinyl Chloride
RN	Ribonucleic Acid
TAC	Technical Advisory Committee
TDRC	Tropical Disease Research Centre
WHO	World Health Organization
WHOPES	World Health Organisation on Pesticides Evaluation S
WP	Wettable Powder
ZEMA	Zambia Environmental Management Agency

Executive summary

The Ministry of Health (MOH) through the National Malaria Elimination Centre (NMEC) in collaboration with the United States Presidential Malaria Initiative (PMI) supported Vectorlink, Bill and Melinda Gates Foundation (BMGF) supported Malaria Control and Evaluation Partnership in Africa (MACEPA), Tropical Disease Research Centre (TDRC) and Macha Research Trust with technical support from the University of Zambia (UNZA) and the World Health Organisation (WHO) country office, reviewed and developed the National Insecticide Resistance Monitoring and Management Plan. The IRMMP aims at detecting insecticide resistance among the malaria vectors against insecticides used for vector control in the country and assessing the levels and mechanism(s) of resistance when detected in order to prevent or delay the development of resistance by rotating insecticides every two years.

Having had recorded gains in the fight against malaria in the past, the National Malaria Elimination Strategic Plan (NMESP) has focused on strategies including vector control to accelerate the elimination of malaria in Zambia. Despite the gains, malaria still remains a major leading cause of morbidity and mortality in the country. One of the contributing factors is the widespread insecticide resistance observed among the malaria vectors against pyrethroids, organochlorines and carbamates. Although no resistance has been detected against organophosphates, its prolonged use exposes this class of insecticides to possible resistance.

Recent sensitivity data shows restoration of susceptibility to some insecticides mentioned above, giving hope to the effectiveness of insecticides used for vector control. This coupled with the introduction of next generation insecticides, in the neonicotinoids class with new modes of action, to public health, enables the rotation of insecticides for the implementation of the IRMMP.

The MOH/NMEC and various partners will ensure resources are mobilised to implement the IRMMP effectively. The IRMMP will provide evidence-based guidance to the program on the selection of appropriate vector control interventions and products. The MOH will thus prioritise informed procurement of affordable vector control products with long residual efficacy.

Acknowledgement

The process of developing the Insecticide Resistance Management and Monitoring Plan (IRMMP) was collaborative, interactive and consultative. All Units at NMEC were involved. I wish to recognize the significant role and valuable input provided by NMEC members of staff during the development of the IRMMP. Allow me to also thank all the partners and stakeholders who contributed to the successful production of the IRMMP meant to be used by Entomologists and implementers of Entomological Surveillance at national, provincial, district and community levels.

The NMEC through the Ministry of Health would like to further recognize the Vector Control Team at NMEC and Environmental Health Practitioners from all the ten (10) Provincial Health Offices for providing invaluable inputs during the development of this document.

Special thanks go to the Global Fund to Fight AIDS, Tuberculosis and Malaria for providing financial resources to support the process of developing and printing of the Insecticide Resistance Management and Monitoring Plan in Zambia.

Finally, I wish to express my gratitude all individuals who worked tirelessly and contributed in one way or another to ensure the successful development of the IRMMP.

Dr. Mutinta Mudenda
Acting Director –National Malaria Elimination Centre
MINISTRY OF HEALTH

2. Situation analysis

A.2.1 Malaria epidemiology in Zambia

Malaria remains major cause of morbidity and mortality in Zambia accounting for close to 4 077 547 confirmed cases and over 3,257 deaths (WHO, 2013). In Zambia, the majority of malaria cases are caused by *Plasmodium falciparum* whilst *P. malarie, P. ovale* and *P. vivax* are extremely rare. The main Anopheline species are the *Anopheles gambiae* complex (namely *An. gambiae* s.s. and *An. arabiensis*) and *Anopheles funestus s.s* (Chanda *et al.*, 2016; Fornadel *et al.*, 2010; Norris and Norris, 2011; Mukonka, *et al.,* 2014; Seyoum, 2012; WHO, 2015).
Figure 1.

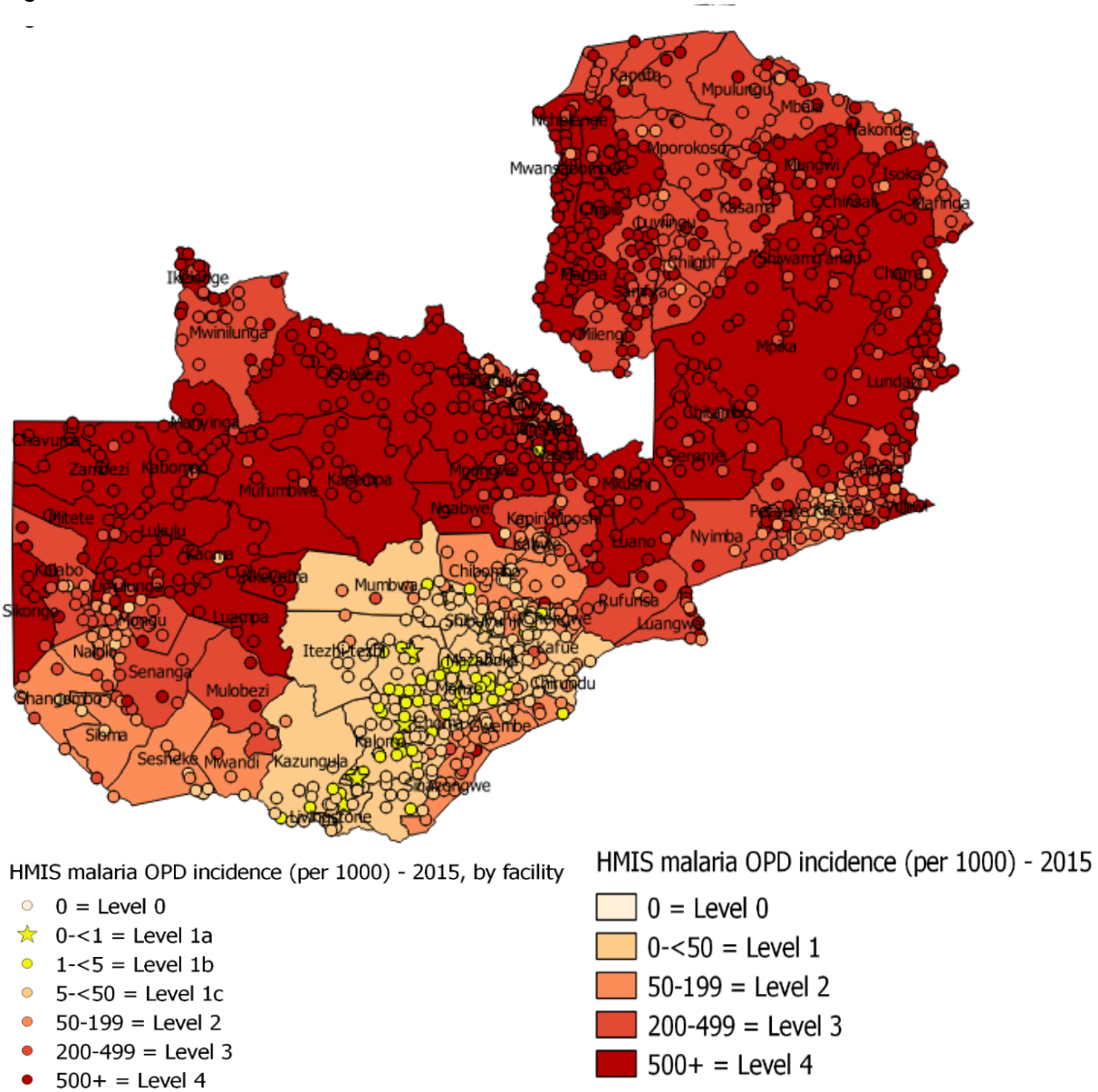


Figure 1. *Malaria (all cases) distribution in provinces, districts and health facility catchment areas based on 2015 HMIS data, Zambia (Source: HMIS, Zambia)*

Currently, Zambia has an average malaria incidence rate of 335/1000 persons. However, substantial variations exist from province to province (Figure 1). The 2015 Malaria Indicator Survey (MIS) and the Health Management Information System (Ministry of Health, 2015) indicates that the highest incidences occur in North Western province (more than 806.9 per

1000 persons) whilst the lowest occur in in Southern province (26.7 per 1000 persons). Both incidence rates and prevalence rates of malaria reveal a regional variation which is seemingly limited to geographic or ecologic influences. Rural populations, compared to urban populations or peri-urban areas are more at risk. Also at risk are populations at lower altitudes and possibly living near water bodies that provide mosquitoes with potential breeding sites e.g. in Eastern province in the Luangwa River valley.

Also noteworthy is that all provinces in Zambia recorded higher incidence of malaria among children under the age of 5 years compared to persons aged 5 years and older. Pregnant women are also at high risk of malaria, partially due to lowered immunity during pregnancy (Guyatt and Snow, 2001). This lends support to the current ITN policy which targets young children and pregnant women through mass distribution campaigns and routine distribution to pregnant women during antenatal care (ANC) clinics so as to increase in ITN ownership and utilization (Chizema-Kawesha, 2010; Sikambale *et al.*, 2013).

Despite the high incidence rates, retrospective data shows that malaria incidence rates have been on the decline. Inamboa *et al.* (2017) show that incidence rates increased by 6% between 2013 and 2014, and then decreased by 18% between 2014 and 2015, resulting in an overall decrease of 12% for the 2013-2015 time periods. Further, annual numbers of reported inpatient cases and deaths due to malaria have been reduced by 52% and 65% respectively since 2010 (Ministry of Health, 2015). The World Malaria report (WHO, 2015) also reveals significant gains made in the fight against malaria. Deaths due to malaria have plummeted from 9,369 in 2001 to just above 3000 in 2014. This marks over 65% reduction in malaria in the past 15 years. Whilst these gains are commendable, it is needless to say malaria still remains among the top killers of children in Zambia.

A2.2 Vector Control interventions

There are three (3) key vector elimination strategies planned for malaria elimination in Zambia; indoor residual spraying (IRS), long-lasting insecticide-treated mosquito nets (LLINs) and larval source management as a supplementary optional intervention (National Malaria Elimination Strategic Plan, 2017). Larval source management (LSM) is done on a low scale in selected mining towns and sugar plantations on the Copperbelt/North Western and in Southern Provinces of Zambia respectively (Chanda *et al.,* 2008).

2.2.1 Implementation

The current insecticide used for the Indoor Residual Spraying (IRS) programme is pirimiphos-methyl capsule suspension (PM-CS). The dosage of the insecticide sprayed is one gram of active ingredient per meter squared (1g a.i/m^2). Timing of the spray campaign varies from district to district within the country depending on the onset of rain season. which is generally between September and December prior to peak Malaria transmission season during and just after the rains. The target for the elimination strategy is to achieve an operational coverage of over 90 percent of eligible structures which would benefit at least 80% of the population of Zambia (Hamainza *et al.,* 2016). Table 1 below shows population protected with IRS intervention between 2014 to 2017 (NMEC, 2017).

Table 1: *Percentage of population protected by Indoor Residual Spraying in Zambia, 2014-2017 (Source: National Malaria Elimination Centre).*

Year	Population	Number of people per structure	Number of Structures sprayed	Population Protected
2014	15,023,315	4.47	1,239,835	5,538,574 (36.9%)
2015	15,473,905	4.43	1,339,775	5,930,141 (38.3%)
2016	15,933,883	4.07	1,655,542	6,737,918 (42.3%)
2017	16,405,229	4.32	1,915,821	7,800,704 (50.9%)

Also integral to the fight against malaria has been effective vector control mainly by mass distribution of Long Lasting Insecticide-treated Nets (LLINs). Whilst IRS provides chemical protection through lethal exposure to toxic active ingredients to mosquitoes resting on the walls before and/or after a blood meal, LLINs offer protection by both as a physical barrier as well as chemical barrier to human hosts. Both these methods have shown community-level suppression of vector population density and infection prevalence (Hamainza *et al.*, 2016; Stuckey *et al.* 2016).

The type of ITN currently in use in Zambia are LLINS, and are in two forms namely PermaNet 2.0™ and Olyset net™ which are made of 100% polyester and polyethylene respectively. The 2015 Malaria Indicator Survey (MIS, 2015) showed an increase in national LLIN ownership from 64% in 2010 to 68% in 2012 and 75.7% in 2015. Significant progress recorded with more than half of the households (64%) reportedly having sufficient LLINs to cover all sleeping spaces compared to 34% in 2010. The LLIN usage rates among children under five increased from 50% in 2010 to 57% in 2012 from to 59% 2015 and the LLIN usage rates among women of reproductive age increase from 46% in 2010 to 58% in 2012 and 60% in 2015.

Figure 2 below shows the cascade of net usage in Zambia, from possession of a single ITN to possession of ITNs to cover every sleeping space. Notably, the percentage difference between those in possession of a single ITN (77.6%) to those in possession of ITNs for every sleeping space is very large (77.6% for those households with single ITNs and 49.7% for those with adequate ITNs to cover all sleeping spaces.).

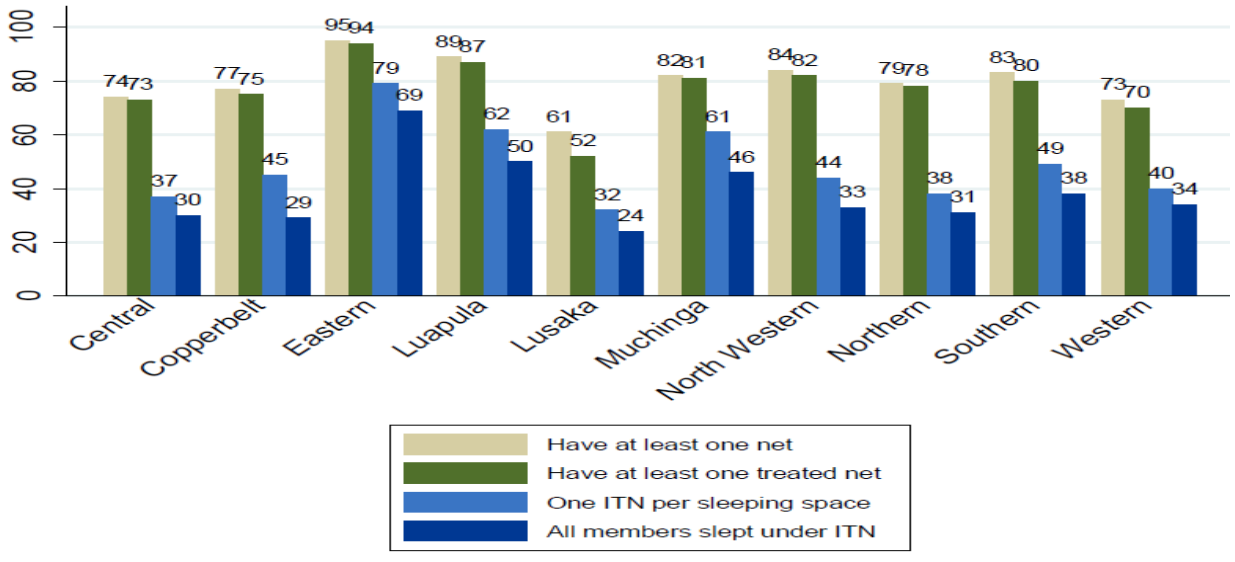


Figure 2: *Net ownership, coverage and usage (Source: MIS, 2015)*

Due to the costs and logistics of running IRS campaigns, IRS is largely viewed as a complementary vector control strategy to the more widespread coverage obtained with ITNs. Figure 2 shows the availability of at least one vector control method (IRS or ITNs) at household level as well as the combined coverage (ITNs and IRS) at household level. IRS was scaled up in rural areas during the 2014 spraying campaign and urban and rural areas are now nearly equal in terms of dual coverage. The rationale for dual coverage is to address resistance challenges especially that IRS uses an organophosphate while LLINS are embedded with pyrethroids.

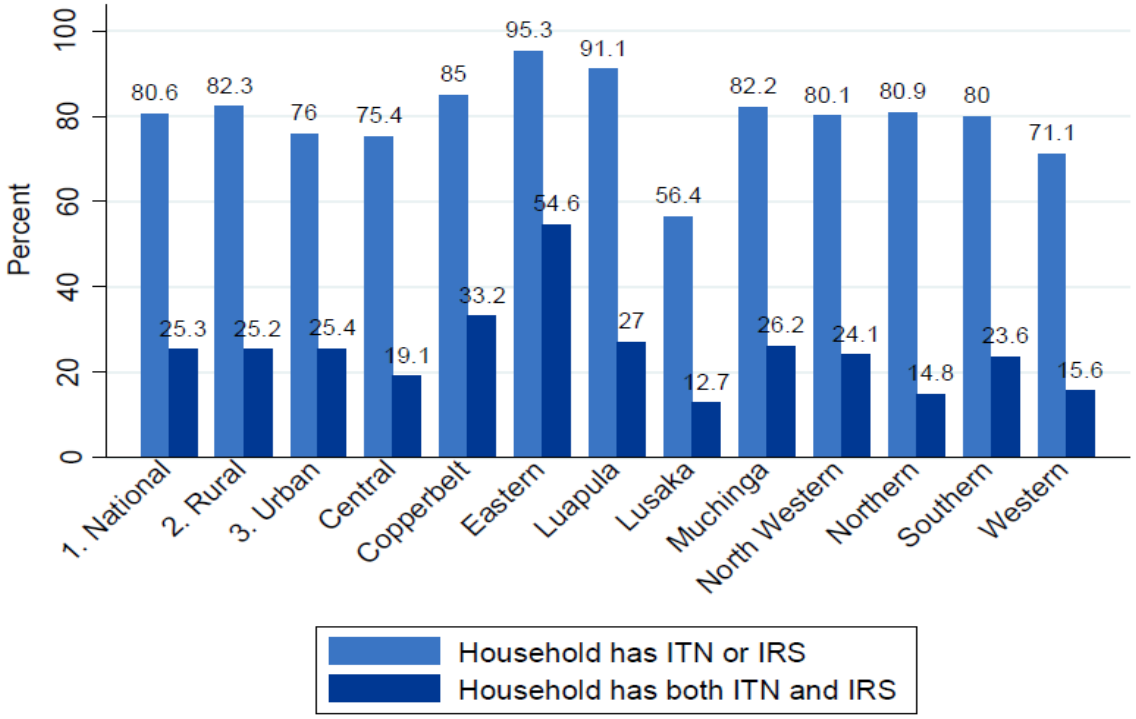


Figure 3: *Percentage of households with at least one insecticide-treated net (ITN) and/or indoor residual spraying (IRS) (MIS - Zambia 2015)*

Prior to 2005, IRS was conducted primarily on the Copperbelt Province in surrounding mining communities. IRS was scaled up to include 15 districts in 2005, 36 in 2008, 54 in 2010, and 72 in 2011. Until 2007, spraying was targeted in urban and peri-urban zones, but since then it has expanded to more rural areas to better align the intervention with malaria burden. DDT or pyrethroids were sprayed in the original 15 districts from 2005-2010. Districts added in 2008 and 2010 were sprayed with pyrethroids (λ-cyhalothrin, deltamethrin, or alpha-cypermethrin).

The insecticides used in each area of the country were modified in 2011 (figure 4). During the 2013-2014 transmission season, Northern, Muchinga, and Eastern provinces were sprayed with an organophosphate insecticide while other provinces were sprayed with carbamates and pyrethroids. In 2014 to 2017, the whole country migrated to organophosphate (Pirimiphos-methyl CS) based on entomological surveillance findings.

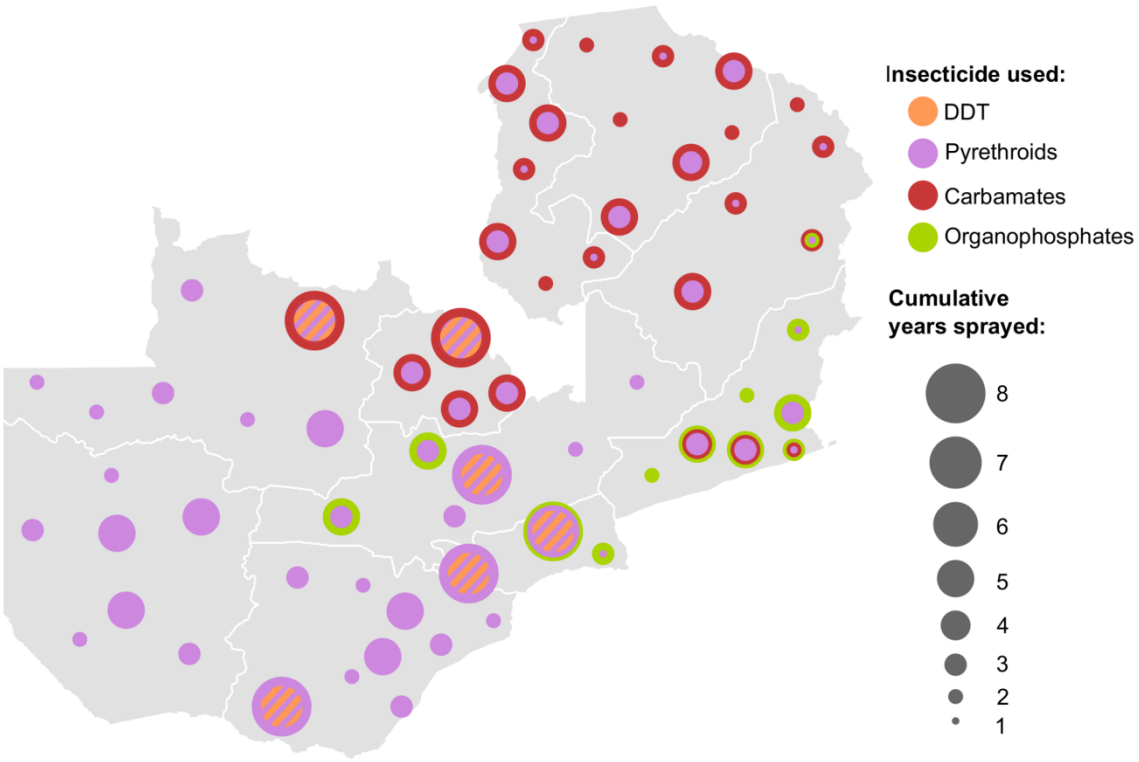


Figure 4. Spatiotemporal pattern of insecticide use for IRS in Zambia from 2005-2012.

Each dot represents the insecticide history for a single district or cluster of districts with similar history (Copperbelt Province). The earliest insecticide used is indicated in the centre of each dot. Subsequent insecticides are added as layers, with the thickness of the layer representing how many years the insecticide was used. Different colours represent different insecticide classes. The size of the dot indicates how many years IRS has been active. Hashed areas indicate times and locations where DDT and pyrethroids were used concurrently, with the former on mud homes and the latter on painted surfaces. In 2013, only Northern, Muchinga, and Eastern provinces were sprayed with organophosphate (Source: National Malaria Elimination Centre, Zambia).

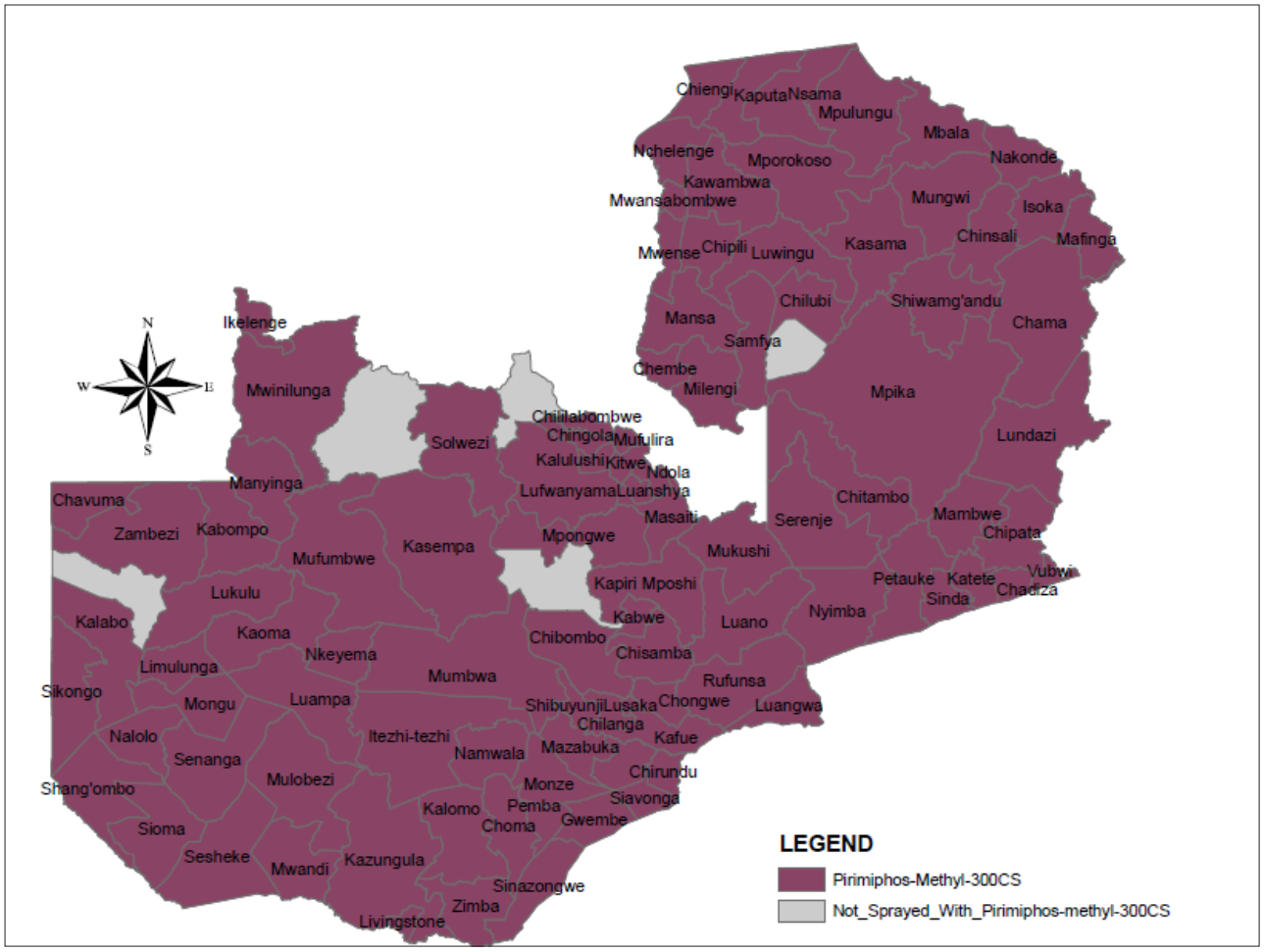


Figure 5 Map showing insecticides used from 2014 to 2017.

2.2.2 Monitoring

Quality assurance and insecticide decay rate 2016-2017 have been done as shown in figure 3 below.

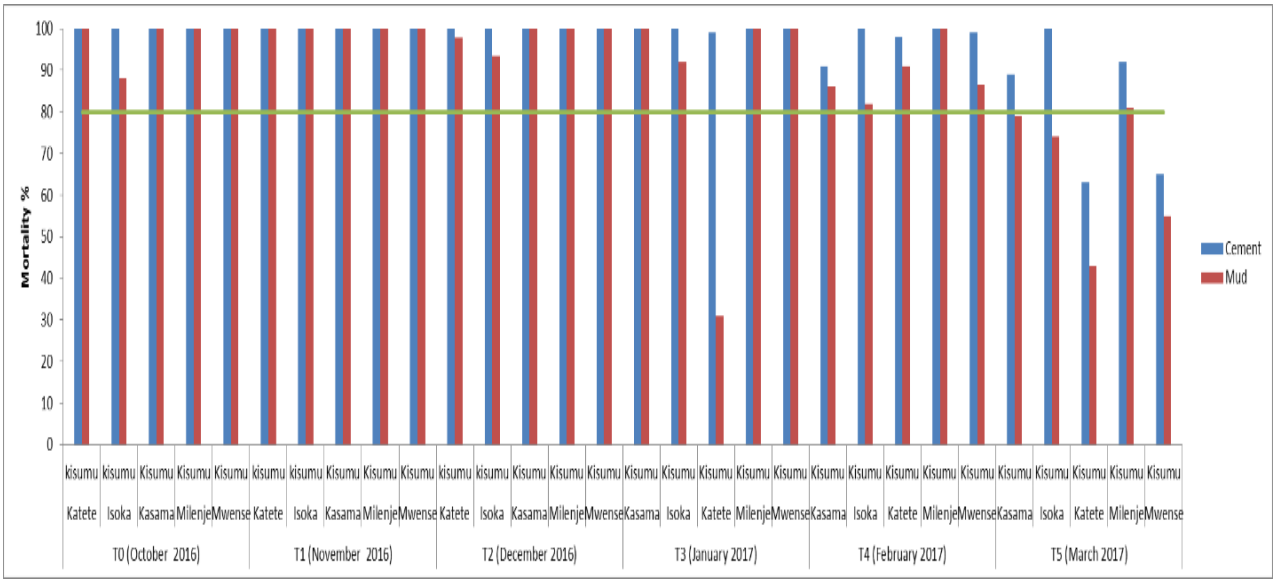


Figure 6: *Quality assurance and insecticide decay rate 2016-2017 (Source: PMI AIRS ZAMBIA 2016 entomology final report).*

According to Tan *et al.* (2016), eight districts in Luapula and Northern province showed that insecticidal activity trended downwards by 2 years of age, and Olyset™ nets seemed to have a significantly lower insecticidal activity at 12 months than PermaNet™ nets. The true threshold for determining when insecticidal protection is inadequate has not been well defined.

Table 2: *Cone bioassay on Olyset R and Permanet 2.0 results using Anopheles gambiae s.s (Kisumu strain) by net type and age (Source: Tan et al. 2016).*

Net age at follow-up	PermaNet®	Olyset®	All nets
Geometric mean of % of mosquitoes knocked down at 60 min of exposure (n) GM 95 % CI			
12 months	(18) 92.6 87.6–97.8	(18) 79.4 71.6–88.0	(36) 85.7 80.9–90.9
24 months	(18) 29.6 12.3–69.1	(20) 42.2 27.2–65.3	(38) 35.7 22.3–56.7
Average % mortality at 24 h of exposure			
12 months	(18) 89.1 83.2–95.4	(18) 47.1 33.6–65.7	(36) 64.8 54.7–76.8
24 months	(18) 31.9 14.9–67.0	(20) 51.4 38.7–68.2	(38) 41.0 27.9–60.1

GM geometric mean, *CI* confidence interval

2.2.3 Evaluation

Assessments on the impact of vector control interventions are mostly based on entomological surveillance. An example is shown below.

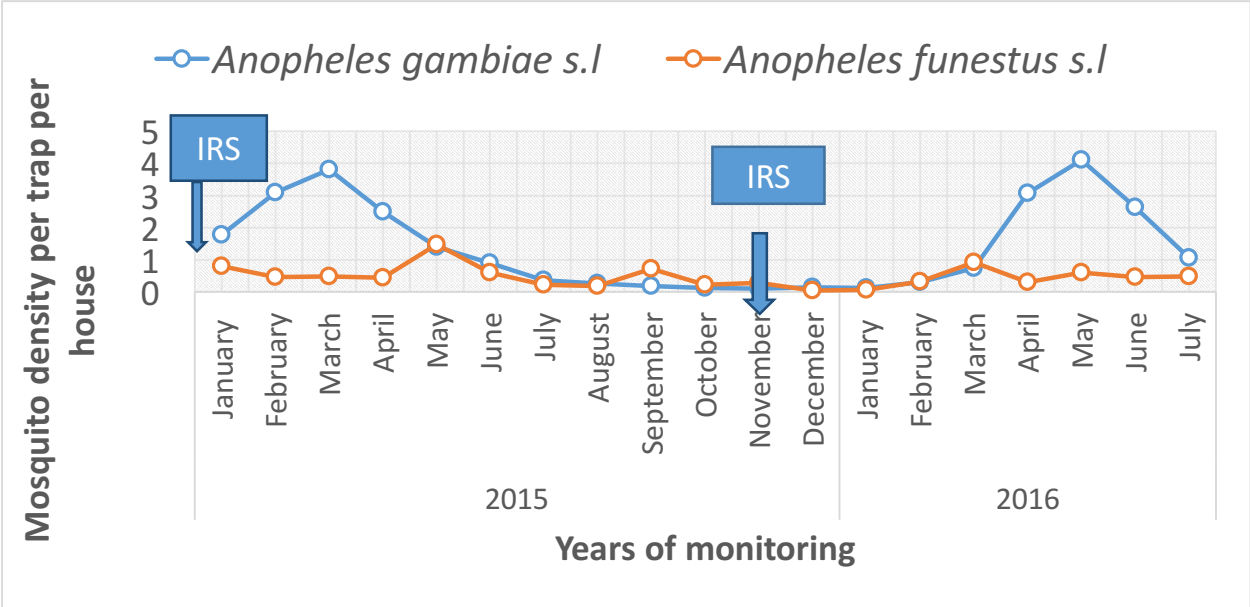


Figure 7: Monthly abundance of An. gambiae s.l, and An. funestus from Southern province Zambia as measured by CDC Light traps, January, 2015 – July, 2016 (Source: Personal communication from Javan Chanda).

As shown above, population densities of *Anopheles funestus* s.l are rapidly reducing while populations for *Anopheles gambiae* s.l seem to persist following the deployment of IRS with pirimiphos methyl (ACTELLIC) in Southern Zambia. The suppression of *An.funestus*, a primary vector of malaria suggests high possibility of eliminating malaria in this region of Zambia.

The above is an example of the effects that IRS can have on a mosquito population and hence malaria transmission. The deployment of IRS with pirimiphos-methyl in December, 2015 (combined with the distribution of LLINs earlier) suppressed both *An. gambiae s.l.* and *An. funestus* indoor host seeking densities to minimal levels even though persistence of *An. gambiae* s.l. was observed. The above findings suggest that species of *An. funestus* are more amenable to control using IRS and LLINs than *An. gambiae* s.l. In addition, the behavioral characteristics of *An. funestus* s.s to bite and rest predominantly indoors makes these species more vulnerable to insecticide contacts than *An. arabiensis* which bites and rest more outdoors and are less dependent on human blood.

A2.3 Registration of Insecticides

The Zambia Environmental Management Agency (ZEMA) is the national institution mandated to register all pesticides in Zambia. use. The main services provided by the agency include:

- Enforcement of regulations and standards on all aspects of the environment.
- Advising government on the formulation of policies, standards and regulations related to environmental management.
- Administration of the Environmental Assessment processes.
- Environmental education and awareness programme to educate and raise awareness on the role of the public in the protection of the environment.
- Collection, production and dissemination of environmental information.

The current processes of insecticide registration involve applying for pesticide and toxic substances licenses to allow the importation of any chemical product into the country and application for hazardous waste management. See annex for details of the forms.

There are various pesticides registered for both public health and agriculture in Zambia, the table below contains insecticide classes that are used for public health and agriculture. The full list of agriculture insecticides can be obtained from the annex as supplementary material.

Table 3: *Registered insecticides in Zambia.*

Insecticide Class	Insecticide Type	Use (IRS, LLINs), Larvicide, or Agriculture	Formulation	Date Of Registration
Organophosphate	Pirimiphos-methyl	IRS	SC	
Organophosphate	Pirimiphos-methyl	Larvicide	EC	
Pyrethroids	Lamdac-cyhalothrine	IRS	EC	
Organophosphate	Abate	Larvicide	GR	
Pyrethroids	Permethrin	LLIN	Incorporated permethrin	
Pyrethroids	Deltamethrine	LLIN	Coated	

			polyester	
Organophosphate	Pirimiphos-methyl	Agriculture	EC	
Organophosphate	Pirimiphos-methyl	Agriculture	SC	
Organophosphate	Methamidophos	Agriculture	SC	
Organophosphate	Fernithrothion	Agriculture	EC	
Organophosphate	Malathion	Agriculture	EC	
Pyrethroids	Cypermethrine	Agriculture	EC	
Pyrethroids	Alpha-cypermethrine	Agriculture	EC	
Pyrethroids	Zeta-cypermethrine	Agriculture	EC	
Pyrethroids	Lambda-cyhalothrines	Agriculture	EC	
Pyrethroids	Plallethrine	Agriculture		
Pyridine	Pyripproxylene	Larvicide	EC	
Synergist	Piperonyl Butoxide	Industrial		
Carbamate	Carbofuran	Agriculture	GR	
Organophosphate	Chloropyriphos	Agriculture		
Organo-chlorine	Dichloropropane	Agriculture	WP	
Acaricide	Chromizine	Larvicide		

DDT, Dichloro diphenyl trichloroethane; WT, wettable powder; GR, granule; SC, capsule suspension; EC, Emulsifiable concentrate, LLINs, long lasting insecticide treated net; IRS, indoor residual spraying.

A2.4 Entomological surveillance, including insecticide resistance monitoring

A.2.4.1 Main vector species

Distribution

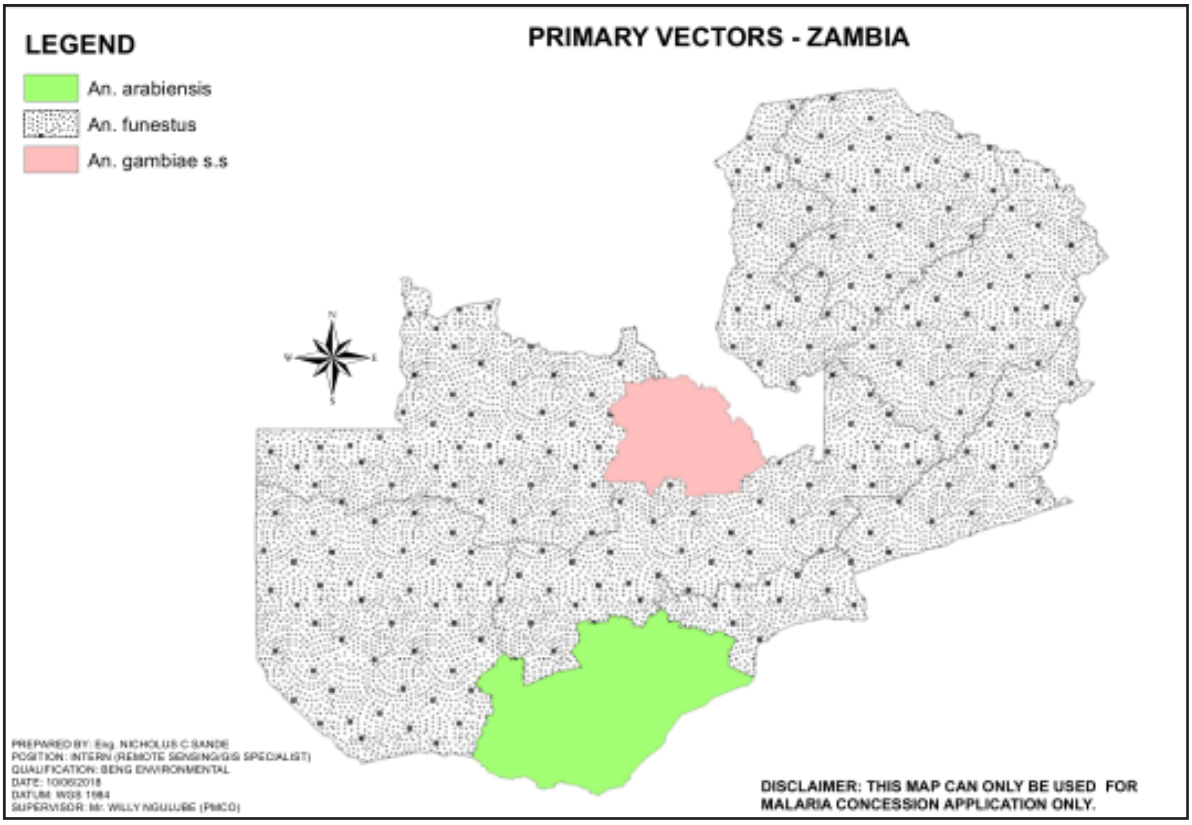
Zambia has three main malaria vectors. *An. funestus s.s., An. gambiae s.s*, and *An. arabiensis.* While all three vector feed on humans, *An. arabiensis*, is also able to feed on animals. There are also a number of other anopheline species that have been shown to occasionally feed on people and have been found harbouring *Plasmodium falciparum* parasites (Lobo et al 2016). Mosquitoes that feed and rest in indoors can be targeted by ITNs and IRS, those feeding and resting outdoors cannot.

Table 4: *below summarises the data available on each species.*

Species	Vector status	Method used to confirm species	Geographical distribution	key larval habitat	Vector behavior
An. funestus s.s.	Main	Morphology/ PCR	Nationwide dominating in most of the province except with restricted pockets in Southern Province and Lusaka provinces	Limited reports due to the difficulty in finding breeding sites. Reports from stream edges in Luapula	Highly anthropophilic, feeds indoors and outdoors. Rests indoors, no data on outdoor resting. Feeding peaks in the middle of the night but starts as early 18hrs up to 06hrs
An. gambiae s.s.	Main	Morphology/ PCR	Found in large numbers on the Copperbelt, North-Western but not as numerous in Central Eastern, Luapula, Northern, Muchinga, Western No found/limited in Southern and Lusaka provinces	Rain water puddles, hoof prints, garden ponds and fish ponds, boats, dams, stream edges etc	Highly anthropophilic, feeds indoors and outdoors. Rests indoors, no data on outdoor resting. Feeding peaks in the middle of the night but starts as early 18hrs up to 06hrs
An. arabiensis	Main	PCR	Dominates in Southern, Central, Western and possibly Eastern, Lusaka provinces	Rain water puddles, hoof prints, garden ponds and fish ponds, boats, dams, stream edges etc	Anthropophilic from indoor collections and zoophilic from outdoor collections. Feeds indoors and outdoors. Rests indoors and no data on outdoor resting. Feeding indoors peaks in middle/late night but starts from 18hrs to 06hrs. Outdoors, feeding starts as early as 16hrs and peaks early about 20hrs .
An. leesoni	Potentially secondary	PCR	Reports from Luapula province	not known	Mainly zoophilic

An. squamosus	Potentially secondary	Morphology/ PCR/ sequencing	Reports from Southern, Northern, Eastern, Central, Luapula Provinces, Northwestern	Report from dams, rain water puddles	Mainly zoophilic and exophagic
An. rufipes	Single reports	Morphology/ PCR	Reports from Copperbelt, Southern, Lusaka, Eastern	Report from rain water puddles	Mainly zoophilic and exophagic
An. coustani	Potentially secondary	Morphology/ PCR/ sequencing	Nation wide	Report from dams, rain water puddles	Mainly zoophilic and exophagic. Early outdoor biting
An. pretoriensis	single reports	Morphology/ PCR	Reports from Copperbelt, Lusaka, Eastern, Southern, Western	not known	Mainly zoophilic and exophagic. Early outdoor biting
An. quadriannulatus	single reports	PCR	Reports from Eastern, Lusaka, Southern Provinces	not known	Mainly zoophilic and exophagic

The spatial distribution of the three main malaria vectors has been modelled by the Malaria Atlas Project (www.map.ox.ac.uk) However, this is very generic and lacks fine resolution; several areas deemed to have low probability of occurrence of species are incorrect. It is not suitable for guiding the control programme. Using the data available maps by district of the dominating species and other main vectors are shown in figures xx and xx. Areas in grey depict where there are no available data. Note that much of the data per district may be single/seasonal collections and some may date back several years. More data are required to update these maps regularly and ensure what is presented reflects seasonal changes. Having accurate maps of combination of vectors species and insecticide resistance can guide the targeting of different insecticides.



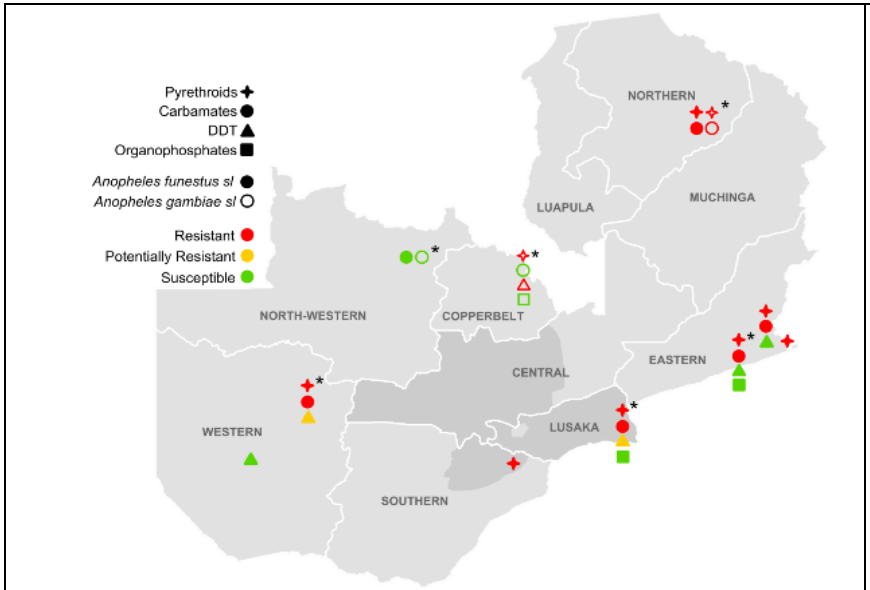
A2.4.2. Vector Insecticide Susceptibility Status

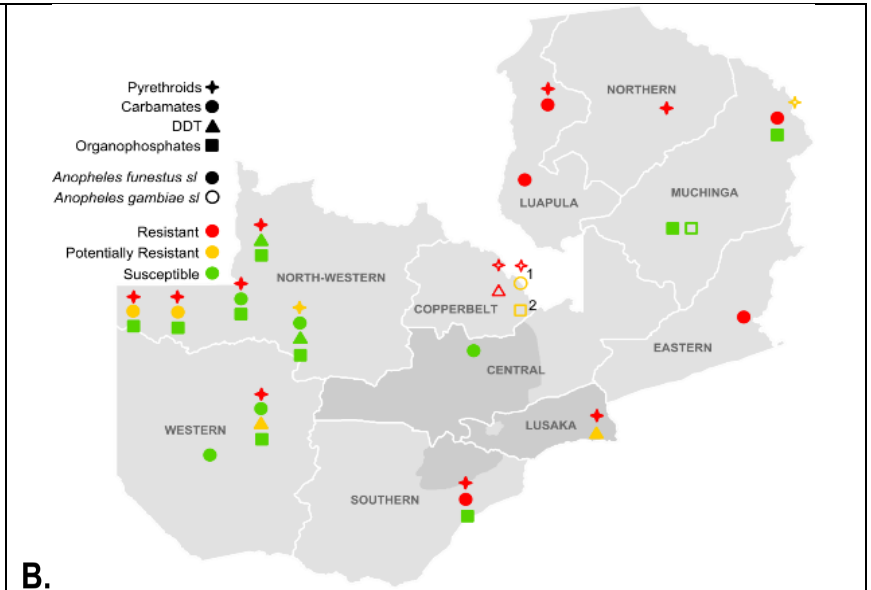
The insecticide resistance status of *An. funestus* and *An. gambiae s.l*. to the four classes of insecticides since 2011 is presented in figure 9.

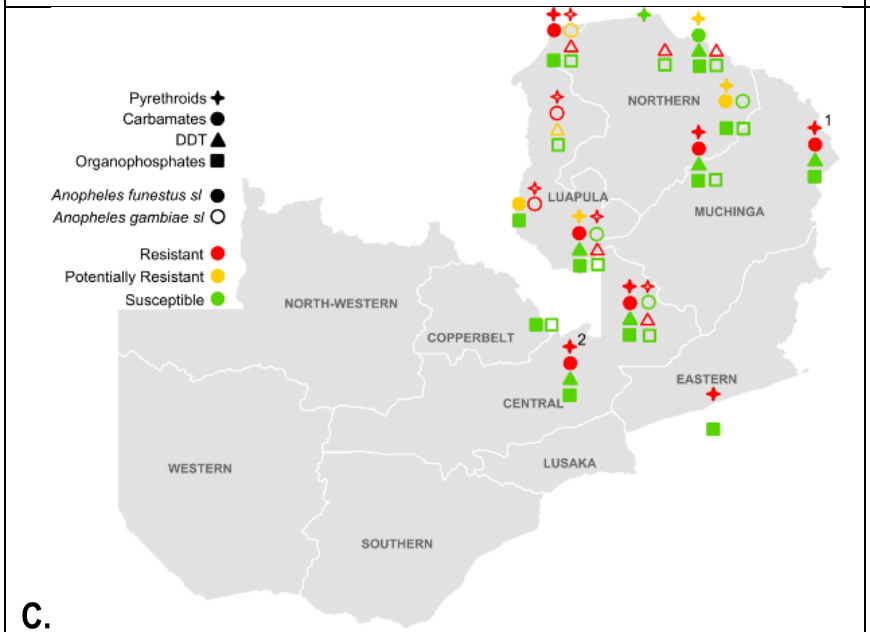
An. gambiae s.l. was resistant to Bendiocard between 2013 and 2015 in most of the districts. Signs of carbamate susceptibility restoration have been observed recently 2016-2017 mostly in Eastern province but not the case with Nchelenge in Luapula. No resistance against Organophospahates has been reported in *An. gambiae s.s.* so far however the borderline mortality (98%) in the 2016-2017 data from Nchelenge should be monitored closely. Pyrethroid resistance continues to be reported in many parts of the country. Susceptibility to pyrethroids however has been reported *An. arabiensis* in southern Zambia.

Anopheles funestus resistance to carbamates was confirmed in 2013 which remained consistent up to 2015 -2016. Though resistance still persisted the mortality rates in *An. funestus* from Kasama and Milenge improved to between 97 and 100% during the latest evaluations data. Pyrethroid resistance in An. funestus continued to be reported. Susceptibility to DDT continues to be recorded but recent data showed reduced %mortality in Milenge and Nchelenge in Luapula province. Generally no resistance has been reported against the Organophosphates in *An. funestus*.

Figure 9: Insecticide resistance maps, 2011 to 2014.





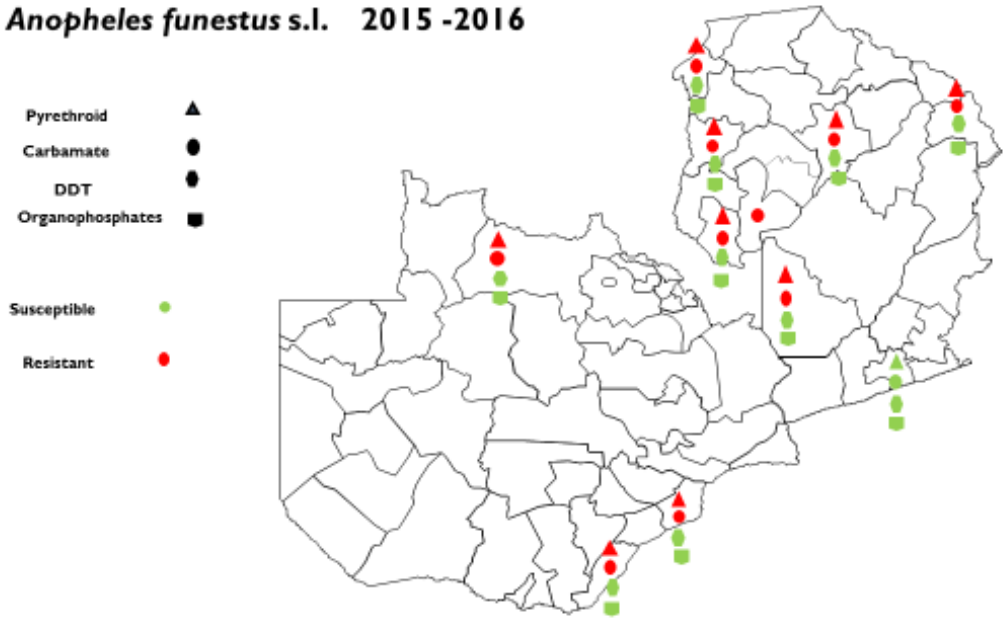


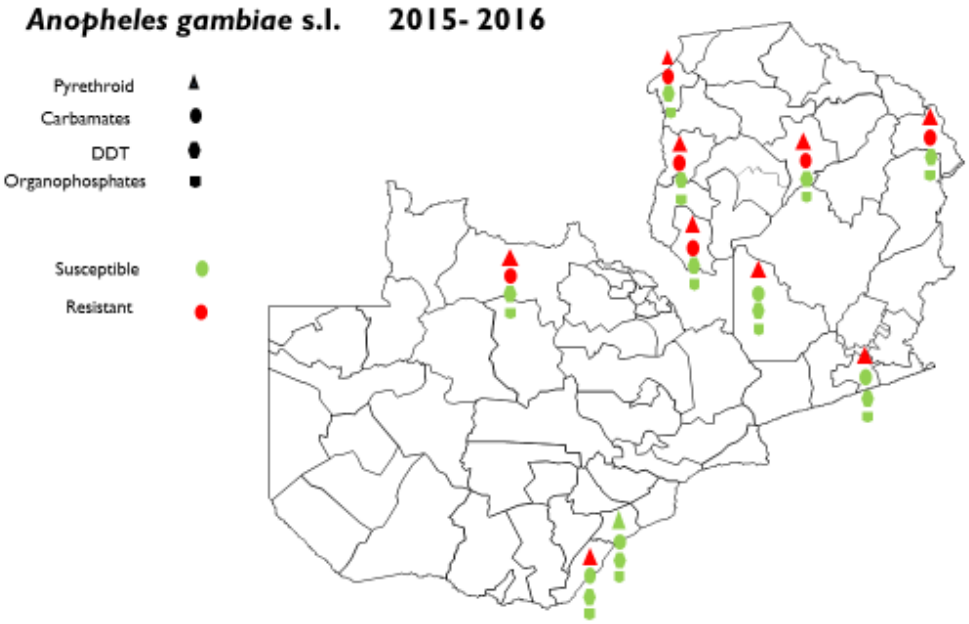
A.
Insecticide resistance in collections from March 2011 – April 2012. Darker gray shading indicates areas surveyed in Chanda 2011. *locations with microarray data.

B.
Insecticide resistance in collections from March 2012 – April 2013.

C.
Insecticide resistance in collections from May 2013 – April 2014. Bordering districts with the same resistance profile were represented with a single set of symbols. [1] Resistant to deltamethrin but susceptible to permethrin. [2]Resistant to permethrin but susceptible to deltamethrin.

Figure 10: Insecticide resistance maps, 2015-2016.





Vector insecticide resistance mechanisms

In combination with the bioassays, Zambia has some data on insecticide resistance mechanisms. As well as detection of point mutations using PCR, synergist assays have been conducted on some samples from some areas to demonstrate whether resistance is conferred by elevated expression of oxidases.

***An. gambiae*.**
The target site of pyrethroids and DDT is the sodium channel. Data prior to 2014 showed a high frequency of the of the *kdr* west allele (1014F) (91%), that confers resistance in *An. gambiae* in Kitwe and Kasama Districts (Copperbelt and Northern Provinces). The *kdr* east (1014S) mutation, which also confers resistance, was not detected from studies conducted at the time. In 2012 in *An. gambiae s.s.* from Luapula the presence of kdr was responsible for resistance to pyrethroids and DDT.

The target site for carbamates and organophosphates is acetylcholinesterase. The insensitive form of this (*iACE*) that confers resistance was not detected (Chanda et al. 2011).

Metabolic resistance genes were also detected in these *An. gambiae* populations. This included cytochrome P450's that give rise to pyrethroid and potentially carbamate resistance and glutathione *S*-transferase that potentially gives rise to DDT resistance (Hamainza et al. 2016).

In 2015 in Southern Province PBO synergist assays for *An. arabiensis* indicated that oxidases were responsible for resistance to pyrethroids (pre-exposure to the synergist PBO recovered susceptibility NMEC, Personal Communicaton).

An. funestus
As yet no alteration in the insecticide target site has been detected in *An. funestus*. However, the cytochrome P450's that give rise to pyrethroid resistance and carbamate resistance were detected (Choit et al. 2014). This is the same mechanism that led to the failure of the South African malaria control programme in the 1990's and has been detected in Malawi [19] and Mozambique [20].

Data from 2012 exist to show that for *An. funestus* in Luapula, resistance to deltamethrin is due to increased oxidase expression (pre-exposure to the synergist PBO recovered susceptibility) and synergist assays for *An. funestus* indicated that for bendiocarb, more than one mechanism is responsible; susceptibility was only partially rescued when mosquitoes were exposed to PBO prior to exposure to the carbamate (Choi et al. 2014).

Mechanisms of resistance need to be monitored to determine how the population is changing. Monitoring of resistance mechanisms should occur at each sentinel site and be in combination with the bioassays.

Intensity of resistance
Intensity of resistance has been monitored recently by PMI/AIRS and by the ICEMR project through TDRC.

TDRC with colleagues from University Witswatersrand studied intensity of resistance of *An. funestus* Intensity assays were conducted in Nchelenge using deltamethrin and carbamates using the following methods*:*

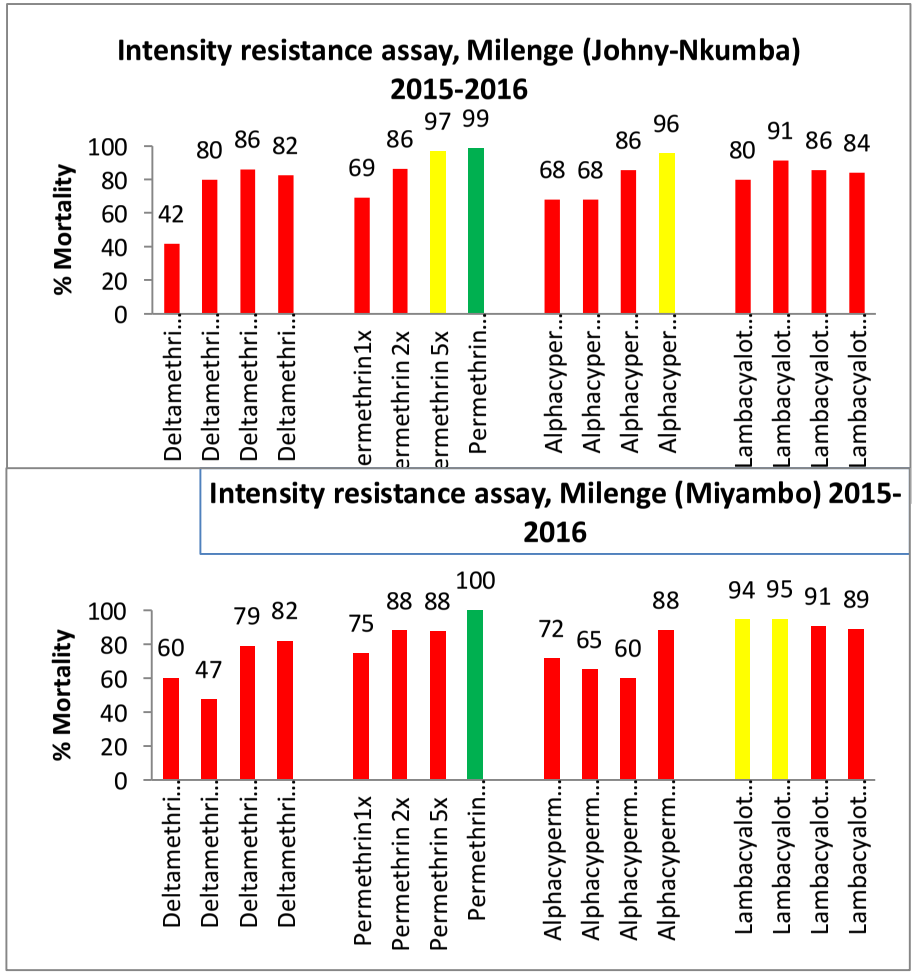
- 2-3d old, unfed female F1s *An. funestus s.s.* clade I and II
- Exposed for 8 hours in WHO tubes to 0.05% deltamethrin and 0.1% bendiocarb.
- Tubes placed on side to allow continuous exposure after knock down
- Knock down measured every 5-10 minutes of 1st hour, at 80min, and then every hour 2-8 hours

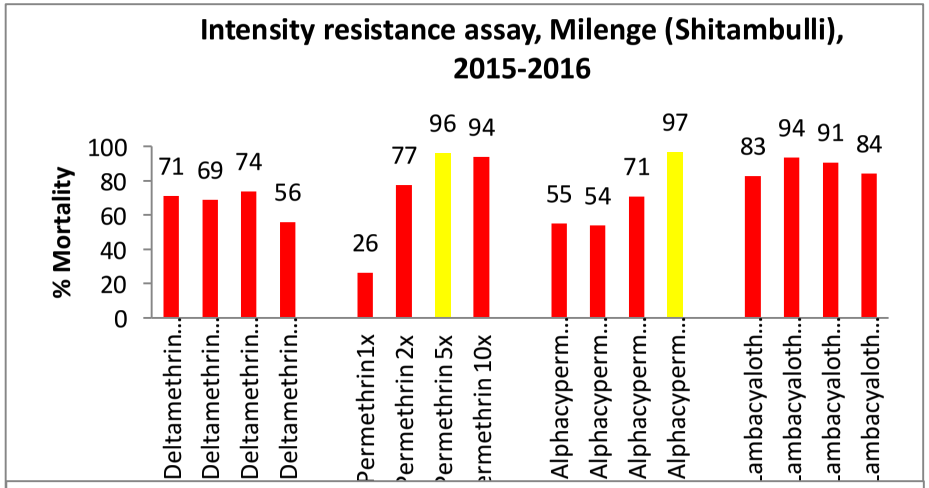
Results showed there to be high intensity of resistance to deltamethrin for both clades of *An. funestus* with 20% or less being killed after almost 2 hours of exposure to the diagnostic dose.

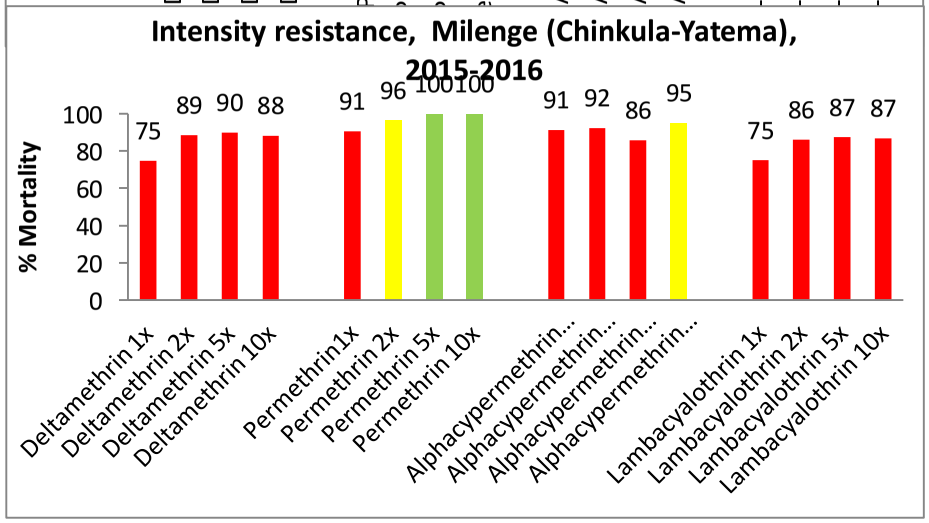
For bendiocarb, intensity of resistance was not high and most mosquitoes were killed after 60 minutes of exposure. Recently the resistance intensity studies were conducted using the new WHO guidelines. The insecticides included were 5x and 10x Deltamethrin and Permethrin. Results showed intense resistance to 5x but less so on 10x.

Under AIRS in 2015-2016 adult female *An. funestus s.s.* were collected from four villages in Milenge and two villages in Samfya. Additional tests were done in November and December 2016 on *An. funestus s.l.* collected from Samfya. Based on November- December data, there is no difference between the two selected villages in terms of intensity of resistance to pyrethroids (figure xx) below.

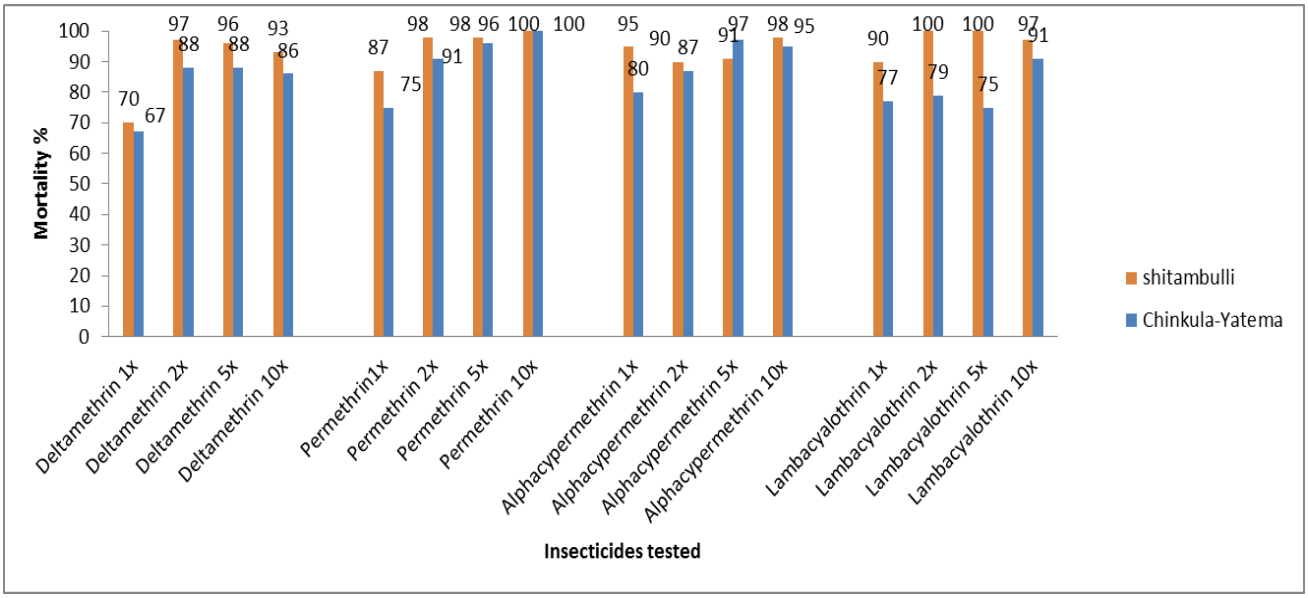
Intensity resistance assay in 4 sites in Milenge district against *An. funestus s.l.*

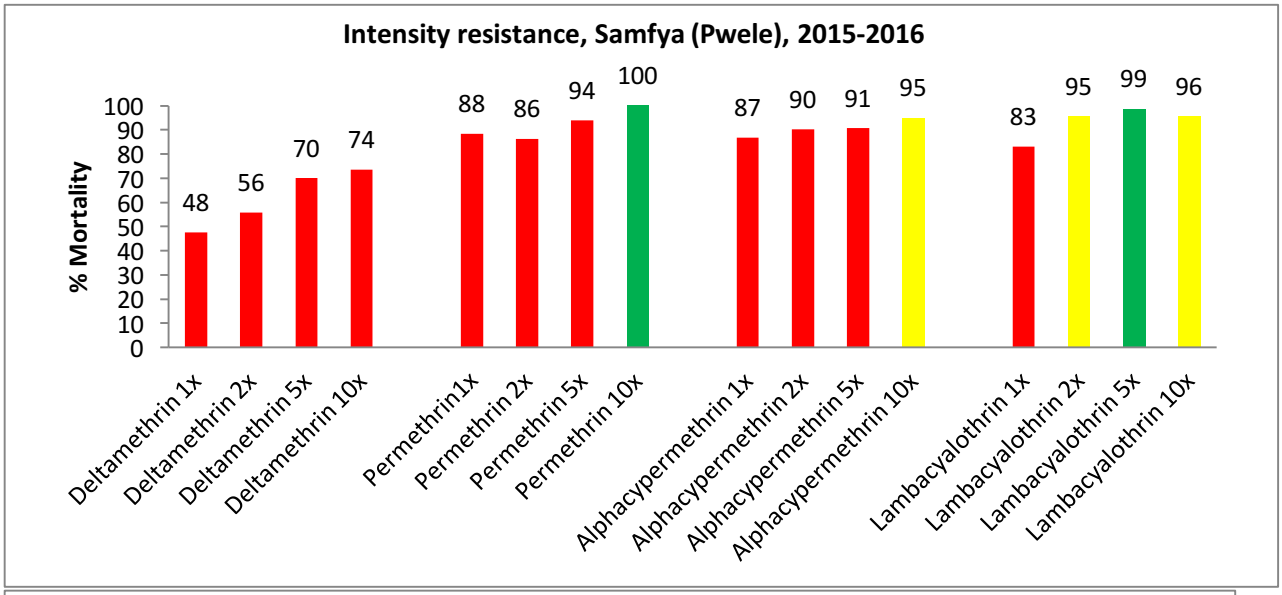


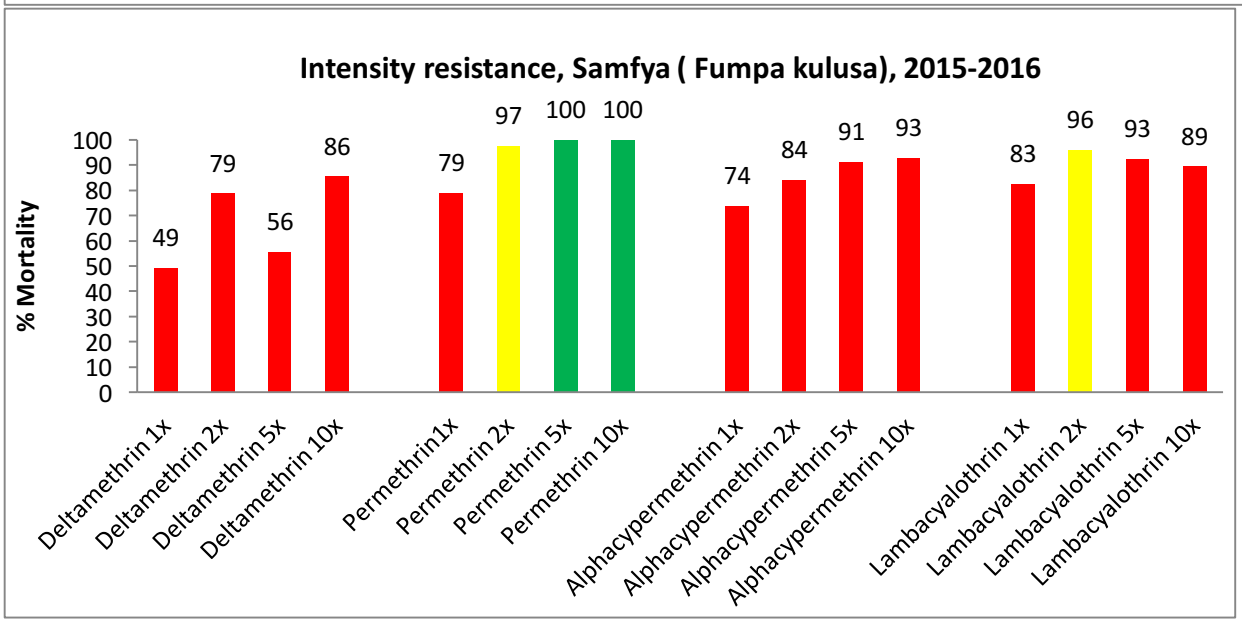




Intensity resistance assay in Milenge District November –December 2016

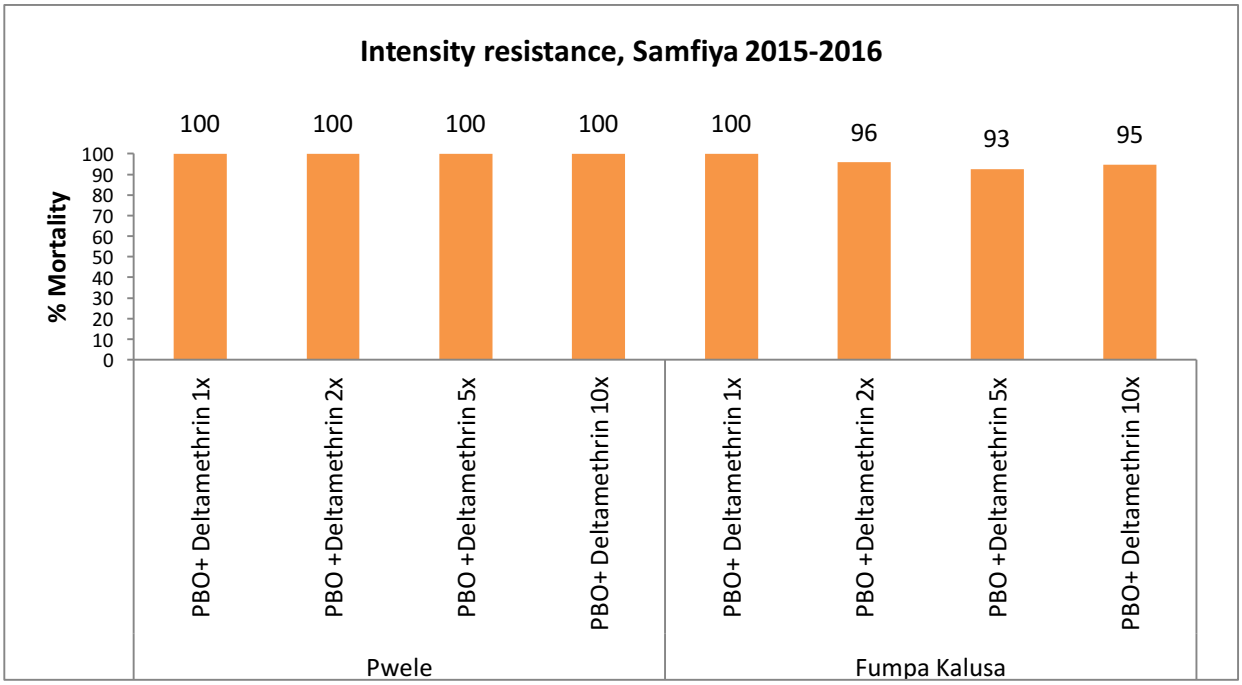






Pre-exposure to PBO restored susceptibility of *An. funestus* to deltamethrin in both villages of Samfya (figure xx below). This indicates that the oxidases are responsible for low and high levels of resistance intensity.

Pre-exposure of *An. funestus* with PBO synergist at various dosages of deltamethrin



A2.5 Data management and dissemination

The NMEP are responsible for data collection in different areas of the country. The following are the partners and their areas of operation: PMIVectorlink (Eastern, Luapula, Muchinga, Northern Central provinces), MACEPA (Southern and Western provinces), Macha Research Trust (Southern province), Tropical Diseases Research Centre/Wits University (Copperbelt, Luapula, North-Western provinces). The rest of the provinces will be supported by the Global Funds through NMEC.

The collected data are shared through organized Technical Working Group meetings coordinated by the National Malaria Elimination Centre. The meetings are organized annually and are chaired by the University of Zambia.

There is a central repository (National Database) managed by the National Malaria Elimination Centre under a project called disease data management system (DDMS). Current data are reviewed by the Technical Advisory Committee (TAC) of Insecticide Resistance Monitoring Technical Working group composed of local, international, research and training institutions. Depending on the prevailing data the TAC advices the program on what insecticides to use.

Ideally the meetings are supposed to be conducted bi-annually but due to financial constraints these are held at least once a year. Sometimes the procurement process and the timing of the decision for switching the insecticide are not well synchronized. The decisions to either switch or maintain the insecticide for vector control are mostly based on phenotypic resistance using WHO/CDC assays as opposed to determination of the actual resistance mechanisms.

A2.6 Evidence and Knowledge gaps

The data collections is driven by partners with specific geographical areas of interest and therefore data for decision making are missing from certain areas of the country. There is urgent need for insecticide resistance data from the Copperbelt, Central and Western provinces. Measures are in place to address this gap in order to have data collected across the whole country.

There is need for procurement of appropriate molecular reagents such as resistance biomarkers. Training in the use and interpretation of data is criticalto facilitate the timely generation of insecticide resistance mechanism.

With current evidence for potential secondary vectors in some areas of the country there is need in the long term to establish their role in malaria transmission on a wider scale. Lack of knowledge on actual breeding sites for the primary vector *An. funestus* need to be addressed. Limited data on outdoor resting and outdoor exposure to the bites of the major vectors in the country need to be addressed.

A2.7 Human resources

The NMEP has human capacity at national, provincial and district levels devoted to insecticide resistance monitoring and management

Administration level	Role	Qualification	Number engaged	Proportion of time for current Insecticide resistance monitoring and management activities	Total for current insecticide resistance monitoring and management activities in FTEresistance	Observations
National	chief entomologist/Senior entomologist	MSC	4	40%	0.4	1 Environnmental Health Officer is currently acting chief entomologist supported by 3 Entomologists from partners
	Insectary manager	BSC	2	30%	1	The current insectary manager is hired with PMI support
	Insectary technicians	BSC	9	100		3 FTE supported by partners

				75%		
	Field technician	BSC	9	25%	4	All the 9 field technicians are hired by partners.
	Laboratory technician	BSC	8	25%	4	The 4 laboratory technicians are hired by partners
	Laboratory scientist	BSC	2	15%	2	The 2 lab scientists are hired by partners
	Epidemiologist	MSC,BSC	3	15%	3	The 2 epidemiologists are full time government employees and one full time hired by partner
	statistician	BSC	1	10%	1	Full time government
	Database manager/GIS specialist	BSC	1	10%	1	1 FTE hired by partners
Provincial	Chief Environnmental Health Officer	MSC	10	5%	10	10 FTE GOVERNMENT
District	Environnmental Health Officer	BSC	110	25%	11	12 EHO FTE government are actively involved in the IRMM
Health Center	Environnmental Health Technician	Diploma			5	

A2.8 Partner contributions

Partners	Field of activity
CDC	• Technical support • Training • Equipment
Macha Research Trust	• Technical support • Support for laboratory analysis (species identification, Kdr and Ache-1R determination?)
PMI AIRS	• Technical support • Human resource • Financial support • Equipment
MACEPA	• Technical support • Human resource • Equipment
TDRC	• Technical support • Research
First Quantum Mineral	• Financial support to research Institute
Welcome Trust	• Technical support • Human resource
John Hopkins	• Technical support • Training
Witwaters rand	• Technical support • Training
Liverpool School	• Technical support • Training
AKROS	• Technical support • Training
Global Funds	• Financial support
PMI USAID	• Financial support
Bill and Melinda gate	• Financial support

A.2.9 Current constraints

- Gap of Government Human resource and skills at all levels to monitor and manage the Insecticide resistance.
- Insufficient sentinel sites across the country to provide a full view of the malaria vectors distribution and their susceptibility to the insecticides used.
- Insufficient equipment and supplies to monitor the susceptibility of malaria vectors to the insecticides
- Gap in financial resource to monitor and manage the insecticides resistance
- Limited skills to conduct PCR to determine the mechanism of resistance
- Lack of awareness and implication of insecticide resistance on the programme.
- Lack of involvement of agriculture and private sectors in the insecticide resistance management

A3. Implementation Framework

Overall objective:

To ensure efficient vector control for malaria elimination in Zambia through prudent management of insecticide resistance

Specific objectives

- Perform timely entomological monitoring of the malaria vectors control interventions from xx to xx
- Carry out insecticide resistance monitoring across the country from xx to xx .
- Build country capacity to collect and interpret resistance data from xx to xx
- Support the development of new and or innovative vector control tools.
- Fill the gaps in knowledge of insecticide resistance and the impact of current resistance management strategies.
- Manage the resistance of malaria vectors

Entomological monitoring of malaria vector control interventions

- ✓ **Insecticide residual efficacy** of IRS testing by WHO cone bioassay will be done in targeted selected sites across the country to assess the quality of the spraying and to follow the decay rate of the insecticide applied.
- ✓ LLINs are distributed during mass campaign and routinely. There is also need to follow the LLINs durability and efficacy using WHO guidelines
- ✓ **Species composition and malaria vectors distribution** can be monitored using CDC-light trap, Human Landing catch or Pyrethrum Spray Catch.
- ✓ **The vectors behaviors** can be assessed by PSC and HLC
- ✓ Provision of information on malaria transmission is key to follow the infectivity of malaria vectors. Sub-samples of mosquitoes collected will be tested. to determine the presence of sporozoites

A3.2. Insecticide Resistance Monitoring

To follow the trend in resistance, the phenotypic resistance is recommended to be assessed using the WHO standard diagnostic assay in all sentinel sites. The CDC bottle assay can be also performed.

At least one insecticide from each of the four insecticides classes will be tested each six months at all sentinel sites. The new insecticides approved by WHOPES can also be tested. The genotypic resistance determination is also recommended as well as the metabolic resistance.

A3.2.1 Sentinel Sites

Currently there are 51 sentinel sites in Zambia where entomological surveillance (species composition at a minimum) are being or will be conducted. These include sites that are supported by NMEC, as well as those that are part of the PMI/AIRS and those that are being monitored by partners. However, only 26 of these are doing quality assured M&E.

- Under PMI/AIRS 12 sites that fall into control (i.e. no IRS) and intervention (i.e. IRS ongoing) exist. Sites are located in Northern, Muchinga, Eastern, Luapula and Central Provinces. Here CDC light trap collections are conducted monthly in 4 houses per site for 4 nights, HLC are conducted in 4 houses per site for 4 nights and PSCs are conducted in 15 houses each month.
- TDRC conduct monthly light trap collections from 24 households in Nchelenge each month.
- MACEPA conducts entomological surveillance in Siavonga, Kalomo and Gwembe districts.
- Macha Research Trust conducts light trap collections each month in index case households, both within houses and next to livestock.

Current sentinel sites for monitoring insecticide resistance:

- At 12 PMI/IRS sites, insecticide susceptibility is determined twice per year, when numbers allow. In addition, there are several extra sites in Katete, Samfya and Milenge districts. This is where resistance is determined twice annually and intensity of resistance using CDC bottle bioassays are conducted.

- TDRC conducts insecticide resistance monitoring in Nchelenge once per year. In Copperbelt TDRC conducted its last assays in 2012. They will begin sampling in 2017 in North-western Province, monitoring resistance once per year in Kalumbila Districts.

- MACEPA monitors resistance in 12 sites of 4 districts in Southern Province annually.

As with entomological surveillance sites, there are xx other identified sentinel sites supported by Global Fund but the protocols are yet to be established.

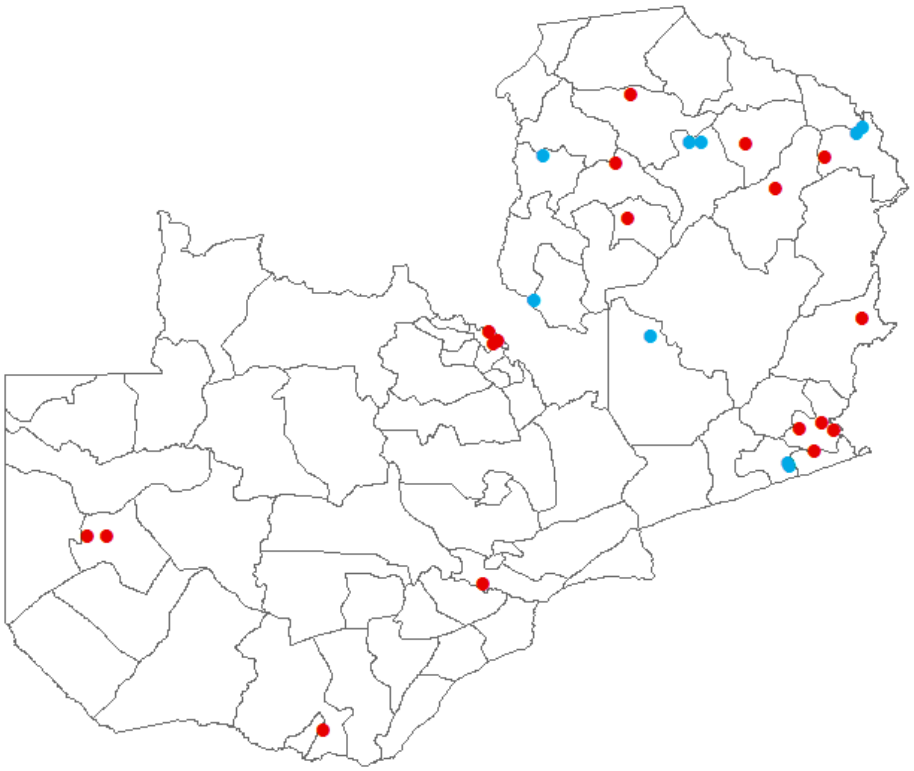
Sites created under Global Fund include 25 but the sites are not yet operational.

Province	District	Sentinel site	Status	Date established	Proposed frequency of collections	Additional information
Northern	Kasama	Kalonga	Established	2014	Each 6 months	
		Simeo Mwaba	Established	2015	Each 6 months	
Muchinga	Isoka	Nsalamba	Established	2014	Each 6 months	
		Namyala	Established	2015	Each 6 months	
Luapula	Milenge	Miyambo	Established	2014	Each 6 months	
		Lunga	Established	2014	Each 6	

					months	
Central	Serenje	Chishi	Established	2014	Each 6 months	
		Chibobo	Established	2014	Each 6 months	
	Itezhi Tezhi	Buunga	proposed		Each 6 months	
		Bushinga	proposed		Each 6 months	
Eastern	Katete	Mbalani	Established	2014	Each 6 months	
		Robert	Established	2014	Each 6 months	
Southern province	Siavonga	Manchavwa	Established	2014	Each 6 months	
		Chaanga	Established	2014	Each 6 months	
		Sianyoolo	Established	2014	Each 6 months	
	Gwembe	Chabbobboma	Established	2014	Each 6 months	
		Sinafala	Established	2014	Each 6 months	
	Kalomo	Masempela	Established	2014	Each 6 months	
	Sinazongwe	Chiyabi	Established	2014	Each 6 months	
		Siatwinda	Established	2014	Each 6 months	
		Buleya malima	Established	2014	Each 6 months	
		GVDC	Established	2014	Each 6 months	
		Sikaneka	Established	2014	Each 6 months	
		Solwegonde	Established	2014	Each 6 months	
North Western	Solwezi	Kazomba	proposed		Each 6 months	
		Kimasala	proposed		Each 6 months	
	Kasempa	Nselauke	proposed		Each 6 months	
		Kivuku	proposed		Each 6 months	
	Mufumbwe	Lubilo	proposed		Each 6 months	
		Boma Clinic	proposed		Each 6 months	
Copperbelt	Ndola	Chipulukusu	proposed		Each 6 months	
		Commando	proposed		Each 6 months	
	Masaiti	Masaiti Council	proposed		Each 6 months	
		Chondwe	proposed		Each 6 months	
Lusaka	Chirundu	Chikanzaya	proposed		Each 6 months	
		Chipepo	proposed		Each 6	

					months	
	Rufunsa	Chinyunyu	proposed		Each 6 months	
		Kankumba	proposed		Each 6 months	
		Chitemalesa	proposed		Each 6 months	
Western	Mongou	Mabumbu	proposed		Each 6 months	
		Sefula	proposed		Each 6 months	
		Mabumbu	proposed		Each 6 months	
	Kaoma	Mutondo	proposed		Each 6 months	
		Urban	proposed		Each 6 months	

Figure 11. Distribution of sentinel sites.



KEY
Red dots. CDC light traps only
Blue dots. CDC light traps, insecticide resistance and pyrethroid spray catches

Robust and reliable data are critical for monitoring the progress of Zambia in achieving malaria control and elimination targets. This needs to include measuring epidemiological and

entomological targets, and ensuring that these two data sets are as closely matched in time and space as possible.

A3.2.2 Mosquitoes to be tested

For the WHO susceptibility tubes tests, the tests should be carried out using female adult malaria vectors 3-5 days old and reared from larvae collection or F1 progeny of wild female mosquitoes. If these options are not available, the test can be run with wild caught unfed females.

At least 120–150 active female mosquitoes should be tested in 4 replicates of 20 – 25 mosquitoes with two controls. The collection efforts have to be made to get enough mosquitoes for the test. The mosquitoes tested will be morphologically identified to specie using identification key. All or the sub-sample of the mosquitoes tested will be identified to specie through Polymerase Chain reaction (PCR). The subsamples should include live and dead mosquitoes.

Specie morphologically identification will be performed by trained insectary technicians supervised by NMEP or partner senior entomologist. The mosquitoes will be properly preserved in silica gel or RNA later for molecular assays.

A3.2.3 Insecticide Susceptibility Testing

WHO susceptibility tube test and CDC bottle bioassay are recommended to monitor the insecticide resistance.

WHO susceptibility test

At least 120–150 active female mosquitoes will be aspirated into six holding tubes and leave in upright position for one hour. At the end of this time, any damaged insects are removed. The mosquitoes will be transferred in the exposure tubes for a period of 1 h. At the end of the exposure period the mosquitoes will be transferred to the holding tubes and will be provide 10% of sugar solution. Mosquitoes are maintained in the holding tubes for 24 hours.9. After 24h the number of dead mosquitoes is counted and recorded. At the end of the test, mosquitoes will be transferred in clearly labelled Eppendorf tubes (separating dead and alive)

CDC bottle bioassay

The bioassay can be performed with the bottles in an upright position or with the bottles lying on their sides. The important thing is to be consistent and follow the same procedure each time. At least 10–25 mosquitoes will be introduced into the control bottle and the test bottles. The bottles have to be coated 24 hours before the test. Examine the bottles at Time 0 and count the number of dead and/or live mosquitoes;

Record how many mosquitoes are dead or alive; whichever is easier to count, every 15 minutes until all are dead, or up to 2 hours. It is not necessary to continue the bioassay beyond 2 hours.
For detecting changes in resistance over time and for detecting metabolic mechanisms the WHO or CDC intensity resistance protocols can be used.

A3.2.2.4 Resistance Mechanisms Identification

Monitoring of resistance mechanisms should occur at each sentinel site where the phenotypic resistance is found. The mechanism can also indirectly be identified using the WHO or CDC bottle assay with synergist.

Biochemical analysis is recommended as need for metabolic resistance determination.

A3.2.5 Coordination with Supporting Institutions

NMEC is in charge of the coordination of monitoring and evaluation of insecticide resistance activities with support of the implementing partners as PMI AIRS, MACEPA and research institutes (Macha, TDRC). The tests and the morphologically identification of the samples are performed by the implementing partners and the molecular assays by Macha Research Trust and TDRC with financial support from PMI, MACEPA and other donors. It is recommended to build the national capacity and procured the required equipment to conduct the molecular assays for resistance mechanism identification.

A.3.2.6 Data Recording and Reporting

The NMEP partners are responsible for data collection in different areas of their operations. The collected data will be shared with NMEC each six months in harmonized template. There is a central repository (National Database) managed by the National Malaria Elimination Centre under a project called Disease Data Management System (DDMS). The data will be entered in DDMS. DDMS will be managed by NMEC database manager under supervision of Epidemiologist and Chief Entomologist. The data will be reviewed each quarter during the IRMTWG and will be analysed annually by the Technical Advisory Committee (TAC) of Insecticide Resistance Monitoring Technical Working group composed of local, international, research and training institutions. The details will be used to provide guidance regarding insecticide selection for IRS and for insecticide resistance management. The data will be shared annually with E8 and WHO.

A3.2.7 Procurement and Supplies

Procurement of commodities prior to the implementation of any vector control intervention is critical. Procurement and delivery of the commodities should be done early to avoid late implementation of the planned activities. The normal procurement process involves the following steps:

a) Establishing the required quantities through needs assessment
b) Requisition of the required commodities through the Permanent Secretary
c) Approval of request by the Permanent Secretary
d) Advertisement for Invitation for bids through the public and private print media. The advertisement run for six to eight weeks.
e) Opening and evaluation of bids
f) Presenting evaluation report and Seeking authorisation to proceed with the procurement through the Ministerial Procurement Committee (MPC)
g) Conveyance of draft contracts to Ministry of Justice for legal opinion
h) Signing and award of the contract to successful bidders for supply and delivery of the requested commodities.
i) In country distribution of the commodities to all the districts

The commodities that are critical for successful implementation of vector control intervention are:

i. Insecticides for IRS and larviciding
ii. Sprayers
iii. Spares for sprayer
iv. Long Lasting Insecticidal Nets (LLINs)
v. Personal Protective Equipment (helmet, goggles/face shield, respirators/face masks, mutton cloth, cotton overalls, socks, gumboots with steel toe cap, PVC glove-elbow length and face towels)
vi. Washing detergent

vii. Bathing soap
viii. SBCC materials (Poster, flyers, sticker/spray card)
ix. Food for breakfast for spray teams
x. Stationery (A4 80grams reams of paper, box file, hard cover books, calculators, staplers, staples and Toner)
xi. Rolls of Polythene sheets
xii. Fuel
xiii. Vehicles to carry spray teams and distribute commodities
xiv. Fire safety equipment (fire extinguishers, buckets of sand)
xv. First Aid kits
xvi. Data collection tools (SOP forms, tablets, smart phones and supervisory tools,
xvii. Repairing tool (pipe wrenches, pliers and screw drivers)
xviii. Equipment for Larval Source Management (Wheel barrows, shovels, rakes, machets and slashers)
xix. Bags for carrying insecticides

A.3.3 Insecticide Resistance Management

A.3.3.1 Decision -Making Body

In order to have a successful IRMM plan. Zambia has established two committees, the Technical Advisory Committee (TAC) and the Insecticide Resistance Management Technical Working group. The TAC advises the Insecticide Resistance Management Technical Working Group coordinated by the National Malaria Elimination Centre in line with GPIRM, this mechanism engages partners with a vested interest in insecticide resistance (Table 1).

However, to be successful these committees need to be supported by the Ministry of health. The developed plan will require the broader group to work together for delivery. The collected data will continue to be shared through the Technical Working Group meetings. The meetings will be organized bi- annually.

Table 5. *The Insecticide Resistance Management Technical Working Group Composition.*

Technical Advisory Committee	**Resistance Management Technical Working Group**
Academic	**Ministries;**
Centre for Disease Control	Health
Liverpool School of Tropical Medicine	Agriculture
Tropical Disease Research Centre, Zambia	Environment
Johns Hopkins Malaria Research Institute	Entomological Society of Zambia.
Macha Research Trust	
Implementing partners	Chemical suppliers
National Malaria Elimination Centre	Pest control operators
CDC PMI	NGO's
African Indoor Residual Spraying Project	Technical Advisory Group
ZEMA	
MACEPA	
Mines	

Interpretation of results and policy implication

Increased vector population monitoring through bioassays revealed that in *An. gambiae s.s.*, pyrethroid resistance is ubiquitous and is always accompanied by resistance to DDT, confirming a prior report of this resistance profile in the central part of Zambia [1]. Recent data showed that An. gambaie s.l. is broadly resistant to pyretroid and DDT. This is a similar profile to that seen in Uganda [24], Kenya [25] and East Africa. Carbamate resistance in this species is present in many parts of West Africa [26-28], and is also reported here in Zambia. All populations were susceptible to organophosphates.

In *An. gambiae s.s.*, the resistance profile is partially mediated by target-site mutations. The *kdr* west allele (1014F) was found at very high frequencies in Nchelenge, and was fixed in one population. This allele confers cross-resistance to both pyrethroids and DDT, which share the same target site. If this allele becomes fixed, the potential fitness cost of carrying the allele in the absence of insecticide would no longer be effective, and susceptible alleles would not be able to spread back through the population.

Target-site resistance alone may not result in operational failure of vector control [29] The Kdr resistance in concert with metabolic resistance, can be a major threat . In Benin, where pyrethroid resistance is conferred by both target-site and metabolic mechanisms, sleeping under an ITN in an area with a resistant population provided little protection against being bitten [30]. In Zambia, metabolic resistance has been selected for in *An. gambiae s.s.* as well, involving an over expression of P450s involved in pyrethroid resistance and GSTs involved in DDT resistance both of which have been associated to resistance in Africa [31-34].Oxydase metabolic resistance was found in Sinazongwe and Siavonga in Southern province and in Milenge in Luapula province in 2015-2016.

In *An. funestus s.s.*, pyrethroid resistance is common and is usually accompanied by resistance to bendiocarb. This is the same resistance profile as in Mozambique [35] and Malawi [19]. In Western Provinces, however, resistance to bendiocarb was unconfirmed. Combined, this pattern of resistance in *An. funestus s.s.* may indicate that the mechanism underlying pyrethroid and carbamate resistance has recently spread to the western side of the country and may be selected for by extensive use of pyrethroids in Agriculture, in IRS and LLINs. This conclusion is supported by the pattern of over expression of P450s involved in pyrethroid resistance in this area. *An. funestus s.s.* was susceptible to DDT in most of areas in Zambia but resistance to DDT was found in Milenge and Mwense in 2016 in Luapula province.

In *An. funestus s.s.*, the resistance profile is partially mediated by metabolic mechanisms, namely an over expression of the P450s involved in pyrethroid metabolism. Although the incrimination of P450s in the metabolism of carbamates has yet to be shown directly, bioassays with piperonyl butoxide, an inhibitor of P450s, implicate this class as the causal mechanism behind carbamate resistance in *An. funestus s.s.* from southern Africa [36]. This mechanism may explain the cross-resistance seen between pyrethroids and carbamates in *An. funestus s.s.* from Zambia. This is the same resistance mechanism that resulted in the failure of South Africa's malaria control programme in 1996.

The level of pyrethroid resistance in both *An. gambiae* and *An. funestus*, suggest that at this point in time that this class of insecticide should not be used for IRS. Unfortunately, this is the only class of insecticide available for LLIN's and cannot therefore be omitted totally. LLIN's should continue to be utilised as they act as a barrier. It may be advantageous, to pilot some of the new generation of ITN's that would in theory mitigate the p450 metabolic resistance.

However due to the potential for an antagonistic effect between PBO and organophosphates, PBO LLINs should not be used in areas programmed for IRS with Pirimiphos-methyl CS. For the pilot distribution of PBO LLIN, it should be performed in the areas where prevalence of malaria in children aged 2–10 years is > 20% and mosquito mortality in bioassay with pyrethroids is < 80%. Data should be collected on the presence, level, intensity and mechanisms of resistance to all insecticide classes at representative sentinel sites;

DDT has been used in the Copperbelt until 2010 when significant resistance was first reported [1], and should not be used to control *An. gambiae* there. However, both *An. funestus* and *An. arabiensis* are still susceptible to this insecticide and both species have been controlled in southern Africa successfully for some time [37]. In areas of Zambia where *An. gambiae* is not the dominant species, DDT could be considered a viable insecticide where An. *funestus* is not resistant to DDT.

Carbamate resistance has been recorded at higher levels each year in *An. gambiae* and *An. funestus*. It is recommended that this insecticide class ceases to be used for the next two years to determine if resistance will recede and it can be rotated into an IRS programme.

Organophosphate, in particular Pirimiphos-methyl, is currently a viable insecticide for all of Zambia as all vectors are susceptible. However, potential resistance was reported in one population of *An. gambiae* in 2014 (Liverpool publication). No further study was conducted in Copperbelt to confirm the trend up to date. This is the final class of insecticide with no broad resistance as yet reported in Zambia. To rely on this class of insecticide for all IRS in Zambia continuously has two major issues, one there is the potential for the selection of insecticide resistance and two there is a significant increase in cost to what has been budgeted. Pirimiphos methyl is the insecticide used for IRS since 2014. It can be rotated with the new insecticides that will show a good residual efficacy overtime. Pilot studies with the new insecticides are recommended.

Larvae source management is carried out by the private sectors using Abate and Pirimiphos methyl. For insecticide resistance management, Pirimiphos methyl should not be used for larviciding when it is used for IRS.

A3.3.2 Regulatory requirements and procedure

The Zambia Environmental Management Agency (ZEMA) is the regulatory body that is tasked to register all insecticides recommended by WHOPES or WHO pre-qualified. A licence for toxic and pesticide substances has to be obtained from ZEMA prior to importation. This should then be followed by another application for the hazardous waste management. See annex for details of the forms.

A3.3.3 Quality control for vector control products

The quality control for vector control products should is done with the National Malaria Elimination Programme (NMEP) in collaboration with the Zambia Environmental Management Agency (ZEMA) before importation of the product into the country. For LLINs randomly selected samples from LLIN consignment for mass distribution campaigns and routine should be tested for quality control before distribution in order to ensure that vector control products meet the target product profiles claimed by the manufacturers.

A3.3.4 Monitoring of Interventions

The NMEP through various partners should have monitoring activities for vector control in randomly selected districts in the country. IRS monitoring is done by looking at changes in

mosquito densities, behaviour, longevity and EIR rates before and after implementation. The timing of IRS implementation should rely on existing entomological surveillance as evidenced in 2016. Therefore, spraying campaign will be done so that it coincides with the peak malaria transmission. IRS coverage should be assessed and all efforts should ensure at least 85% coverage.

Mass distribution campaigns for LLINs should be conducted every after 2.5 years based on the recent durability study (Tan et al,2016) and assessment should continue. The 2017 distribution campaign should be aligned with a routine assessment for net attrition, integrity and insecticide content overtime following WHO standard procedures. Periodic surveys such as the MIS have also been used to assess the utilisation rates and coverage rates at national level.

LSM activities especially larviciding should assed before and after implementation to provide information on decision making.

A3.3.5 Operational Research to Support IRMMP

- What is the effectiveness of next generation of LLINs containing PBO and PPF in insecticide resistance management?
- Can Sumishield and other novel vector control insecticides be alternatives to Pirimiphos-methyl?
- What mechanisms are driving insecticide resistance?
- Is there a link between bioassays and mechanisms impacting on vector control?
- Is selection pressure due to public health, agriculture or industry (mining)?
- What is the impact of insecticide resistance management strategies such as combination, rotations, mixtures and mosaic on vector control?

A3.4
Outlined tasks objectives and activities with responsibilities

TASK	OBJECTIVES	ACTIVITY	RESPONSIBLE	YEAR 1				YEAR2				YEAR 3				YEAR 4				YEAR 5			
Capacity building	To enable appropriate insecticide resistance monitoring and management through human and infrastructure capacity enhancement	Recruit necessary personnel to fill identified gap	NMEP			X	X																
		Conduct training of established and new personnel (followed by on-going mentorship)	NMEP/partners			X	X						X	X									
		Improve laboratory and Insectary facilities and procure necessary equipment	NMEP/research institutions			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Insecticide resistance monitoring	To conduct annual evaluations of insecticide resistance at all sentinel sites to support evidence-based decision-making	Establish sentinel sites	NMEP																				
		Carryout field collections of larvae (or blooded adults as required	NMEP			X			X	X			X	X			X	X			X	X	
		Rear larvae to adult mosquitoes in field Insectary	NMEP				X	X			X	X			X	X			X	X			X
		Conduct morphological identifications (and using other techniques as required)	NMEP/research institutions			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Conduct bioassays with discriminating and intensity concentrations	NMEP						X			X				X					X		
		Conduct synergist-insecticide bioassays	NMEP							X			X				X					X	
		Enter data electronically	NMEP						X		X		X		X		X		X		X		X

		into standard spreadsheet																					
Quality Assurance and control	To conduct periodic evaluations of vector control interventions at selected sites to support evidence-based decision-making	Develop and implement monitoring and evaluation plan for interventions (resistance efficacy, durability)	NMEP/partners			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Conduct monitoring and evaluation activities	NMEP/partners			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Enter data electronically into standard spreadsheet	NMEP/partners						X		X		X		X		X		X		X		X
Data management and dissemination	To streamline systems for reporting of insecticide resistance monitoring as well as quality monitoring data	Develop standard national spreadsheets for data reporting, and disseminate to partners involved in data collection	NMEP/WHO			X																	
		Establish and maintain national insecticide resistance database	NMEP			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	To streamline system for reporting of insecticide resistance monitoring as well as quality monitoring data	Establish and maintain national database for intervention monitoring information	NMEP			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Compile available data on insecticide resistance, interventions implementation and quality, and link with epidemiological information	NMEP				X				X				X				X				X
		Report data to WHO and implementing partners	NMEP				X				X				X				X				X

Insecticide resistance management	To leverage available information to tailor vector control interventions to local situations, and communicate outcomes with partners	Convene the Technical Advisory Committee/Insecticide Resistance Monitoring Technical Working Group for insecticide resistance management decision-making	NMEP			X	X				X				X				X				X
		Evaluate data to determine impact of resistance management strategies on (a) disease outcomes, and (b) resistance profiles of local malaria vectors	NMEP/WHO			X				X				X				X				X		
		Generate recommendations for changes in vector interventions or their implementation based on decision-making framework	TAC/IRMTWG				X				X				X				X				X
		Update national malaria strategic plan as required	NMEP							X								X						
		Implement changes	NMEP					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coordination	To strengthen collaboration and communication within the health sector and with other sectors (e.g. agriculture, education)	Convene broad stakeholder meeting to report outcomes	NMEP				X				X				X				X				X
		Actively engage with Zambia Environmental Management Agency (ZEMA) to identify ways to optimize registration process for vector control products	NMEP			X			X	X			X	X			X	X			X	X	
		Engage with partners involved in the development of new vector control tools to encourage and support development and registration as appropriate	NMEP/ZEMA			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Operation research	To address gaps in evidence and knowledge on insecticide resistance and its management	Establish or update research agenda for insecticide resistance management	NMEP/research institutions			X			X	X							X	X					
		Collaborate with research institutes as required to evaluate the impact of insecticide resistance and management strategies	NMEP			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

A3. 5 Human resources

Specifications			**Current personnel (under government not partners)**	**Required personnel**					
Administrative level	Role	Minimum qualifications	Total for current insecticide resistance monitoring and management activities, in FTE	Total required for IRMMP implementation, in FTE	Capacity gap	Annual gross salary per person ($) (Note does not include DSA. This needs to be included for those roles that require travel to study sites)	DSA per person per year ($)	Training per year	Estimated cost to fill capacity gap
National	Chief entomologist/Senior entomologist	MSc	0	1	1	27586.21			27586.21
National	Insectary manager	BSc	0	1	1	17931.03			17931.03
National	Insectary technician	BSc/Diploma	0	2	2	17931.03			35862.07
National	Field technician	BSc	0	4	4	17931.03			71724.14
National	Laboratory technician	BSc	0	2	2	17931.03			35862.07
National	Epidemiologist	MSc/BSc	2	1	0	0.00			0.00
National	Statistician	BSc	1	1	0	0.00			0.00
National	Database manager/ GIS specialist	BSc	0	1	1	17931.03			17931.03
Provincial	Chief environmental health officer	MSc	10	10	0	0.00			0.00
District	Environmental health officer	BSc	110	19	0	0.00			0.00
Health centre	Environmental health technologist/CHA	Diploma/ Grade 12	??	49	0	0.00			0.00

A3.6 Budget and potential sources of funding

Budget Line	Year 1	Year 2	Year 3	Year 4	Year 5	Total (US $)
Insectary	116210.7	27382	127831.77	27382	140614.947	439421.417
Laboratory	2118920	2034020	2330812	2237422	2563893.2	11285067.2
Field	693829	445250	763211.9	489775	839533.09	3231598.99
Data management and Dissemination	25990	4590	28589	5,049	31447.9	95665.9
Personnel	5681855.35	6250040.885	6875044.974	7,562,549	8318804.418	34688295.1
Meetings	13794	15173.4	16690.74	18359.814	20195.7954	84213.7494
Office	67741.41	74515.551	81967.1061	90,163.82	99180.19838	413568.0822
Additional costs for response to resistance	436000	436000	479600	527560	580316	2459476
External testing	342000	376200	413820	455202	500722.2	2087944.2
Technical support	56105	61715.5	67887.05	74675.755	82143.3305	342526.6355

A3.7 Risks and mitigating measures

The major risk to national insecticide resistance management programme is the untimely availability of required financial resources to successfully run the programme. With the set target of eliminating malaria within the next 5 years (by 2021), resource mobilization for supporting the IRM plan should be done in quickest possible time.

The other risk is that of potential emergence of resistance to organophosphates. Currently resistance to major insecticides recommended by WHO except for organophosphates (which include Pirimiphos-methyl) has been reported. However, since its deployment on a wider scale in 2013 the coverage has been increased to cover more and more areas of the country but without immediate plans to rotate it with an effective insecticide. Therefore, the risk for resistance against Pirimiphos-methyl is high and measures for inclusion of new products into the vector control programme needs to be put in place. The NMEC should engage with ZEMA and product manufacturers to enhance the evaluation and registration of new proven efficacious compounds that can be rotated with the organophosphates and other older insecticides that record restoration of susceptibilities. The impact of high pyrethroids resistance and the efficacy of LLINs has not been measured in different epidemiological settings within Zambia. There is urgent need to quantify the impact of pyrethroids resistance to enhance the management of resistance.

A3.8 Annexes

SECTION B: Annual Work plan for National Insecticide Resistance Monitoring Insecticide Resistance Monitoring & Management Plan Implementation

B1. Annual tasks, activities and timelines

Table 6: *Annual work plan activities*

Activity	Responsible	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Recruit necessary personnel to fill identified gap	NMEC												
Conduct training of established and new personnel followed by ongoing mentorship	NMEC/Partners												
Improve laboratory and insectary facilitates and procure necessary equipment	NMEC/Partners												
Establish sentinel sites	NMEC/Partners												

Carry out field collections of larvae (of blood fed adults as required)	NMEC/Partners												
Rear larvae to adult mosquitoes in field insectary	NMEC/Partners												
Conduct morhological species identification (and using other techniques as required)	NMEC/Partners												
Conduct bioassays with discriminating and intensity concentrations	NMEC/Partners												
Conduct synergist insecticide-bioassay	NMEC/Partners												
Activity	**Responsible**	**Jan**	**Feb**	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Enter data electonically into standard spreadsheets	NMEC/Partners												
Develop and implement monitoring and evaluation plans for interventions (residual efficacy and durability)	NMEC/Partners												
Coduct monitoring and evalaution activities	NMEC/Partners												
Develop standard national spreadsheets for data reporting and dissemination to partners involved in	NMEC												

data collection													
Establish and maintain national insecticide resistance database	NMEC/Partners												
Establish and maintain national database for intervention monitoring information	NMEC/Partners												
Complile availbale data on insecticide resistance, intervention implimentation and quality and link with epidemiological information	NMEC/Partners												
Report data to WHO and implementing partners	NMEC/Partners												
Convene insecticide decision making body for resistance management	NMEC/Partners	X											
Evaluate data to determine impact of resistance management strategies on a) disease outcomes, and b) resistance profiles of local malaria vectors	NMEC/Partners	X											
Generate recommendations for changes in vector control interventions	NMEC/Partners	X											

or their implementation based on decision-making framework													
Update national malaria strategic plan as required	NMEC/Partners	X											
Implement changes	NMEC/Partners												
Convene broad stakeholder meeting to report outcomes	NMEC/Partners												
Actively engange with national regulatory body to identify ways to optimize registration process for vector control products	NMEC/Partners												
Engage with patners involved in development of new vector control tools to encourage and support developement and registration as appropriate	NMEC/Partners	X											
Colloborate with research intitutes as required to evaluate the impact of insecticide resistance and management strategies	NMEC/Partners												

B1.1 Insecticide resistance monitoring

Table 7: *Locations, test, assay types, priorities and frequencies for insecticide tests in Zambia*

Locality Name			Test Type	Assay Type	Insecticide and Concentration to be Tested, Ordered by Priority	Month to be Tested											
						Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sep	Oct	Nov	Dec
Province	**District**	**Facility**															
Western	Mongu	Mabumbu	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Sefula	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%,	X	X	X	X	X				X	X	X	X

					Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%												
	Kaoma	Mutondo	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Urban	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

Southern	Kazungula	Sikaunzwe	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Mukuni	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

	Monze	Chikuni	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Kaumba	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

Lusaka	Chirundu	Chikanzaya	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Chipepo	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

	Rufunsa	Chinyunyu	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Kankumba	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Chitemales a	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
Central	Itezhi Tezhi	Buunga	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Bushinga	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
Copperbelt	Ndola	Chipulukusu	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Commando	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
	Masaiti	Masaiti Council	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Chondwe	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
North-Western	Solwezi	Kazomba	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Kimasala	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
	Kasempa	Nselauke	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Kivuku	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
	Mufumbwe	Lubilo	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Boma Clinic	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
Southern	Siavonga	Manchavwa	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Chaanga	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Sianyoolo	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

	Gwembe	Chabbobbo ma	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Sinafala	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

	Kalomo	Masempela	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
	Sinazongwe	Chiyabi	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Siatwinda	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Buleya malima	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		GVDC	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
		Sikaneka	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Solwegonde	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
Eastern	Katete	Mbalani	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Robert	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
Muchinga	Isoka	Nsalamba	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Namyala	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
Northern	Kasama	Kalonga	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Simeo Mwaba	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
Luapula	Mwense	Shibesa	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Chebele	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
	Milenge	Lunga	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Miyambo	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X
Central	Serenje	Chibobo	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

		Chishi	WHO susceptibility test	Adult assay and larvae assay	Pirimiphos-methyl 0.25%, Bendiacarb 0.1%, Propoxur 0.1%, DDT 4%, Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin,0.05%, Etofenprox 0.5%, Malathion 5%	X	X	X	X	X				X	X	X	X

B2. Annual budget

Table 8: *Annual budget*

Item description			Units	Unit cost (US $)	Total (USD $)
Insectary	**Building, Colony rearing and consumables**	Building maintenance	1	1000	1000
		Adult mosquito cages (bugdorms)	100	50.5	5050
		Water (Nuclease-free Water, 25 ml)	100	2	200
		Larval tray (small =24.8 x 19.7cm)	120	9	1080
		Larval tray (large= 45.7 x 31.8cm)	90	17	1530
		Mice Cage-Critter Nation Double Unit with Stand	6	239.95	1439.7
		Mice Cage (IVC cage)	12	35	420
		Larval foods (bottles) (50 per month)	50	3	150
		Blood meal source (e.g. mice) (per mouse at every 3 months)	60	5	300
		Sugar (per 2kg) (per quarter)	15	3	45
		Grade 40 filter paper, diameter 15cm (10 packs)	90	50	4500
		Grade 50 filter paper, diameter 11cm (10 packs)	90	70	6300
		Mosquito netting materials/ yard	120	3.1	372
		Cotton wool (500 g)	270	5	1350
		Pipettes (50 /pkt)	540	6	3240
		Paper cups (per packet of 10 cups)	1200	1	1200
		Petri dishes (500 per box)	30	0.5	15
		Wash bottles	100	5.59	559
		Mouth aspirators	1500	29	43500
		Temperature/Relative Humidity USB Field Dataloggers	100	82	8200
		Latex gloves (per box of 50)	150	12	1800
		Rubber bands (per box)	1200	2.99	3588
		Fisher Scientific Temperature/Humidity Meter **(ASIN: B004VWB0DY)**	12	331	3972
		Emegence cups (breeders)	1200	12	14400
		Forceps	600	5	3000
		Ziploc double zipper bags (small) 10 bags 20cm x18 cm or 16x16 cm (10 pieces/pkt)	1200	3	3600
		Tower self adhesive labels (small: 13x19mm) (10 pieces/pkt)	1200	3	3600
		Tower self adhesive labels (large: 75x37mm) (10 pieces/pkt)	600	3	1800
		Data forms		0.5	0
	Subtotal				**116210.7**

Laboratory	Building	Building maintenance (cleaning, repairs, plumbing etc)	1	1000	1000
	Fixed equipment	Stereo/Binocular microscopes	60	200	12000
		Compound microscopes	30	300	9000
		PCR machine	3	3000	9000
		Equipment for gel electophoresis	3	700	2100
		Freezers	3	6000	18000
		Centrifuge machine	3	2000	6000
	Other equipment	Insecticde susceptibility test kits	9	1200	10800
		Insecticde testing papers	60	18	1080
		Insecticide synergist	30	20	600
		Hand held magnifying glass	120	15	1800
		Forceps	600	5	3000
		Pipettes	1200	6	7200
	Consumables	Insceticide papers	120	18	2160
		Silica gel (per kg)	6	30	180
		Specimen tubes (Ependorf tubes 500/bag)	15000	120	1800000
		ELISA reader	3	2000	6000
		ELISA Reagents (primers, taq polymerase etc) (every 3 months)	4	12000	48000
		Pippette tips (960 tips per pack)	1200	140	168000
		Latex gloves (per box of 50)	1000	10	10000
		Data forms	6000	0.5	3000
					0
	Subtotal				**2118920**
Field collections	Transport	Vehicle lease/maintenance (hire per day)	9	150	1350
		Fuel/day	60	50	3000
		Driver (DSA per day)	60	60	3600
	Mosquito collection equipment	Larval dippers/laddles (350ml)	120	22.35	2682
		Adult mouth aspirators	600	29	17400
		Larval collections vials	1000	12	12000
		Adult cages (bug doms)	300	50.5	15150
		Torches + batteries (or solar lamps)	300	6	1800
		CDC Light traps	600	100	60000
		CDC- Extra collection cups	600	8	4800
		CDC collection cup and cloth (fine mesh)	600	15	9000
		CDC replacement lamps (bulbs)	750	1	750
		CDC Light traps batteries (6 Volts)	1200	20	24000
		Sugar (per 2kg)	800	3	2400

		Coffee	800	4	3200
		Backpack baterries (12 Volts)	120	70	8400
		Fine mesh collection cups (backpack cups)	120	5	600
		Backpack aspirator	60	550	33000
		Solar panels (12 Volts)	300	20	6000
		Forceps	300	5	1500
		Buckets (each)	120	3	360
		Work suit (overalls)	300	30	9000
		Rubber gloves	600	5	3000
		Rubber boots	300	23	6900
		Shapies (pens)	120	18	2160
		Ethyl Acetate (Mosquito killing/preservation)(6 x 500ml) per box	120	30	3600
		Paper cups (per packet of 10 cups)	1200	1	1200
		70% Ethanol 500 ml Bottles (Preservation of Larval Specimens)	60	7.95	477
		Cool boxes	150	40	6000
	Data collection tools	GPS units	50	250	12500
	Communication	Mobile phones	240	100	24000
		Airtime (bundles/month)	120	50	6000
	Consumables	Specimen tubes (Ependorf tubes 500/bag)	3000	120	360000
		Silica gel	1500	30	45000
		Emergence cups (breeders)	100	12	1200
		Data forms	3600	0.5	1800
	Subtotal				**693829**
Data management and dissemination	Office information technology hardware	Laptop	12	600	7200
		Printer	10	500	5000
	Office information technology software	Data processing software	12	200	2400
		Mapping software	2	695	1390
	National insecticide resistance datatbase	IR database establishment	1	8,000	8000
		IR database maintenance	1	2,000	2000
	SUBTOTAL				**25990**
Human Resource	Chief entomologist/ Senior entomologist	Salary, benefits and overheads	1	27,586.21	27586.21
	Insectary manager	Salary, benefits and overheads	1	17,931.03	17931.03

	Insectary technicians	Salary, benefits and overheads	2	17,931.03	35862.06
	Field technician	Salary, benefits and overheads	4	17,931.03	71724.12
		Per diems for fieldwork	4	12,413.79	49655.16
		Community Health Assistants	49	689.66	33793.34
	Laboratory scientist	Salary, benefits and overheads	2	17,931.03	35862.06
	Epidemiologist	Salary, benefits and overheads	1	27,586.21	27586.21
	Statistician	Salary, benefits and overheads	1	17,931.03	17931.03
	Database manager/GIS specialist	Salary, benefits and overheads	1	17,931.03	17931.03
	Chief enviornmental health officer	Salary, benefits and overheads	10	27,586.21	275862.1
	Environmental health officer	Salary, benefits and overheads	110	17,931.03	1972413.3
	Environmental health technologist	Salary, benefits and overheads	220	13,793.16	3034495.2
	Training courses	Field collection techniques and mosquito identification	30	114.95	17242.5
		Insectary management and mosquito rearing	30	114.95	17242.5
		Laboratory management, insecticide susceptibility testing, resistance mechanisms testing	10	114.95	5747.5
		Insecticide resitance management (including sound management of public health pesticides)	20	114.95	11495
		Other as needed (e.g. data management, GIS, programme managemnet)	20	114.95	11495
	Subtotal				**5681855.35**
Meetings	Meetings of decision making body	First meeting	20	114.95	6897
		Second meeting	20	114.95	6897
	Subtotal				**13794**
Office	Overheads	Administration feeds, maintenance, electricity, water			16,172.41
	Stationery	Paper, pens, photocopying			30,000.00
	Communication	Internet, phone, talk time			21,569.00
	Subtotal				**67,741.41**
Additional	Commodities	Trainings on new formulation of insecticides	2	2000	4000

cost for response to resistance		Equipment and materials	72	6000	432000
					0
	Subtotal				436000
External testing	Specimen assessments by external institution	Species identification (3000 samples from the three institutes, namely PMI-AIRS, TDRC & MACEPA @ $5.5/sample)	6	16500	99000
		Sporozoite detection (3000 samples from the three institutes, namely PMI-AIRS, TDRC & MACEPA @ $5.5/sample)	6	16500	99000
		Testing for insecticides resistance mechanism in malaria vectors (3000 samples from the three institutes, namely PMI-AIRS, TDRC & MACEPA @ $8/sample)	6	24000	144000
	Subtotal				342000
Technical support	Specialist assistance	**Training on genotying and morphological identification**			
		Consultant (fee @ 7days, air tickets, accomodation, local transport etc)	1	5000	5000
		Transport refunds (may includes fuel refunds)	2		0
		Accomodation (per diem per day for trainees)	10	60	600
		Training fees for Macha	1	50505	50505
	Subtotal				56105
Approximate total cost for the year					**9,552,445.46**

Annex 5. Members of the IRMMP Guidelines Review Committee

NAME	DESIGNATION	ORGANIZATION
EMMANUEL H. KOOMA	VCS	NMEC
PRESLEY MUSONDA	OPS MAN	VL-PMI
SHEILA MWACHILENGA	ECO	VL-PMI
CHRISTOPHER MULENGA	PEST CONTROL SPT	LUSAKA CITY COUNCIL
ALICK CHIRWA	SEHO	KABWE - DHO
TEDDY WAKUNUMA	CEHO	PHO-CENTRAL PROVINCE
MORGAN SANKA	CEHO	PHO- NORTHERN PROVINCE
BERNARD MWANSA	CEHO	PHO-MUCHINGA
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MATEYO MOYO	CEHO	PHO-MANSA
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BENJAMIN MASUMBA	DRIVER	MOH/NMEC
DINGANI CHINULA	ENTOMOLOGIST	NMEC
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ANTHONY YETA	D DIRECTOR	NMEC
JENNIFER STEVENSON	SCI. DIRECTOR	MACHA
CONSTANCE NJOVU	REGIONAL COORDIANTOR	IFCBMI
NSOFWA FRANCIS	CEHO	PHO - NWP
ELIZABETH CHIZEMA	DIRECTOR	NMEC
MUTINTA MUDENDA	DEPUTY DIRECTOR	NMEC
MESELI HASTINGS	DRIVER	NMEC
FREDDIE MASANINGA	NPO-MAL	WHO
CHRISTOPHER KANEMA	PRINICIPAL INSPECTOR	ZEMA
NELSON WAITOLO	PHO	COPPERBELT
NDUKA IWUCHUKWU	COP	VECTORLINK
MAXWELL NKOYA	DOP	ZEMA
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BENJAMIN CHANDA	ENTOMOLOGIST	NMEC
MWANSA MWENYA	ENTOMOLOGIST	NMEC
TRESFORD KANIKI	ENTOMOLOGIST	NMEC
BRENDA SICHONE	SECRETARY	NMEC
DORIS BUSAKA	OFFICE ASSISTANT	NMEC

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