

PMI /IRS OPERATIONAL RESEARCH IN BENIN

REPORT 2: JANUARY 2009

A. EVALUATION OF INDOOR RESIDUAL SPRAYING IN OUEME AND OUIDAH-KPOMASSE-TORI BOSSITO HEATH ZONE, BENIN: PROJECTS A AND B

Introduction

The entomological monitoring is still going on in the areas of IRS and LLINs in Ouémé and in the sanitary Zone of Ouidah-Kpomasse-Tori bossito according to the established program based on: the effectiveness of the walls treated with bendiocab, night capture of *Anopheles gambiae* to assess the reduction of mosquito bites, the behaviour of *Anopheles* through the treated walls.

Our last report was focus on the data collected before and after the implementation of IRS strategies.

Few data were collected from December 2008 to January 2009 to conclude on the entomological monitoring and the effectiveness of the bendiocarb during this period.

However, significant efforts were made on laboratory screening tests for the presence of circumsporozoite using ELISA techniques with specific monoclonal antibodies, and sociological study to collate the impression of Communities on the implementation of IRS and LLINs strategies. As regards the PCR analysis on the dynamics population of species of *Anopheles gambiae* and the forms M and S, many *Anopheles* were stored in the freezer, but not yet analyzed due to the lack of reagents. Purchase order from CREC to Eurogentec (Belgium) since August 2008 have not yet been delivered despite the payment of the invoice. This report is mainly based on the ELISA analysis and the sociological study.

I-Results of a sociological study on the use of PMI LLIN and IRS implementation

In Oueme (Project A) and in Ouidah-Kpomassè-Tori Bossito Health Zone (Project B)

1. Justification

A total number of 1436 bed rooms were treated in 7 hamlets at sanitary Zone of Ouidah-Kpomasse-Tori bossito districts in July 2008, representing 81% of rooms. One month later, an additional IRS was organized particularly for Manguevier hamlet and other villages to increase IRS covering rate. Today, 90% of houses are covered by the IRS strategy. In October 2008, Permanets were graciously distributed in the same hamlets. Each household has received 1 or 2 Permanets, depending on the size of the family. In total, 1000 LLINs were distributed. In another hamlet, in the department of Oueme, populations from 4 districts were protected against mosquito bites by indoor residual spraying (IRS) and freely received LLINs (Permanets). In December 2008 and January 2009, a sociological survey was conducted in the two zones to collate information on the community's perception about malaria, their point of view about IRS and the use of LLINs.

General objective

To collate information on the use of Permanets distributed by PMI and other bed nets from the communities and their perception on the implementation of indoor residual spraying in Oueme and in Ouidah-Kpomassè-Tori Bossito Health Zone after 6 months of trial

Specific objectives

- To get feed-back information from communities on the success or the failure of the IRS strategy

- To appreciate the real use and coverage of PMI LLINs and other mosquito nets in Oueme and Ouidah-Kpomassè-Tori Bossito Health Zone and the origin of mosquito nets used in the two areas.

Material and method

To conduct the study, 100 houses were randomly selected per hamlet out of 4 in Ouidah-Kpomasse-Tori Bossito Health Zone and 800 in the department of Oueme (200 in the IRS area, 200 in LLINs area X 2 districts). During the survey, treated houses were checked for certainty. When a house was not treated then it was excluded and we move to the next house of the random list. If a house is treated and the owner agreed, we include the house in the study and we start the focus group. The survey was conducted with emphasis on individual and focus group discussions addressed to the head of households particularly women and local leaders. Discussions were based on a quantitative and qualitative questionnaire. These discussions were completed by a direct field observation

The protocol was articulated on getting sociological data on:

- the rate of untreated homes and the reasons behind it
- untreated places for sleeping (the place can be a room, hall or lounge)
- the impression of communities on the advantages of the IRS
- Disapproval or disadvantages of IRS
- the impression of communities on the renewal of IRS
- Other individual and collective protection against mosquito bites used in the IRS area
- Number of impregnated nets of PMI; PNLP and others recorded in the houses
- Status of the impregnated nets distributed at Tori and Ouémé (torn, good condition etc...)
- Origin of the nets distributed in the different areas
- Frequency of the use of impregnated nets

- Position of impregnated nets distributed

Data analysis

Analysis using computer software Epi info and Excel were performed on sets of data collected from the different sites

Results

1- Means of protection against mosquitoes before and after the IRS

Insecticides bomb, mosquito burner, medicinal plants and impregnated nets are the common means used against mosquito bites before the IRS strategies with high percentage of bed nets in the two areas. However, after the implementation of IRS strategies, the impregnated nets are still being used by most of the communities of Tori (95%) and Ouémé (90%).

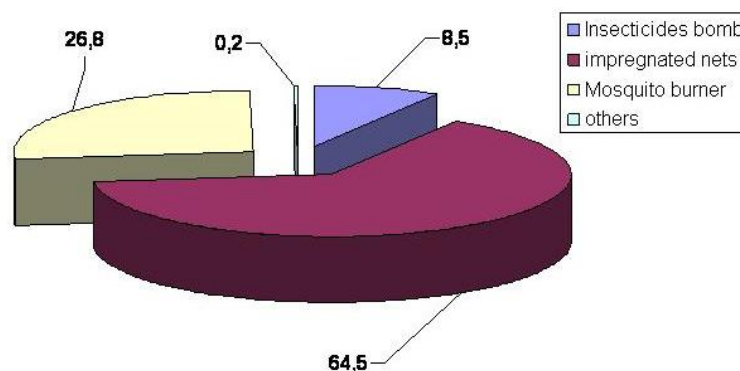


Fig1 : Means against mosquito bites before IRS in Tori and in Ouémé

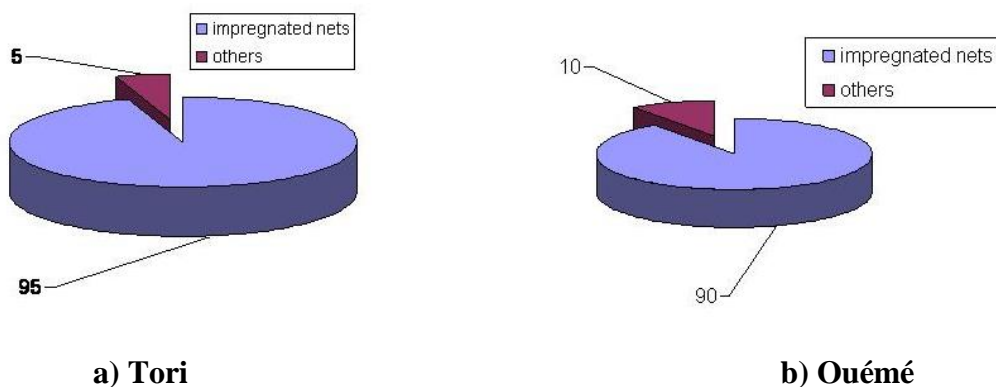


Fig2 : Means against mosquito bites after IRS in Tori and in Ouémé

2- IRS Coverage

At Tori, over 95% of the houses were treated. The remaining 5% of untreated homes were due, either to the refusal of the head of households or to their unavailability to receive impregnation agents. In Ouémé, the coverage rate was 70%, which is lower than the expected rate of 80% (Fig3).

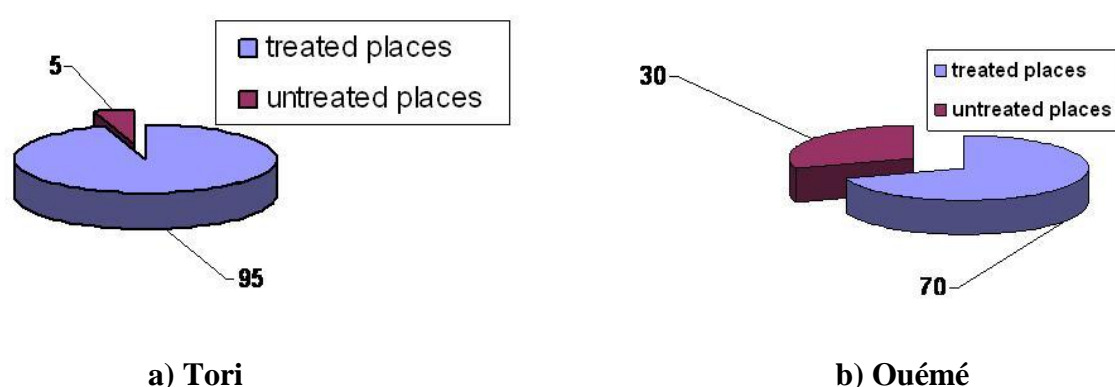
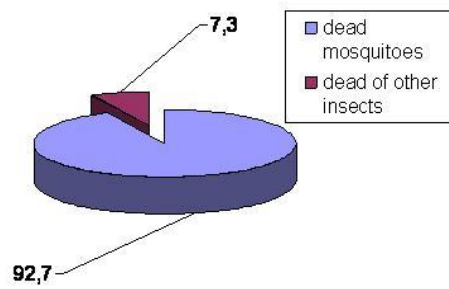


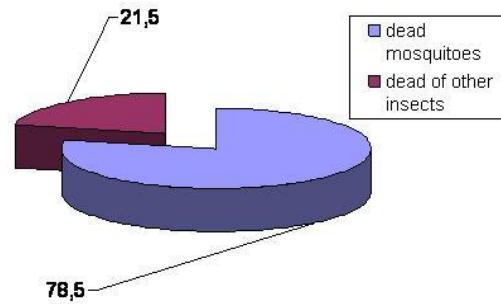
Fig3 : IRS Coverage in Tori and in Ouémé

3- Communities impression on the IRS: IRS advantage

Six months after the IRS trials in Ouidah Kpomassè-Tori Bossito districts and in the department of Ouémé, people continue to appreciate the effectiveness of this strategy. In fact, 92.7% of respondents in Ouidah Kpomassè-Tori Bossito believe that IRS kills mosquitoes. In Ouémé, it's the same reaction (78%). The others (7.3% in Tori and 20% in Ouémé) indicate that the IRS has effect only on mosquitoes, but also in other insects that live in houses (fig3). In addition, 82.2% of respondents in Ouémé think that IRS has no side-effect on human health. The same reaction was observed in Tori with 97.5% of respondents.



a) Tori

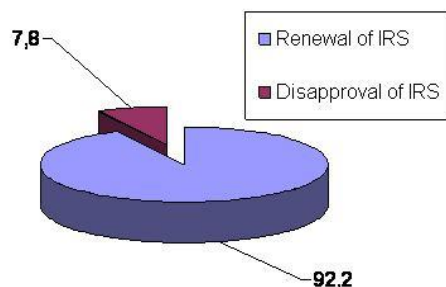


b) Ouémé

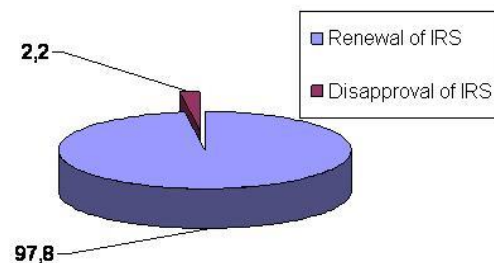
Fig4- Communities impression on the IRS in Tori and in Ouémé

3-IRS renewal

All the people approached during the survey wanted the renewal of the IRS: 92.2% in Ouémé and 97.8% in Tori-Bossito. However, in Ouémé, 7.8% required IRS operations to be stopped because of its bad smell. This percentage of respondents is very low in Tori (2.2%) (Fig5).



a) Ouémé



b) Tori

Fig5 : Renewal of IRS according to Tori and Ouémé communities

4-Strategy desired by Tori-Bossito communities against mosquito bites.

At Tori-Bossito, more than half of the population (52.8%) think that the best way to avoid or reduce mosquito bites is to combine IRS and impregnated nets (Fig6)

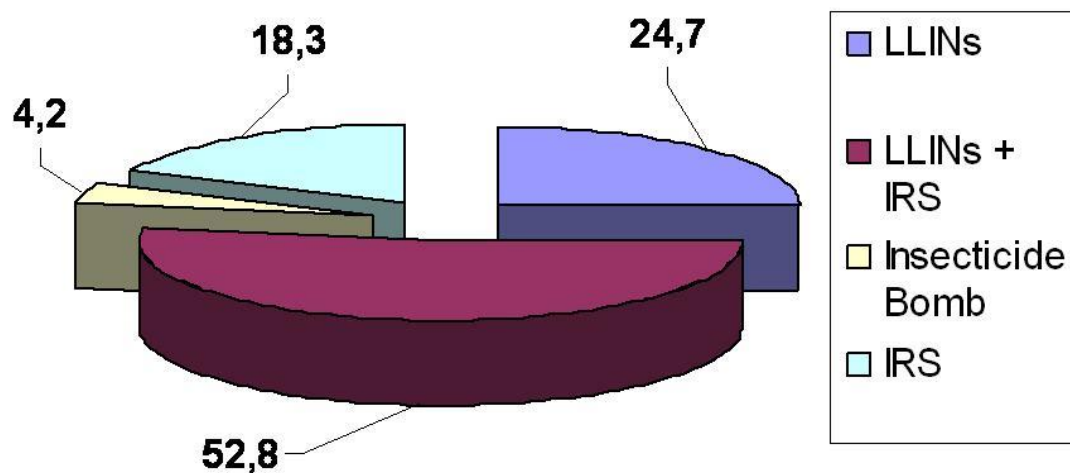


Fig6: Strategy desired by Tori-Bossito communities against mosquito bites 6-Origin of impregnated nets

6-Origin of impregnated nets

57.14% of the impregnated nets used by people came from PMI; 13.37% from the national campaign, 11.04 were either from hospitals or pharmacies, 16.42% from markets and 2.02% elsewhere (Fig7).

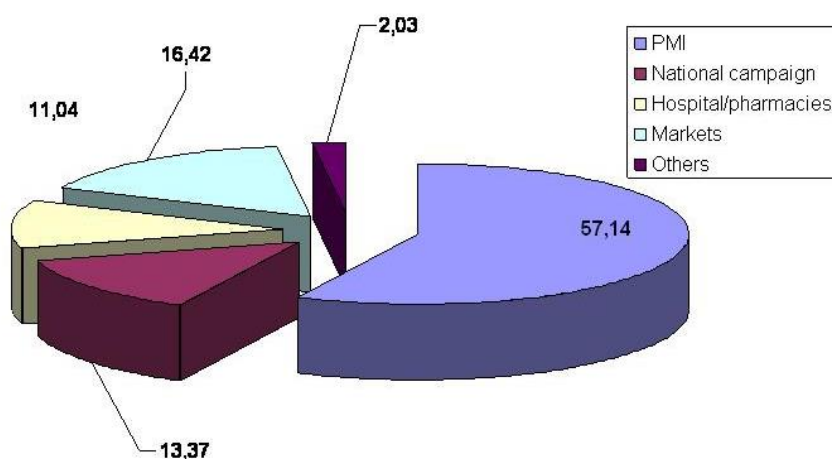


Fig7 : Origin of impregnated nets in Ouémé

7-Frequency of the use of nets

During our discussion and focus group in Tori and Ouémé, most of the populations declared spending the night under impregnated nets to avoid or reduce mosquito bites. Observations made in houses have showed that 90% of families actually sleep under mosquito nets in Ouémé. It's the same in Tori with 93% (Fig 8)

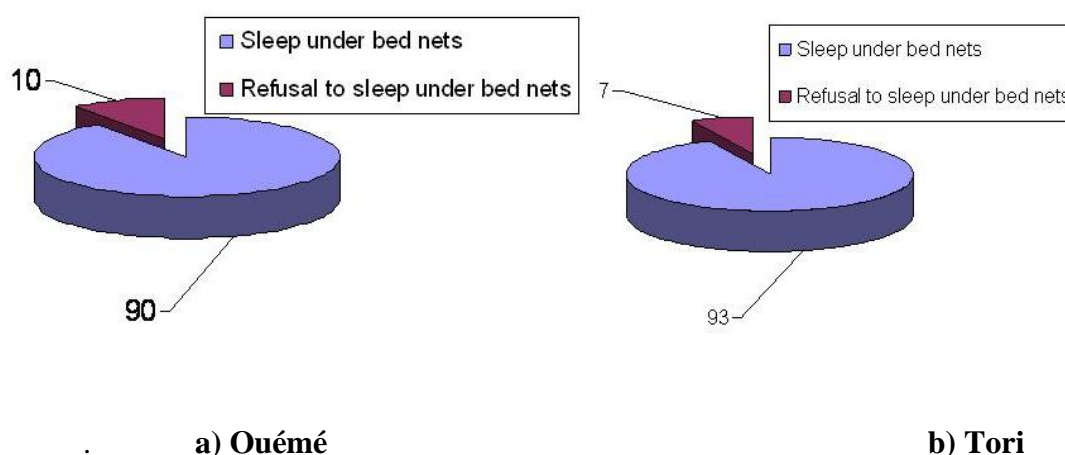


Fig8: Frequency of use of nets

8 – Position of impregnated nets in the houses.

Most of the nets were found hung. The result of the survey conducted shows that 93% of impregnated net in Tori were hung on the mat and 92% in Ouémé (Fig9).

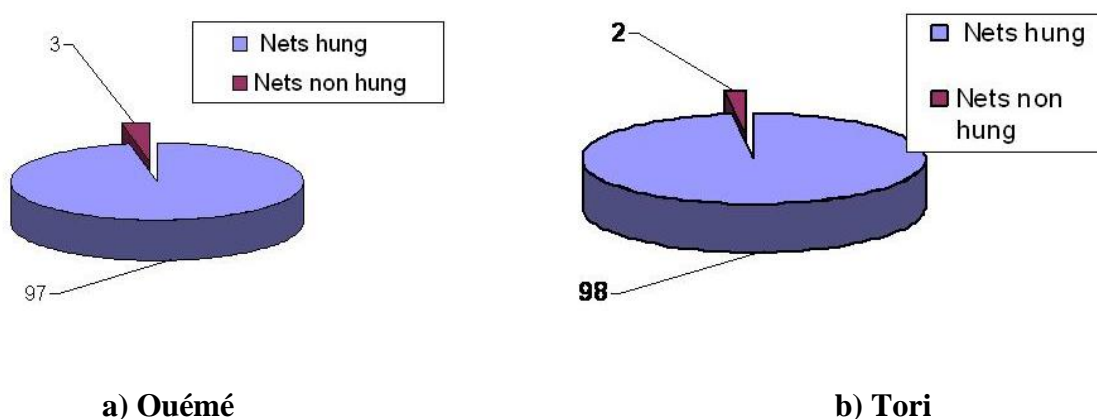


Fig9: Position of impregnated nets in the houses in Tori and in Ouémé.

9-Status of impregnated nets

In Ouémé and Tori, most of the nets were still in good shape. However, 9% of these nets were torn in Ouémé against 5% to Tori (Fig10)

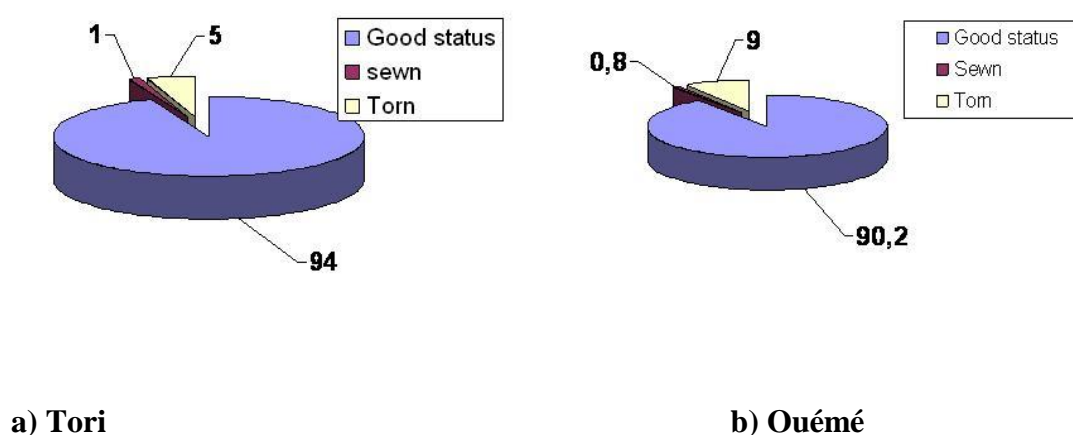


Fig10 : Status of impregnated nets

Conclusion.

Six months after the IRS trials in Ouidah Kpomassè-Tori Bossito and in the department of Ouémé, people continue to appreciate the effectiveness of this strategy.

Most of people who were interviewed during our study declared that the IRS didn't have any effect on their health and would be very happy to see the renewal of this strategy yearly.

In the sanitary zone of Ouidah Kpomassè-Tori Bossito, more than 50% of the respondents think that the best way to control mosquito bites is to combine IRS and LLINs.

In Ouémé, most of the impregnated nets came from PMI, despite those from the national campaign, markets and pharmacies.

II. Variation of sporozoitic index (%CS+) and Entomological Inoculation Rate (EIR) in the IRS zone

Introduction

Four strategies are implemented in the Ouidah-Kpomasse-Tori Bossito's Health Zone:

- (i) Protect children aged 0 to 5 years old sleeping under LLINs against mosquito's bites (This strategy is currently used nationwide by the National Malaria Control Program): Arm 1
- (ii) Protect the whole community sleeping under LLINs against mosquito's bites: Arm 2
- (iii) Protect the communities sleeping in houses lined with Insecticide Treated Plastic Sheetings (ITPS, treated with a non-pyrethroid: bendiocarb) against mosquito's bites: Arm 3
- (iv) Protect the community sleeping in houses where the walls are treated (IRS) with bendiocarb (a non-pyrethroid) against mosquito's bites: Arm 4

Only the 4th one was funded by the Presidential Malaria Initiative (PMI). In total, the 4 interventions cover 28 villages/hamlets. The main characteristic of Ouidah-Kpomasse-Tori Bossito's Health Zone is the low density of *Anopheles* assuring malaria transmission. Data obtained from October 2007 to April-May 2008 before IRS and from July to October-November 2008 are mentioned on the following table. The mean number of bites per man per night (ma) is 0.30 for *An. gambiae* + *An. funestus* before IRS. After IRS, this number decreases to 0.11. The impact of the IRS was spectacular on *An. funestus* which is a very endophilic mosquito. Indeed, after IRS, a considerable reduction of 75% of its population (16 females of *An. funestus* before IRS against 4 after IRS) was recorded. 6.25% to 23.9% were found harboring the CS protein of *P.falciparum* before IRS. The high percentage (23.9) was due to the infected mosquitoes in July 2008, when the spraying took place, and was recorded among the specimens collected after IRS. This result was wrongly recorded, since the extrinsic incubation of *Anopheles* requiring a period of about 2 weeks, *gambiae* and *funestus* in July took their blood meal in late June or early July. In this regard, the positive of CS antigen increase from 13% (19 Thorax+ / 145) before IRS to 14% (2 thorax + / 14) after IRS. As such, the entomological inoculation rate (EIR) decreases from 0.04 to 0.015. These values indicate 62.5% reduction of transmission through the implementation of the IRS. Before indoor residual spraying with bendiocarb, each inhabitant living in IRS hamlets was receiving 1.2 bites infected by *An. gambiae* or *An. funestus* per month. After IRS, the rate has dropped to 0.45 bites per month.

Conclusion

Six months after the IRS trials in Ouidah Kpomassè-Tori Bossito and in the department of Ouémé, the reducing of malaria transmission is obvious and the density of Anopheles' threshold has become very low. Actually, the more the threshold of vector density is low, the lower the impact of strategies is visible. In spite of the situation, after the IRS in the sanitary zone of Ouidah Kpomassè-Tori Bossito, the density of *An. gambiae* and *An. funestus* was reduced by 63.3%.

An. funestus density was reduced by 75% and its transmission level was reduced by 63.3%

| Entomological data obtained before and after interventions in Tori, Benin | | | | | | | | | |
|---|--------------------|----------|----------|--------------|------------|--------------------|----------|----------|-----------|
| | | Before | | Intervention | | After intervention | | | |
| | | Oct.2007 | Nov.2007 | Jan.2008 | March 2008 | April 2008 | Jul.2008 | Aug 2008 | Oct. 2008 |
| NMCP strategy | Total An.gambiae | 29 | 2 | 11 | 12 | 16 | 39 | 12 | 6 |
| | Total An. Funestus | 14 | 3 | 8 | 12 | 8 | 20 | 5 | 2 |
| | ma | | | | | | | | |
| | Thorax | 29 | 1 | 11 | 12 | 8 | 46 | 7 | 35 |
| | CS+ | 6 | 0 | 1 | 0 | 1 | 4 | 0 | 10 |
| | % CS + | 20,7 | 0 | 9,1 | 0 | 12,5 | 8,7 | 0 | 28,6 |
| | he | | | | | | | | |
| LLINs | Total An.gambie | 19 | 7 | 4 | 2 | 11 | 18 | 28 | 18 |
| | Total An. Funestus | 70 | 52 | 114 | 107 | 32 | 19 | 22 | 13 |
| | ma | | | | | | | | |
| | Thorax | 19 | 5 | 4 | 2 | 11 | 12 | 14 | 6 |
| | CS + | 3 | 1 | 1 | 0 | 1 | 3 | 3 | 0 |
| | % CS + | 15,8 | 20 | 25 | 0 | 9,1 | 25 | 21,4 | 0 |
| | he | | | | | | | | |
| IRS | Total An.gambie | 39 | 12 | 3 | 6 | 29 | 29 | 16 | 5 |
| | Total An. Funestus | 11 | 12 | 15 | 27 | 15 | 5 | 8 | 1 |
| | ma | | | | | | | | |
| | Thorax | 37 | 13 | 3 | 6 | 37 | 49 | 9 | 5 |
| | CS + | 3 | 2 | 0 | 0 | 1 | 13 | 1 | 1 |
| | % CS + | 8,1 | 15,4 | 0 | 0 | 2,7 | 26,5 | 11,1 | 20 |
| | he | | | | | | | | |
| ITP + LLINs | Total An.gambie | | | | | | 20 | 27 | 31 |
| | Total An. Funestus | | | | | | 3 | 5 | 1 |
| | ma | | | | | | | | |
| | Thorax | 60 | 21 | 9 | 7 | 23 | 12 | 11 | 14 |
| | CS + | 5 | 1 | 0 | 0 | 6 | 2 | 3 | 1 |
| | % CS + | 8,3 | 4,8 | 0 | 0 | 26,1 | 16,7 | 27,3 | 7,14 |
| | he | | | | | | | | |

III. MONITORING OF DELTAMETHRIN LEVELS ON LLINs USING A NON-DESTRUCTIVE COLORIMETRIC FIELD TEST (NET TEST): T0 EVALUATION: REPORT C

1. Collaboration:

CDC Atlanta: Mike Green, Ph.D., Chemist, EB, DPD, CDC

Center for Entomological Research – Cotonou (CREC)

Martin Akogbeto, Ph.D., Director / Professor, Univ. Abomey-Calavi

M.Sc./Ph.D. Students, Univ. Abomey-Calavi: Anges Yadouleton, Roseric Azondékon,

Roland Alia

2. Justification

During the first mission of CDC team in Benin, we have evaluated the value of colorimetric test developed by CDC corresponding to $\leq 80\%$ and $> 80\%$ of anopheles' mortality using bioassay- cone method and make a standardized test. The results obtained were very interesting. A high correlation was found between CDC colormetrec test and WHO bioassay cone test. What is important, the CDC colorimetric method offers the possibility to carry out the tests on the field, directly in the houses where LLINs were hung without moving them. This is why we recommended the choice of this technique for the quality control of impregnated mosquito nets distributed by PMI in October 2008 in Ouidah-Kpomasse-Tori Bossito's Health zone and for the monitoring of the degradation of the insecticide in the fiber nets during their use. We also recommended 3 steps of evaluation: at T0 (some weeks after the distribution of LLINs), at T6 (after 6 months of use) and T12 (after 12 months of use). These results show data obtained at T0

3. Objectives:

-To monitor deltamethrin levels on LLINs using a non-destructive colorimetric field test (NetTest).

-Establish baseline levels of deltamethrin on surfaces from recently distributed LLINs and compare with levels at 6 and 12 months of normal usage.

4. Method:

Two villages, Manguevie and Tokoli, were selected as test sites since new LLINs have recently been distributed (~1 month) and had not been subjected to washing. The sample collection team entered each village and asked inhabitants whether they possess a net and if it had been washed. Unwashed nets were then selected for testing. Sample collection was performed on hung nets with minimum disruption to inhabitants. Net surface levels of deltamethrin were obtained by exposure of filter paper to designated locations (bottom left, middle side, upper right, and top) locations of a hung net as described by Green et al.¹. These locations were marked by tying colored threads as to prevent testing of the same area during subsequent sample collections at 6 and 12 months. After all the samples were collected (4 locations per net x 76 nets = 304 samples), the colorimetric assays were performed. All necessary equipment including reagents, filter papers, camera, light box, pipette were packed at CDC in a kit approximately 0.3 x 0.2 m and transported to the site. Surface deltamethrin levels were determined per method described by Green et al.¹ and compared to a threshold value of 0.7 $\mu\text{g}/\text{sample}$ (equivalent to amount of deltamethrin per surface area of 2.7 $\mu\text{g}/\text{m}^2$).

This value was established as the amount of surface deltamethrin equivalent to 80% mosquito mortality (WHO Cone Test) as determined by Green et al.¹ using indigenous mosquitoes (AKRON- a local strain of *An. gambiae* s.s. with some deltamethrin resistance). Any net below the threshold was bioassayed and replaced with a new net.

5. Results:

Samples for 76 nets were collected in 2.5 days with a collection team consisting of four members. The colorimetric testing for deltamethrin on all samples (n=304) were performed in one working day. The results are shown in the table. If the average of all four locations is < 0.7 µg/sample, the net is considered as “failed”. Table 1 shows two failed nets, 42-01 and 52-01. Deltamethrin amounts were below detection limits on all four positions for net 42-01 and trace levels were found on net 52-01. These nets were subsequently replaced with new nets and brought to CREC for bioanalysis using the WHO Cone assay and AKRON and KISUMU strain mosquitoes (Table 2).

Table1

| Deltamethrin amount per Sample (µg) | | | | | |
|-------------------------------------|--------|-------------|-------|-----|---------|
| Net ID | Bottom | Side middle | Upper | Top | Average |
| | left | | right | | |
| 37-02 | 2.7 | 2.0 | 0.7 | 0.9 | 1.6 |
| 01-09 | 2.4 | 1.7 | 2.6 | 2.8 | 2.4 |
| 30-02 | 1.2 | 2.4 | 1.4 | 1.6 | 1.7 |
| 01-14 | 0.5 | 1.9 | 1.8 | 1.3 | 1.4 |
| 01-06 | 1.8 | 2.6 | 2.6 | 1.9 | 2.2 |
| 01-30 | 1.1 | 1.4 | 1.6 | 2.3 | 1.6 |
| 35-01 | 1.1 | 1.9 | 2.2 | 4.0 | 2.3 |
| 42-01* | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 41-01 | 2.4 | 0.7 | 1.8 | 2.0 | 1.7 |
| 62-01 | 1.9 | 2.1 | 1.4 | 3.0 | 2.1 |
| 61-01 | 2.0 | 2.6 | 1.5 | 2.7 | 2.2 |
| 15-01 | 1.1 | 1.3 | 1.2 | 1.8 | 1.4 |
| 01-23 | 2.0 | 1.0 | 1.2 | 1.4 | 1.4 |
| 33-01 | 2.8 | 1.5 | 1.9 | 2.5 | 2.2 |
| 22-01 | 3.5 | 3.2 | 2.5 | 2.9 | 3.0 |
| 09-01 | 0.7 | 1.5 | 0.5 | 2.3 | 1.3 |
| 54-01 | 2.2 | 1.4 | 1.2 | 3.2 | 2.0 |
| 40-01 | 0.5 | 2.9 | 0.9 | 0.7 | 1.3 |
| 26-01 | 3.6 | 3.0 | 0.9 | 3.9 | 2.9 |
| 38-01 | 3.1 | 2.0 | 1.6 | 3.0 | 2.4 |
| 50-01 | 5.7 | 2.2 | 1.3 | 2.9 | 3.0 |
| 33-01 | 2.5 | 1.4 | 1.5 | 2.8 | 2.0 |
| 30-01 | 2.7 | 2.3 | 2.3 | 1.5 | 2.2 |
| 01-08a | 2.5 | 2.4 | 1.1 | 2.0 | 2.0 |
| 03-04 | 2.7 | 3.2 | 1.6 | 2.2 | 2.4 |
| 01-08b | 1.5 | 1.7 | 2.1 | 2.8 | 2.0 |
| 02-08 | 3.3 | 2.2 | 2.6 | 3.6 | 2.9 |
| 01-10 | 2.8 | 0.7 | 1.2 | 0.8 | 1.4 |
| 02-09 | 1.6 | 2.3 | 1.5 | 3.1 | 2.1 |
| 55-01 | 0.0 | 2.7 | 3.0 | 1.5 | 1.8 |
| 01-07 | 2.9 | 1.9 | 1.1 | 2.6 | 2.1 |

| | | | | | |
|--------|-----|-----|-----|-----|------------|
| 47-01 | 1.3 | 1.3 | 1.3 | 2.4 | 1.6 |
| 01-07 | 1.3 | 1.2 | 1.0 | 1.9 | 1.4 |
| 44-01 | 2.9 | 1.6 | 1.9 | 2.3 | 2.2 |
| 31-01 | 3.6 | 2.4 | 2.7 | 3.0 | 2.9 |
| 20-01 | 2.4 | 2.1 | 1.5 | 2.1 | 2.1 |
| 01-11 | 1.3 | 1.1 | 2.2 | 3.3 | 2.0 |
| 03-05 | 2.0 | 1.6 | 2.1 | 1.4 | 1.7 |
| 01-12 | 1.5 | 1.2 | 2.6 | 2.0 | 1.8 |
| 25-01 | 2.1 | 2.4 | 2.1 | 2.7 | 2.3 |
| 31-01 | 5.1 | 2.8 | 2.9 | 4.4 | 3.8 |
| 32-01 | 2.4 | 1.9 | 2.3 | 2.2 | 2.2 |
| 02-04 | 0.9 | 2.0 | 2.3 | 3.1 | 2.0 |
| 31-00 | 3.2 | 1.6 | 1.7 | 3.9 | 2.6 |
| 8-1 | 1.7 | 1.6 | 1.7 | 2.5 | 1.9 |
| 7-1 | 3.6 | 2.6 | 2.7 | 2.7 | 2.9 |
| 48-01 | 1.9 | 1.2 | 1.8 | 4.4 | 2.3 |
| 46-01 | 2.1 | 1.7 | 1.5 | 3.5 | 2.2 |
| 52-01* | 0.0 | 0.3 | 0.5 | 0.0 | 0.2 |
| 16-01 | 4.7 | 3.6 | 3.4 | 4.9 | 4.2 |
| 02-07 | 2.8 | 3.4 | 2.6 | 2.6 | 2.8 |
| 19-01 | 2.5 | 1.3 | 2.0 | 2.1 | 2.0 |
| 02-03 | 1.1 | 1.6 | 1.6 | 2.2 | 1.6 |
| 03-03 | 2.3 | 3.6 | 2.5 | 3.3 | 2.9 |
| 10-09 | 1.6 | 3.4 | 1.9 | 3.1 | 2.5 |
| 43-01 | 2.9 | 1.7 | 1.6 | 1.8 | 2.0 |
| 64-01 | 1.6 | 2.5 | 1.3 | 2.6 | 2.0 |
| 11-01 | 3.2 | 3.3 | 2.2 | 2.7 | 2.8 |
| 17-01 | 4.2 | 2.4 | 2.9 | 3.8 | 3.3 |
| 45-01 | 1.6 | 1.3 | 1.5 | 2.6 | 1.8 |
| 14-01 | 3.8 | 3.2 | 2.9 | 2.3 | 3.1 |
| 53-02 | 2.5 | 1.9 | 1.0 | 3.0 | 2.1 |
| 36-01 | 1.5 | 2.1 | 2.7 | 3.3 | 2.4 |
| 6-1 | 2.7 | 2.0 | 1.9 | 2.2 | 2.2 |
| 65-02 | 3.1 | 2.5 | 1.5 | 2.0 | 2.3 |
| 03-07 | 3.2 | 2.3 | 2.4 | 1.3 | 2.3 |
| 32-01 | 1.8 | 1.4 | 0.8 | 1.5 | 1.4 |
| 30-00 | 1.6 | 0.5 | 1.0 | 2.3 | 1.4 |
| 39-01 | 2.0 | 2.2 | 1.8 | 2.7 | 2.2 |
| 59-01 | 1.5 | 0.9 | 0.9 | 2.8 | 1.5 |
| 43-01 | 4.7 | 2.1 | 1.9 | 4.3 | 3.3 |
| 33-01 | 3.2 | 2.5 | 2.7 | 2.5 | 2.7 |
| 45-01 | 2.0 | 2.3 | 2.7 | 2.6 | 2.4 |
| 40-01 | 1.1 | 1.0 | 1.2 | 1.4 | 1.2 |
| 23-01 | 1.6 | 0.6 | 1.4 | 1.7 | 1.3 |
| 7-2 | 3.4 | 2.3 | 2.5 | 3.5 | 2.9 |

* Failed net (< 0.7 µg/sample)

Table 2: Results obtained after WHO biassay cone test on the Permanet 42-01 and 52-01
Results from table 2 confirm net 42-01 to be a failed net since mortality falls below 80% for

| | | Control | | 42-01 | | 52-01 | |
|---------------|----|----------------|--------------|--------------|--------------|--------------|--------------|
| Cône A | | Kis | Akron | Kis | Akron | Kis | Akron |
| | M | 0 | 0 | 5 | 3 | 5 | 4 |
| | V | 7 | 6 | 1 | 3 | 1 | 0 |
| | %M | 0% | 0% | 83,33% | 50% | 83,33% | 100% |
| Cône B | M | 0 | 0 | 4 | 5 | 7 | 5 |
| | V | 7 | 6 | 2 | 1 | 0 | 0 |
| | %M | 0% | 0% | 66,67% | 83,33% | 100% | 100% |
| Cône C | M | 0 | 0 | 3 | 5 | 8 | 4 |
| | V | 6 | 6 | 2 | 1 | 0 | 0 |
| | %M | 0% | 0% | 60% | 83,33% | 100% | 100% |
| Cône D | M | 0 | 0 | 4 | 5 | 8 | 6 |
| | V | 6 | 6 | 1 | 1 | 0 | 0 |
| | %M | 0% | 0% | 80% | 83,33% | 100% | 100% |
| Total | M | 0 | 0 | 12 | 18 | 28 | 19 |

Results from table 2 confirm net 42-01 to be a failed net since mortality falls below 80% for both strains of mosquito. Although, the Deltamethrin surface levels for Net 52-01 was below the threshold for 80% mortality, the cone test showed 97-100% mortality. Examination of net 52-01 revealed that there were no sampling sites marked by thread indicating that this net was not the original net sampled.

Previous data have shown that nets that have been used for a year have shown a gradient of surface insecticide from top to bottom (less insecticide on bottom relative to top of net); likely due to exposure of the net material to people and bedding materials¹. No gradient was observed among positions (top to bottom) in these nets since they have been used for only a month.

6. Conclusion:

The colorimetric field test was successful in finding a failed net (42-01), subsequently confirmed by the WHO Cone test. It was suspected that net 52-01 (failed using colorimetric test but passed Cone Test) was not the actual net that was originally tested, since it did not bear the threads which was used to mark the net during the initial testing. These nets presumably have not been washed and maybe an error in quality control at the manufacturer contributed to the release of a poor quality product. Assuming these two new nets to be failures (2/76), a 2.6% failure rate among newly distributed nets is not acceptable. A large-scale distribution of 10000 net would result in 260 failed nets. Therefore, the NetTest may be used to insure distribution of good quality nets as well as monitor net effectiveness through normal use.

IV. ENTOMOLOGICAL PROFILE AND CARTOGRAPHY OF MALARIA VECTOR INSECTICIDE RESISTANCE IN BENIN: PROJECT D

The results of a large study carried out in about 40 localities through a transect from south to Northern Benin in our last report have shown that *Anopheles gambiae s.s.* species comprises two forms: M and S. The M form prefers the same ecological settings like Mopti chromosomal form (Toure and Coluzzi, 1983). Most strains of *An. gambiae s.s.* in the 40 localities have developed a resistance against DDT and permethrin. However, in rural areas with no cotton field, *An. gambiae* was fully susceptible to pyrethroids. With the deltamethrin, a suspicion of resistance was found in some areas. *An. gambiae* was entirely susceptible to bendiocarb. The main mechanism of resistance found from north to south of Benin is *Kdr* mutation with a high proportion in urban and Cotton-cultivated areas.

Most of these data were found in raining season. According to what was planned, it was found interesting to verify if the level of resistance obtained in the raining season will be the same during the dry season. Then, we have visited the most of the 40 localities to collect *An. gambiae* larvae for rearing and for susceptibility tests using WHO and CDC methods. But most of breeding the sites visited was dry or negative. We continue the study and think we'll get a minimum of result before the end of the dry season. For now, only 5 localities (Malanville, Karimama, Houéyiho, Akron and Bamè) were analysed. The level of resistance

ⁱ Green MD, Atieli F, Akogbeto M. A Rapid Colorimetric Field Test to Determine Levels of Deltamethrin on Permanet Surfaces: Association with mosquito bioactivity (provisionally accepted by Tropical Medicine and International Health)

