Title of thesis: Efficacy of long lasting insecticide treated net technology against *Anopheles culicifacies,* a principal malaria vector in India

Summary

A study on three long-lasting insecticidal nets (LLIN), namely Olyset® Net, PermaNet® 2.0 and K-O Tab® 1-2-3, and a conventionally treated deltamethrin net (CTDN) was undertaken to evaluate their bioefficacy against *Anopheles culicifacies s.l.* (field collected), *An. culicifacies* species A and species C, and *An. stephensi* (Laboratory strains), the vectors of malaria in India. Bioefficacy was determined using standard WHO cone bioassays and ring net bioassays. Wash resistance of the LLINs and CTDN was evaluated by washing the nets with Surf Excel detergent powder by two methods of washing which are generally practiced in India— with washing machine and with hand. All the candidate LLINs and CTDN were washed for twenty times by both means at an interval of 7 days in between each wash starting from 1 April 2006. All the washed nets were dried in shade. To test the regeneration of Olyset nets, one batch of washed nets were dried in sunlight from 0900 to 1700 hrs in direct sunlight for five days continuously. Bioassays were conducted on Day 1, and after 5th, 10th, 15th and 20th wash on all LLINs and on Day 1, and after 1st, 3rd, 5th, 7th and 10th wash on CTDN. All the unwashed candidate LLINs and CTDN produced 100% mortality against all the mosquito species tested in cone bioassays. The cone bioassay results suggested that there is no statistically significant difference between hand wash and machine wash. Dirtiness of Olyset nets and Permanets did not dilute the efficacy in producing mortality against mosquitoes. Mortality of all mosquito species tested remained >70% against 20x machine washed and >80% against hand washed Olyset nets. Regeneration of permethrin on Olyset nets was observed when the nets were exposed to sunlight for five days continuously. More than 60% mortality was reported in all the test mosquito species against machine washed Permanets and more than 80% mortality against hand washed Permanets after 20 washings. More than 67% mortality was reported in all test mosquito species against machine washed K-O Tab 1-2-3 nets and more than 80% mortality against hand washed K-O Tab 1-2-3 nets after 20 washings. No statistically significant difference was observed when the mortality of mosquitoes were compared in between the species for all types of LLINs. Whereas in case of CTDN, efficacy (>80% mortality) remained up to only one machine wash and three hand washes against all the mosquito species tested.

Median knockdown time increased progressively after repeated washings of LLINs. Regeneration of permethrin on Olyset nets was clearly evident in MTKD bioassays also. No impact of dirtiness on the efficacy of Olyset nets on *An. stephensi* and *An. culicifacies s.l.* in the MTKD bioassays was observed. There is a significant difference in between hand wash and machine wash as revealed from differences in MTKD of all test mosquitoes. Hand washed nets performed better than the machine washed nets as revealed from Cone and MTKD bioassays. After 7 washes the insecticide is completely lost in CTDN as revealed from MTKD (there was no knockdown up to 1 h). MTKD of *An. stephensi* was more than that of all three *An. culicifacies* species tested against washed or unwashed LLINs.

Field evaluation of Olyset nets

A village-scale trial of Olyset net was undertaken from August 2003 to August 2006 in Khandera, Beel Akbarpur and Anandpur villages in Dadri PHC, Distt. Gautam Budh Nagar,

Uttar Pradesh, India. Olyset nets were distributed in Khandera village, untreated nets in Beel Akbarpur and Anandpur village was kept as control where nets were not used. Baseline entomological and epidemiological data (August 2003 to July 2004) were collected for distribution of nets. Nets were distributed on 1 August 2004. Usage of nets was more during transmission season (July to November) (>99%) and all inhabitants were using nets to protect from mosquito bites. Indoor resting mosquitoes substantially reduced in Olyset net used village. There were some indications that in untreated net used village also there were reductions in mosquito densities over the control village. Entry of mosquitoes was reduced into the rooms having Olyset nets and there was no landing of mosquitoes on Olyset nets. Use of Olyset nets provided complete protection to the users against mosquito bites. The parity rate of *An. culicifacies* mosquitoes reduced substantially in Olyset net used village. There was interruption of malaria transmission in Olyset net used village as absence of *P. falciparum* cases was observed, where as malaria cases continued in the other two villages including *P. falciparum* cases. Inhabitants expressed overwhelming response to Olyset nets and they did not report any adverse effects due to the use of Olyset nets.

Field evaluation of Permanets

A small-scale field trial was conducted from April to November 2005 to study the efficacy of Permanets in three villages, namely Nawada, Harampur and Durgawali in Loni PHC, District Ghaziabad, Uttar Pradesh, India. Depending on the prevalence of vector species PermaNets were distributed in Nawada village, untreated nets in Durgawali village and Harampur village was kept as control where nets were not used. Nets were distributed to all the inhabitants of the two villages on 1 July 2005. Entomological data were collected using standard procedures. Use of Permanets resulted in substantial reduction of per man hour density of *An. culicifacies, An. stephensi* and all mosquitoes determined by hand catch collections in early morning hours. Indoor resting mosquitoes also reduced in Permanet used village as revealed from total catch collections. Entry of mosquitoes also reduced into rooms having Permanet. High killing action of Permanet was observed as all mosquitoes that landed on Permanet died after 24 h holding. Overwhelming response from the inhabitants was reported using Permanets.

The results of the present study reveals that the three LLINs, namely Olyset net, manufactured by incorporation of Permethrin into the polyethylene fibers during preparation of yarn for nets; Permanet 2.0 coated with deltamethrin on polyester nets at the factory level and K-O- Tab 1-2-3, impregnated with deltamethrin along with a binder which holds the insecticide for longer duration, showed good performance against *An. culicifacies* and *An. stephensi* mosquitoes. All the three LLINs produced by different manufacturing processes are resistant to multiple washes (20 washes) as evidenced by the fact that after 20 washes also the mortality of test mosquitoes remained more than 80%. Hand washed LLINs produced good results than the machine washed nets. Olyset net and Permanet showed their efficacy in reducing the man-vector contact in field conditions. Long- term studies for four to five years are needed to confirm the long-lasting efficacy of these LLINs in reducing mosquito density and malaria prevalence in different eco-epidemiological settings.