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# TECHNOLOGY FOR CONVERSION OF CONVENTIONAL MOSQUITO NETS IN THE FIELD INTO LONG LASTING BED NETS

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**Abstract** A new technology is described for in-field dipping of conventional mosquito nets for long lasting efficacy by adding a polymer binder to the deltamethrin treatment. Various polymer binders vary greatly in their ability to improve the retention of deltamethrin on the fiber and ensure resistance of the treatment over time and after washing. For each individual binder, the optimum ratio between binder and deltamethrin needs to be identified, ensuring resistance of the treatment against numerous washings and at the same time allowing the full bioavailability of the insecticide for fast knock down (KD) efficacy and high mosquito mortality.

The results of bioassay of a type of treated net vary depending on what type of soap is used, and at what concentration. The higher concentrated solutions of Savon de Marseille (SdM) had a higher pH and were much more aggressive than the ones at lower concentration. However, various African soaps had an effect equivalent to SdM although their pH was higher. The new technology for in–field dipping of conventional mosquito nets will be marketed under the name of K-O TAB® 1-2-3 is based on an existing deltamethrin water dispersible tablet, K-O TAB®, at the dose rate of 25 mg deltamethrin / $m^2$ as recommended by WHO. Treated nets show mortality of *Anopheles stephensi* mosquitoes of 97% even after 20 washes, and 100% mosquito knock down is achieved throughout from the first to the twentieth washing.
**Key Words** K-O TAB 1-2-3®, K-O TAB®, deltamethrin, polymer binder, wash resistance

## INTRODUCTION

The majority of mosquito nets currently in use are conventional nets without long lasting treatment. Insecticide treated nets (ITN) for personal malaria protection have to be re-treated with insecticide about once a year. However, re-treatment rates are generally low, threatening the success of anti-malaria campaigns. In order to overcome this problem, Long Lasting Insecticide Treated Nets (LLIN) have been developed. The field use, or conversion of existing nets in the field into LLIN, has recently been adopted as priority number one to reach the Abuja Targets by the 2nd Roll Back Malaria (RBM) WIN Working Group Meeting of the World Health Organisation (WHO), Nairobi, Feb. 2005. A LLIN remains effective over a certain period of time even after several washings. The LLIN currently on the market are pre-treated nets that have been industrially manufactured.

Bayer Environmental Science has developed a new technology for in-field dipping of existing nets to achieve a long lasting efficacy. The technology is simple and consists of only one additional step in the conventional process of treating a net with a K-O TAB® [1], a well established treatment for mosquito nets. The in-field treatment is based on a WHOPES evaluated formulation of a deltamethrin tablet (WT) used at the WHO recommended dose rate of 25 mg A.I. deltamethrin/$m^2$.

Our research objective was to identify an additive to the insecticide formulation that will form protect the A.I. molecules on the fiber surface thus preventing loss of A.I. through washing and daily wear. This type of technology is referred to as type II LLIN by the WHO (WHOPES Evaluation of Long Lasting Insecticidal Mosquito Nets, Draft Guideline for efficacy testing, WHO, 2000).

WHO guidelines for evaluation of LLIN are in wide use, referring to the capacity of a net treatment to induce a fast knock down of mosquitoes, as well as high mosquito mortality, based on the WHO bioassay method. WHO has also drafted recommendations for a standard washing of the LLIN (WHO PVC 3/07/2002). However, the testing procedures in the various laboratories may vary due to practical reasons. In order to evaluate the performance of LLIN, it is required to adopt standardized methods, and to identify evaluation criteria that can be used in any laboratory and under various conditions.

In the following, we describe factors influencing the performance and bio-efficacy of LLIN treated in the laboratory by dipping conventional nets with mixtures of insecticide and binder.

## MATERIALS AND METHODS

**Mosquito Nets.** Nets of the following specification were used: white 100% polyester, 75 DEN, mesh 156, size 14.25 $m^2$, according to WHO specification. Source: Siamdutch Mosquito Netting Co., Bangkok, Thailand.

**Deltamethrin source.** K-O TAB® is a water dispersible tablet (WT, weight: 1600 mg) and contains 25% (w/w) deltamethrin. The deltamethrin content of 1 K-O TAB® is 400 mg A.I. K-O TAB® is based on WHO specified Deltamethrin TG, WHO/SIT/24.R2. The K-O TAB® has passed WHO evaluation and is specified as water dispersible tablet WT, WHO/IS/ 00.1.

**Polymer binders.** Various polymer binders that are being used in the textile industry were tested at different dose rates.

**Detergents and soaps.** Savon de Marseille (Le Chat), soap flakes, Henkel France, source: LIN Montpellier. Ariel, Procter and Gamble, commercial laundry detergent. US Standard Detergent, AATCC 1993 Standard Detergent WOB (without optical brighteners) as specified in ISO 6330: 2000 – 12. EU Standard Detergent, ECE non-phosphate reference detergent without optical brighteners (1998), acc. ISO 105 – C08. A variety of soaps and laundry detergents commercially available in African countries: Surf, Gental, Omo, Ushindi, Jamaa, White Star.

**Insecticide Treatment of A Standard 14.25$m^2$ Polyester Mosquito Net, Laboratory Procedure.**
Entire nets were treated following the original use recommendations as indicated on the K-O TAB® Kit leaflet. Prior to treatment, freshly manufactured nets were washed in a laundry washer at 30ºC without laundry detergent and dried for 24 hours in order to remove any residual of spin finish and to imitate a “used” net. For treatment of one entire standard net (14.25 $m^2$) at a target dose of 25 mg deltamethrin A.I./sqm, and 50 mg/sqm, one or two K-O TAB® was used, respectively. 500 ml of tap water was poured in a bowl of suitable size. Under constant stirring, the respective amount of binder was added. Then one or two K-O TAB® was added, respectively, with continuous stirring of the mixture until the tablet dissolved completely.

The net was then unfolded and placed into a second bowl of suitable size. The entire dip solution was added, and the net was kneaded with gloved hands for 5 minutes until it was thoroughly and evenly soaked. The net was then taken out of the bowl, folded in two and put flat on the floor to dry at ambient temperature, or put on PE lining. After one hour, dripping of the net will have stopped, the net is then hung over a laundry line to dry for another 24 hours until completely dry.

**Three-Minute Exposure Bioassay Tests.**
The bioassay tests were carried out using the WHO Adult Mosquito Susceptibility Test Kit (Figure 1). This type of test can also be done using the WHO plastic cones; however we found it to be too clumsy when handling plenty of nets, and more time consuming than using the cylinders. All bioassays were done using preferably female sugar fed *Anopheles stephensi* mosquitoes. The bioassay testing was continued until the efficacy clearly dropped below 50% mortality. Each test kit cylinder was marked with a red spot, or green spot, indicating its use for holding insecticide treated samples or for holding the mosquitoes after the 3 minute exposure.

Of each treated net, the required number of 12 x 15 cm samples was cut for bioassay. The net samples were attached to suitable rectangles of labeled white chromatography paper using a stapler. These were then rolled up, the bed net side facing the inside, and were placed individually inside a 'red spot' cylinder, and held in place using a circlip. Twenty mosquitoes (females preferred) were collected out of a cage by an aspirator and blown into 'green spot' cylinders lined with clean white chromatography paper, with a movable slide attached. 'Red spot' cylinders were screwed onto the opposite side of the movable slide; the slide was then opened and the mosquitoes gently blown through to the 'red spot' cylinder after which the slide was closed again. Insects were exposed for 3 minutes with cylinders in the horizontal position, then gently blown back into the 'green spot' cylinder. Knock down was recorded after 60 minutes, when the mosquitoes were supplied with a 5% sugar water source for 24 hours. Mortality was recorded 24 hours post exposure. Each test consisted of three replicates from which the mean values were calculated.

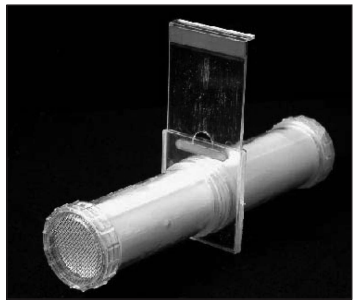

**Figure 1.** WHO Adult Mosquito Susceptibility Test Kit cylinders.

**Washing Procedure, Adapted After WHO Draft Recommendation.**
Washing was carried out at daily intervals. The washing powder was diluted at the desired rate of washing powder per liter in a mixture of one part tap water and one part de-mineralized water at a temperature of 30 ±3ºC in a 5L plastic bucket. 2 liters of the soap solution was being used for washing each net sample.

Each net sample was placed into an individual bucket, mixed twice using a glass rod and allowed to soak for 4 minutes; the samples were then stirred a further six times using a glass rod and allowed to soak for a further 4 minutes. The samples were squeezed using a gloved hand and dipped six times in the washing water and allowed to soak for a further 2 minutes. The pieces were then rinsed in cold flowing water twice before being squeezed again. The net samples were dried flat on aluminum foil for at least 24 hours until the next washing.

## RESULTS AND DISCUSSION

**Improved Wash Resistance by Polymer Binder Treatment**. **Qualitative Impact of Binders.**
A polymer binder for in-field dipping technology has to meet special requirements: it has to be suitable for this type of technology, must be compatible with the insecticide formulation used, and has to yield a good retention of A.I. on the fibers even during washing of the net in the alkaline environment of a detergent solution. Different polymer binders vary greatly in their capacity to bind the A.I. onto the fibers and withstand washing. We have evaluated a variety of existing binders that are currently available on the market and have identified one binder (type 1) for further investigation. Addition of polymer binders to an insecticide treatment may improve the wash resistance of the treated net (Figure 2). It is suggested that binders form a film around the net fibers that keeps the deltamethrin particles bound to the net fibers. Binders of different type result in different washing resistance. The coating properties of type 1 binder resulted in a higher retention of Deltamethrin A.I. on the fibers even after repeated washings as compared to regular deltamethrin WT treatment without polymer binder and with other binders tested. Polymer film of type 1 binder appears to protect the A.I. against the impact of the alkaline environment of the washing detergents.

Any insecticide treatment with Deltamethrin and binder on a mosquito net can only be effective if the insecticide particles are bio-available to the mosquitoes. Therefore, it was important to show that the film formed by the polymer binder does not block or cover the insecticide particles directly after treatment, thus preventing the mosquito from getting the full effective insecticide dosage. Bioassay data showed that a net treated with insecticide and polymer binder type 1 gave the full bio-efficacy of 100% mortality directly after treatment (Figure 3). The better retention of A.I. particles on the fiber after net treatment with insecticide and polymer binder led to better efficacy against mosquitoes as compared to conventional treatment after numerous washings. After a conventional net treatment, the mosquito mortality was lost after only a few washes in detergent solution. By adding the binder to the treatment, the net retained the full bio-efficacy of about 100% mortality against *Anopheles stephensi* for more than 20 washes in the laboratory.

**Impact Of Various Amounts of Polymer Binder Type 1 on Wash Resistance. Quantitative Analysis.**
To determine the optimum amount of binder to be used in combination with a fixed amount of deltamethrin, various amounts of binder type 1 were added to the insecticide formulation for treatment of nets. The binder was used at 100, 250, 500, 750 and 1000 mg/sqm, respectively, together with a standard dose of deltamethrin at 25 mg A.I./$m^2$. If used at a rate of 100 and 250 mg/sqm, respectively, the mosquito mortality was 85% and 98% after 10 washes but dropped to 50% or below after 15 washes. Higher doses of the same binder of 500, 750, and 1000 mg/sqm, respectively, performed much better with little difference between the various doses: even after 20 washes, mosquito mortality remained to be very high at 91%, 89%, and 82%, respectively (Figure 4)

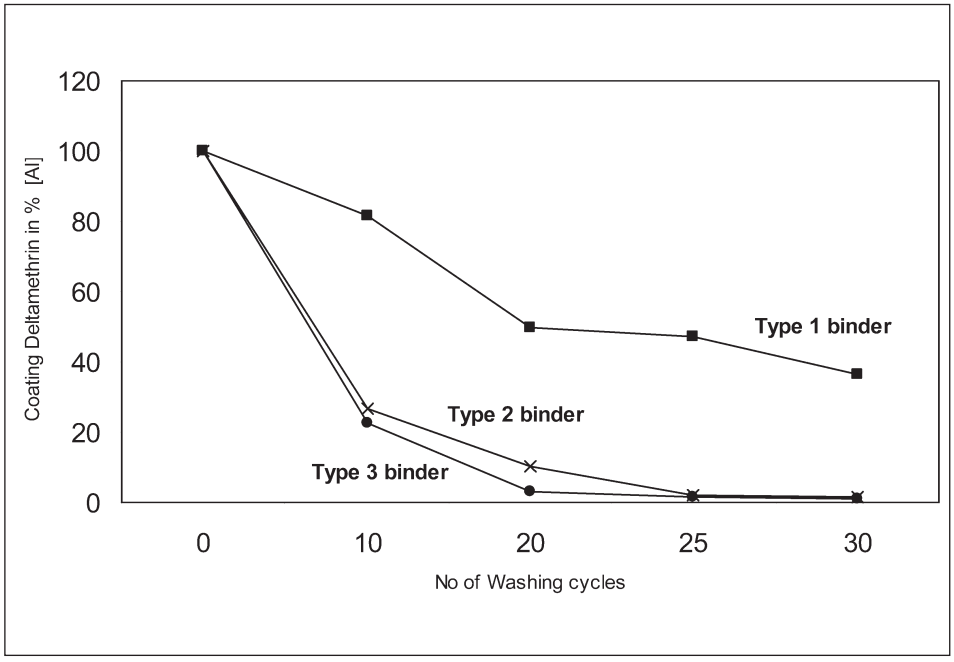


**Figure 2.** Impact of polymer binders on wash resistance of deltamethrin treated nets

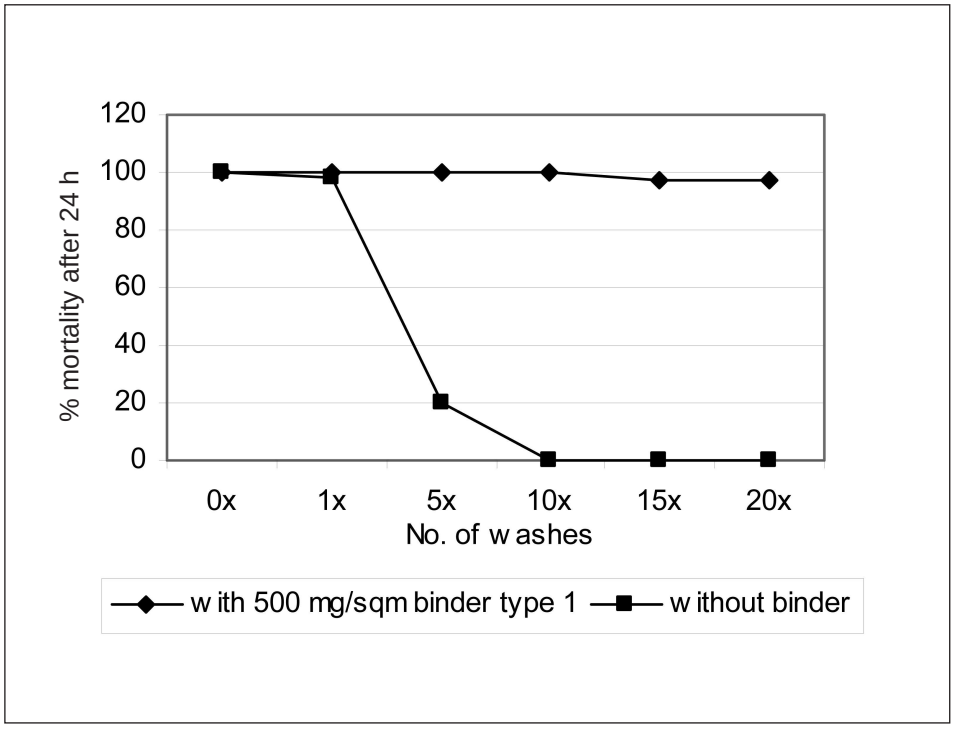


**Figure 3.** Wash resistance of net treated with 25 mg/m$^2$ deltamethrin with or without binder type 1

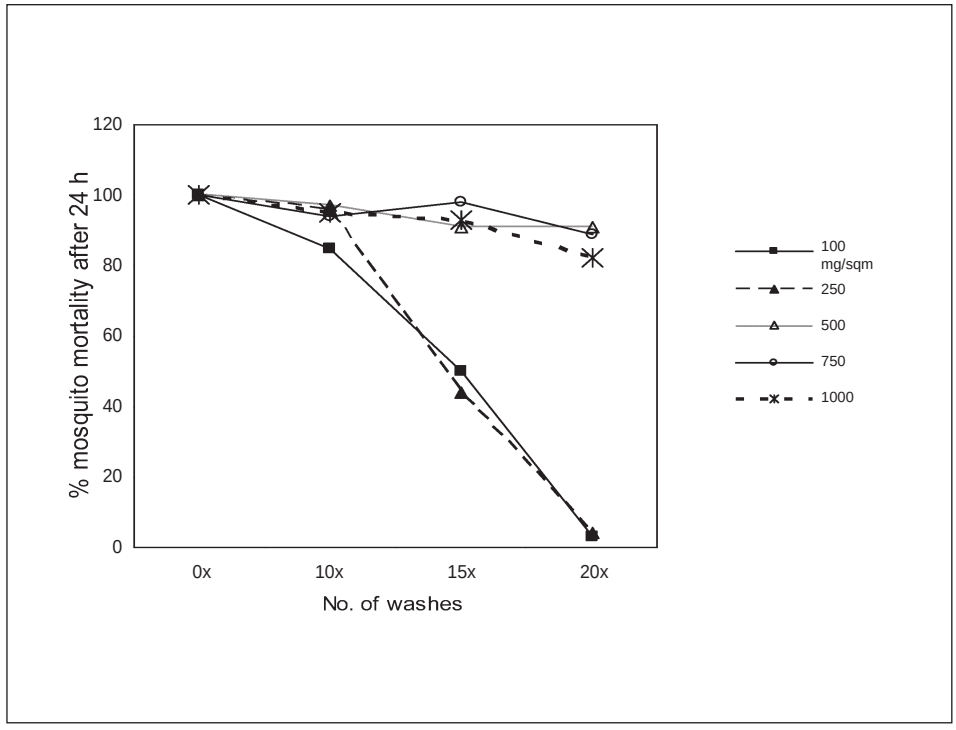


**Figure 4.** Impact of various amounts (mg/m$^2$) of binder type 1 on bio-efficacy (% mortality) of DTM treated nets

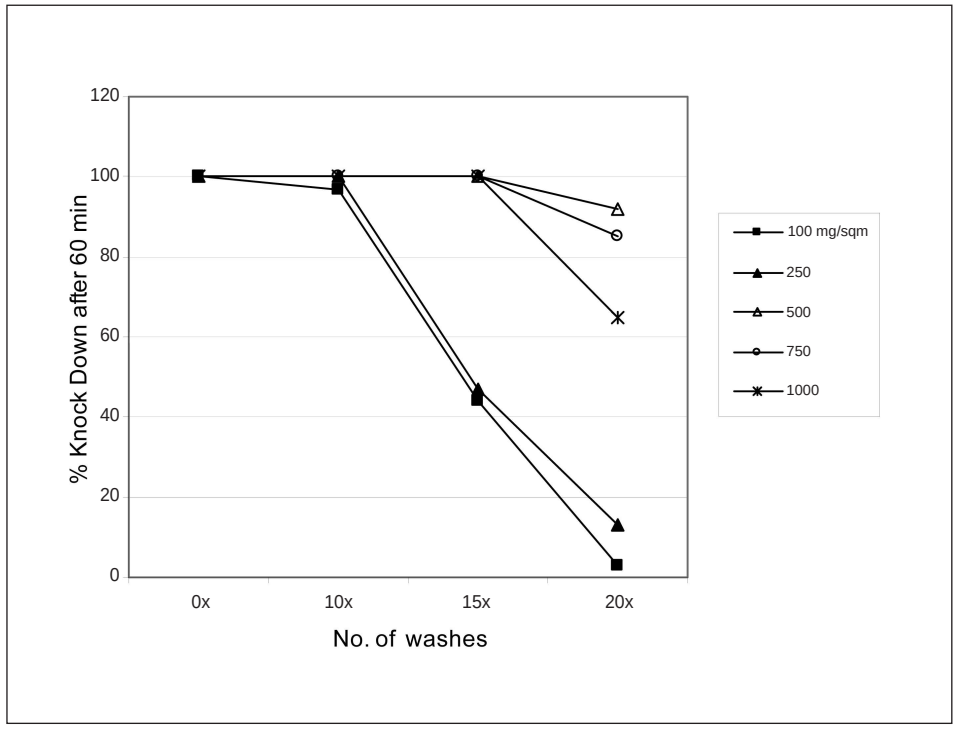


**Figure 5.** Impact of various amounts (mg/m$^2$) of binder type 1 on bio-efficacy (% Knock Down) of DTM treated nets

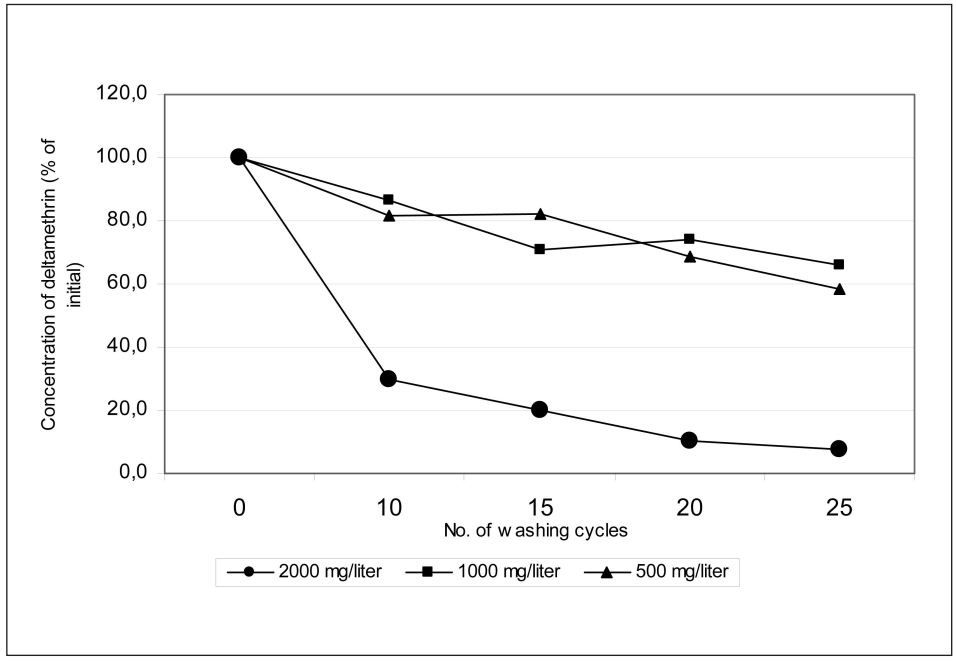


**Figure 7.** Impact of various amounts of Savon de Marseille on wash resistance

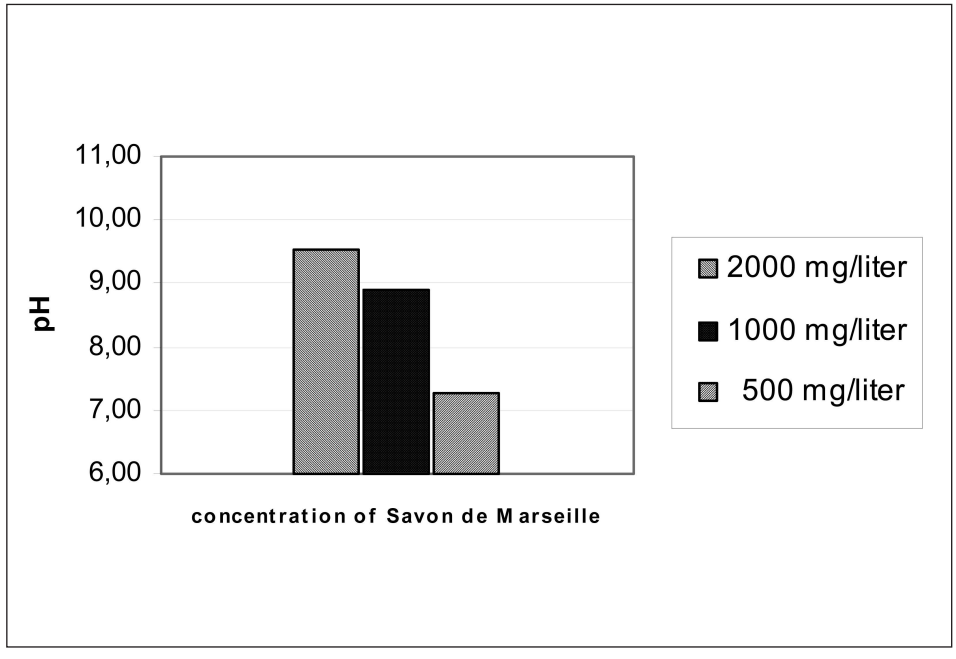


**Figure 8.** pH of different concentrations of of Savon de Marseille

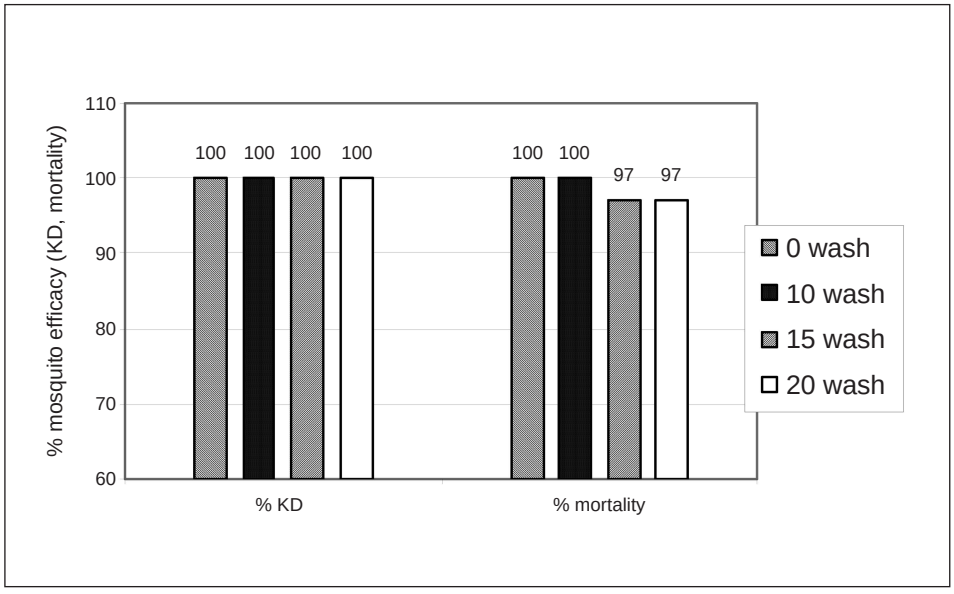


**Figure 9.** Bio-efficacy of K-O TAB® 1-2-3 treated LLIN after washing cycles

The results of mosquito knock down (KD) efficacy showed a similar tendency (Figure 5). Nets treated with polymer binder at 100 or 250 mg/m$^2$ showed that knock down efficacy dropped considerably after 10 washes below 50%, whereas after treatment with higher doses of binder, the knock down remained 100% for more than 15 washes, and after 20 washes is still as high as 92%, 85%, and 65%, respectively. The higher the rate of binder, the slower the KD. However, as seen in Figure 4, mortality was still above 80% even at the highest rate of binder. The results suggests that too low levels of binder were not sufficient to achieve the desired effect of binding the A.I. to the fiber, whereas increasing the binder above an optimum level did not yield an improvement in performance. It may be assumed that there is a range of insecticide-binder ratio for optimum efficacy. This may be characteristic for each binder and may have to be identified for each selective purpose.

**Impact of Detergents On Wash Resistance of Nets Treated with Deltamethrin And Polymer Binder.**

In an effort to standardize the methods for evaluation of LLIN, WHO has established draft recommendations for testing of LLIN including a standard wash procedure developed by LIN in Montpellier (WHO Collaborating Centre for Phase I testing) for testing wash resistance of treated nets. It has to be noted that WHO considers the recommended LIN procedure to be an aggressive one, washing off permethrin and deltamethrin from conventionally dipped nets in 3 to 5 washes. By using it, the risk of overestimating wash resistance would be minimal (WHO PVC, 2002).

Currently, the WHO procedure recommends using Savon de Marseille soap flakes (Le Chat), a commercial laundry detergent made in France. In order to evaluate if the soaps available on the African markets differ in their impact on the wash resistance, and how they compare to the standard washing detergent recommended by WHO, we have tested 6 African soaps and washing detergents alongside Savon de Marseille. Four of the six tested detergents are in the range of Savon de Marseille or slightly less aggressive, resulting in an equal or better wash resistance of the treated nets. Their pH values do not differ greatly from one another (pH 9.57 – 10.00 at 2 g/l), but are generally higher than that of Savon de Marseille as tested in our laboratory (pH 9.54). This was surprising as a more alkaline environment generally is viewed as more unfavorable to pyrethroids. It can be concluded that the African soaps appear to be equivalent to Savon de Marseille if tested under identical conditions. Of the two remaining soaps, one was more aggressive (Jamaa), the other one much less aggressive (White Star).

We also tested the US Standard Detergent AATCC 1993 (without optical brighteners) which is used in the textile industry for washing tests according to an ISO certified standard washing process (ISO 6330: 2000 – 12). When we used it for washing LLIN under identical conditions, the US Standard Detergent proved to be comparable to, or only slightly less aggressive than, Savon de Marseille. The US Standard Detergent has the advantage of being a standardized product of guaranteed quality that would be readily available to any institution.

**Impact of Various Amounts of Savon De Marseille on A.I. Retention of Nets Treated With Deltamethrin and the Polymer Binder Type 1.**

The wash resistance of a net treated with Deltamethrin and a polymer binder can be assessed indirectly by measuring the retention of deltamethrin A.I. on the net after a certain number of washing cycles. When a net treated with deltamethrin and polymer binder type 1 was washed with 2000 mg/liter of Savon de Marseille, the concentration of deltamethrin declined to 30% of the initial loading after 10 washes (Figure 7). When samples of the same net were washed with only 1000 and 500 mg/liter of Savon de Marseille, respectively, the decline of A.I. was much smaller and 86.7% and 81.4% of the initial A.I. load could still be found on the net after 10 washes; even after 20 washes, the deltamethrin concentration was still over 60% of the initial A.I. load.

**Impact of Various Amounts of Savon De Marseille on The pH of the Wash Solution.**

A great difference in pH values exists between solutions of different concentrations of Savon de Marseille: 500 mg/liter (pH 7.27), 1000 mg/liter (pH 8.88), and 2000 mg/liter (pH 9.54), respectively (Figure 8). It can be assumed that the higher concentrated soap solutions, with their higher pH, would be more aggressive to the deltamethrin A.I. concentration on the fiber than the less concentrated soap solutions with a less alkaline pH.

**Bio-Efficacy Against *Anopheles stephensi* Of K-O TAB® 1-2-3 Treated LLIN After Washing.**

As a result of the reported findings, an optimized combination of deltamethrin and polymer binder type 1 was identified for treatement of conventional polyester mosquito nets for long lasting efficacy. The deltamethrin dosage on the treated mosquito net was 25 mg A.I./ $m^2$, as recommended by WHO. The treatment was based on an existing product, K-O TAB®. The polymer binder type 1 was applied at 10 ml/net.

If treated with this optimized combination, the net had a wash resistance of more than 20 washes: the mean mortality of *An. stephensi* mosquitoes after 10, 15, and 20 washes, was 100%, 97%, and 97%, respectively (see Figure 9. Bio-efficacy of K-O TAB® 1-2-3 treated LLIN after washing cycles). Mosquito knock down efficacy was 100% from the first until the last wash.

## CONCLUSION

By combining the effectiveness of K-O TAB® with the polymer binder type 1 it is now possible to treat existing polyester mosquito nets in the field and turn them into long lasting nets that can withstand over 20 washings without losing their efficacy against the malaria mosquito *An. stephensi*. The product will be marketed under the brand name K-O TAB® 1-2-3.

## ACKNOWLEDGMENTS

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K-O TAB® registered trademark of Bayer AG, Leverkusen, Germany

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