

# IMPACT OF WATER QUALITY ON LABORATORY EVALUATION OF INSECTICIDE-TREATED MOSQUITO NETS

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**Abstract** Laboratory testing of long-lasting insecticide treated nets (LN's) includes assessment of bio-efficacy and determination of insecticide retention after washings. The standard WHO washing method recommends the use of deionized water with a hardness of 0 ppm (water without Ca<sup>++</sup> and Mg<sup>++</sup>). In the context of Bayer's LN development we conducted laboratory tests to evaluate the impact of washings with water of varying hardness (0 - 500 ppm) on the bio-efficacy and the active ingredient retention of two Bayer Deltamethrin binder-coated LN's: K-O TAB<sup>®</sup> 1-2-3 for hand treatment of nets in the field, and the industrially treated DAWA Plus net. The results demonstrate that nets washed with water containing any level of Ca<sup>++</sup> and Mg<sup>++</sup> ions showed considerably higher knock down and mortality compared to nets washed with deionized water (0 ppm). The chemical analysis surprisingly showed that this is not a function of a stronger wash loss of Deltamethrin after washing in deionized water, but in contrast suggests that water without Ca<sup>++</sup> and Mg<sup>++</sup> ions may have a detrimental impact on the chemical-physical conditions of the coating technology. The results of our studies imply that the laboratory bio-efficacy evaluation of K-O TAB<sup>®</sup> 1-2-3 or DAWA Plus nets after washing with deionized water may not reflect the full potential activity of bed nets treated by the coating technologies.

**Key words** water hardness, wash resistance, *Anopheles gambiae*

## INTRODUCTION

Long lasting insecticide treated nets are an established tool for personal protection against Malaria. According to WHO, a long lasting net has to retain for at least 20 washings a bio-efficacy of  $\geq 95$  % knock down within 60 minutes, or  $\geq 80$  % mortality after 24 hours when tested with the WHO cone testing method (WHO, 2006). Many laboratory and field data concerning the wash resistance of mosquito nets have been published (Sharma et al., 2006; Yates et al. 2005; Graham et al., 2005), including the influence of net material on the efficacy (Vatandoost et al., 2006). However, there is a lack of knowledge regarding the influence of washing factors such as type of detergent, water hardness etc, on the long lasting efficacy.

The objective of the present paper is to describe the influence of the water hardness on the efficacy of two types of Bayer Deltamethrin binder-coated LN mosquito nets after washing with waters of different hardness. Besides the biological efficacy, the quantity of remaining active ingredient was determined by chemical analysis to evaluate possible relationships between active ingredient content and biological efficacy.

## MATERIAL AND METHODS

### Insects

Non-blood-fed susceptible female mosquitoes aged 2-3 days of the Malaria mosquito *Anopheles gambiae*, susceptible strain Kisumu, were used.

### Insecticides

Hand treated net: Deltamethrin WT (1.6 g) with 25 % Deltamethrin and Bayer activated LN binder. Industrial LN: Deltamethrin SC 10 with 10 g Deltamethrin per liter and Bayer activated LN binder.

## Detergent

Savon de Marseille (“Le Chat” from Henkel France) provided by LIN Montpellier, France, was used for washing. Detergent composition: soap = 86%, phosphates 0.9%, glycerin 1.0%.

## Water Specifications

Tap water from Monheim Agricultural Research Center varies between ca. 200-300 ppm (11-16°DH). For the first tests, one part tap water was mixed with one part deionized water. Water of defined hardness was prepared by mixing known amounts of CIPAC water C or D with known amounts of deionized water (CIPAC waters: Dobrat and Martijn, 1995). Water specifications in ppm (content of Ca<sup>++</sup> : Mg<sup>++</sup> 4 : 1): 500 ppm = CIPAC water D; 324 ppm = CIPAC water C; 250 ppm = approximately 100-150 ppm = 1 part deionized water + 1 part tap water; 125 ppm = 1 part 250 pp water + 1 part deionized water; 62.5 ppm = 1 part 125 ppm water + 1 part deionized water; 0 ppm = deionized water.

**Mosquito Nets.** Specification of untreated mosquito nets for hand treatment:

<b>Supplier</b>	Siamdutch Mosquito Netting, Bangkok	<b>Mesh</b>	156/inch <sup>2</sup> = 27/cm <sup>2</sup>
<b>Material</b>	100 % Polyester	<b>Color</b>	white
<b>Yarn weight</b>	75 Deniers/30 g/m <sup>2</sup> 100 Deniers/40 g/m <sup>2</sup>	<b>Dimensions Size</b>	150 x 180 x 190 cm 14.25 m <sup>2</sup>

Specification of industrially treated long lasting net (LN):

<b>Supplier</b>	TANA Netting Co., Bangkok	<b>Mesh</b>	156/inch <sup>2</sup> = 27/cm <sup>2</sup>
<b>Material</b>	100 % Polyester	<b>Color</b>	white
<b>Yarns weight</b>	75 Deniers/30 g/m <sup>2</sup> 100 Deniers/40 g/m <sup>2</sup>	<b>Dimensions</b>	150 x 180 x 190 cm
<b>Insecticide</b>	treated with 40 mg deltamethrin per sqm.	<b>Size</b>	14.25m <sup>2</sup>

## Methods for Net Treatment

Hand treatment with K-O TAB<sup>®</sup> 1-2-3: One net was hand dipped in 500 ml water containing one K-O TAB and a sachet with Bayer activated LLIN Binder and then air dried. The target dose was 25 mg Deltamethrin per m<sup>2</sup>. Industrial LN: Nets were industrially treated in a post finishing technology using spray application and tumbler drying. The target dose was 40 mg Deltamethrin per m<sup>2</sup>.

## Net Washing Procedure

Washing of the samples was done following the WHO (2006) Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets using Savon de Marseille as detergent at 2 g/liter of deionized water or waters of different hardness for washing in a shaker water bath at 155 strokes per minute for 10 min at 30°C (±3°C).

After rinsing in deionized water for two times ten minutes, the samples were dried hanging vertically, and were stored at 27°C (±2°C) and 80% RH (±10%) for at least 24 hours until the next washing.

## Bioassay

The bioassays were carried out using the WHO Adult Mosquito Susceptibility Test Kit (WHO 2006), consisting of 2 plastic cylinders (4.5 cm diameter, 12 cm height), one of which is for exposure of the mosquitoes to the treated net, the other one is the holding cylinder for holding of the mosquitoes before and after exposure. The net samples (12 x 15 cm) were attached to filter papers (12 x 15 cm) before insertion

into a tube. Mosquitoes were introduced by aspirator from the holding chamber and exposed for 3 minutes before being blown back into the holding chamber. Percentage knock-down after 60 minutes and percentage mortality after 24 hours were recorded. After knock-down evaluation, mosquitoes were provided with a source of sucrose solution.

### pH Measurement

A commercially available pH-meter (Portamess Type 911, Knick company) and pH electrode (SE 102) was used. Calibration was carried out at room temperature with two known buffer solutions at the beginning of the operations. The buffer solutions covered the expected pH range of the waters to be determined.

### Chemical Analysis

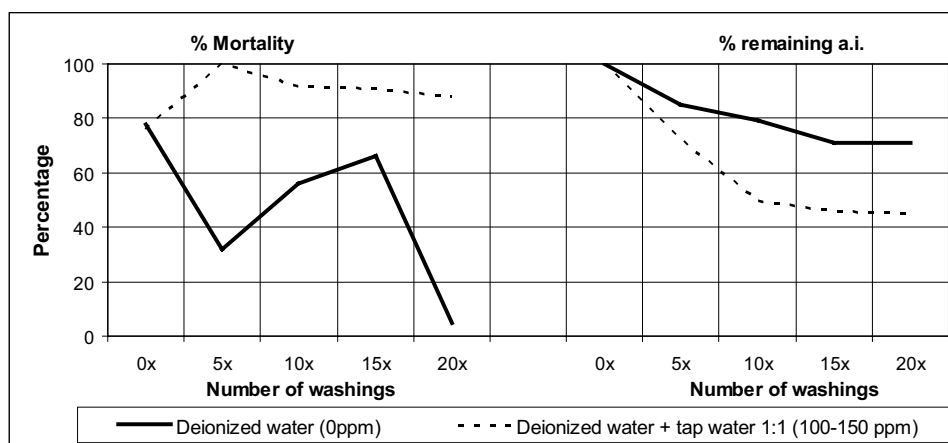
For quantification of deltamethrin in netting samples reversed phase HPLC with external standardization and UV detection at 208 nm was used. Separation followed isocratic elution in water: acetonitrile at 54:46 (v/v) on a Discovery HS PEG column at a flow rate of 2 ml/min. All reagents and solvents were of HPLC grade quality. Retention time for the deltamethrin related signal was approximately 1.1 minutes. For preparation, all samples were cut to suitable sizes and weighed to the nearest milligram. For extraction, a mix of acetonitrile: tetrahydrofuran: 2-propanol (2:3:5 v/v/v) was used in a ratio of about 100 ml per 25 x 25 cm area. Extraction was done by sonification for 1 h in a water bath shaker at room temperature.

## RESULTS AND DISCUSSION

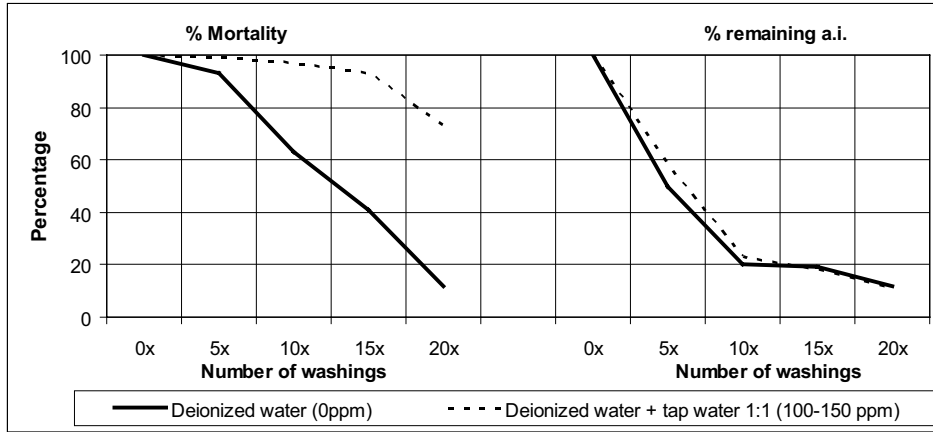
Impact on bio-efficacy of washing with water entirely deplete of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions (deionized water 0ppm) versus washing with water containing high amounts of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions (deionized water + tap water 1:1, 100-150 ppm) (Figure 1 and 2). As shown in Figure 1, the hand dipped unwashed nets revealed the full bio-efficacy of 100% mosquito mortality. When washed with water containing a high amount of ions, the bio-efficacy of the net remained above the WHO threshold of 80% mosquito mortality for more than 15 washes. However, when the nets were washed with deionized water, the bio-efficacy decreased dramatically after as few as 5 washes and had dropped below the threshold after 10 washes.

Unexpectedly, the chemical analysis showed that the remaining percentage of deltamethrin after washing was nearly the same for both washing regimes, no matter the water quality. As shown in Figure 2, the bio-efficacy of the unwashed, industrially treated nets was above the WHO threshold with >80% mosquito mortality.

In nets washed with half tap water and half deionized water, the bio-efficacy of the net remained above the WHO threshold of >80% mosquito mortality for more than 20 washes and even reached 90%-100%. However, in nets washed with the deionized water, bio-efficacy dropped already after the first washes to levels of between 40% and 60 % mosquito mortality and never reached the threshold again after subsequent washes.



**Figure 1.** Hand dipped LN: Bio-efficacy after washing with deionized water (0 ppm) versus deionized water + tap water 1:1 (100-150ppm).



**Figure 2.** Industrially treated LN: Bio-efficacy after washing with deionized water versus deionized water + tap water (1:1).

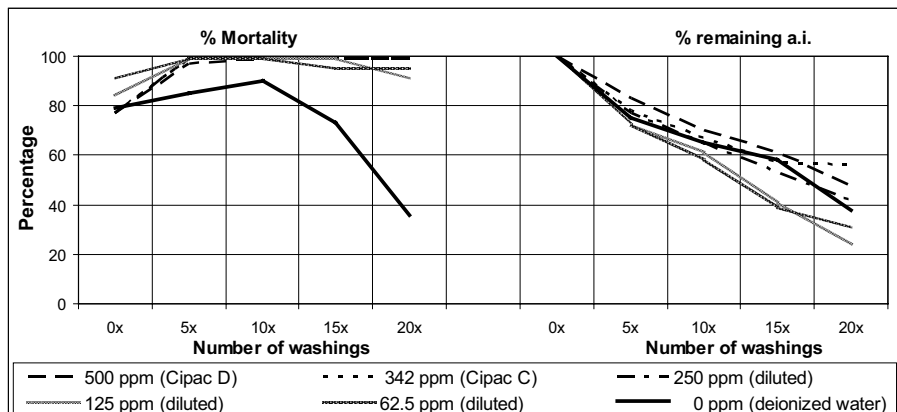
The chemical analysis revealed that after washing with deionized water, which had so drastically reduced the bio-efficacy of the nets, not only the same percentage of Deltamethrin AI, as seen with the hand treated LN, but even a higher percentage than after washing with the water with higher ion content remained on the net.

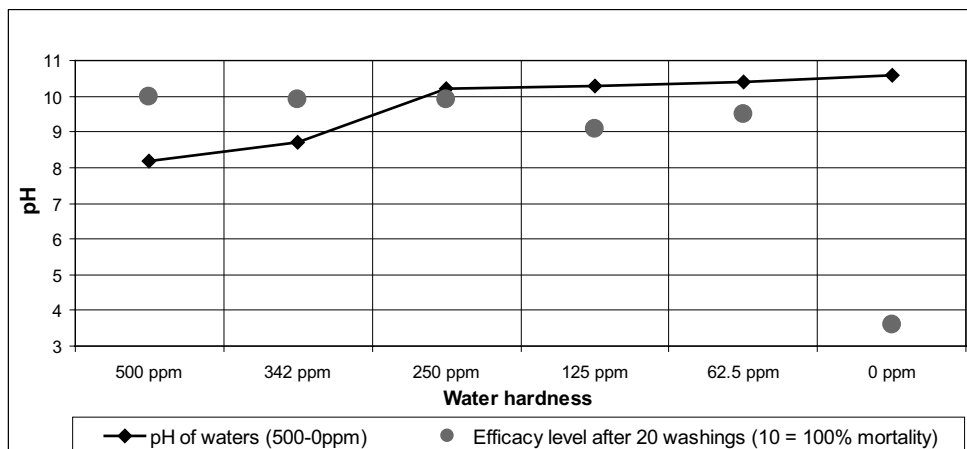
**Impact of washing with water of varying hardness on bio-efficacy of industrial LN (Figure 3).** The significant difference of bio-efficacy of nets washed in deionized water versus of nets washed with water containing high levels of  $Ca^{++}$  and  $Mg^{++}$  ions led us to evaluate the possible relationship between the quantity of ions and the biological efficacy after washing. Industrially treated LN nets were washed in waters of the following hardness levels: deionized water (0 ppm), water with 62.5 ppm, 125 ppm, 250 ppm, 342 ppm (CIPAC C), and 500 ppm (CIPAC D).

As shown in Figure 3, washing with deionized water resulted again in a drop of mosquito mortality below the WHO threshold of >80% mortality after less than 15 washes. However, after washing with any of the other waters containing any quantity of  $Ca^{++}$  and  $Mg^{++}$  ions, the mosquito mortality was always much higher, remaining between 80% and 100% and never falling below the WHO threshold for as many as 20 washes.

As seen in the first tests (deionized water versus deionized water + tap water 1:1), the poor bio-efficacy results of nets washed in deionized water does not seem to be induced by a stronger wash-off of Deltamethrin: according to the chemical analysis, the percentage of Deltamethrin remaining on the net after washing with deionized water was in the medium range as compared with the other water qualities containing various quantities of ions. Washing with water of low hardness (62.5 ppm, 125 ppm) induce a higher wash loss of Deltamethrin and resulted in lower percentage remaining AI on the net, whereas the waters containing 250 ppm, 342 ppm, and 500 ppm induced a wash loss of AI in the range of deionized water.

**Figure 3.** Industrial LN: Bio-efficacy after washing with waters of different hardness versus deionized water.





**Figure 4.** pH and biological efficacy of washing solutions (water +detergent) based on waters of different hardness (500 ppm to 0 ppm).

**Impact of pH (Figure 4).** We investigated if the pH of the washing solutions based on waters with different hardness would reflect the different impact of the waters on bio-efficacy. We measured the pH of the washing solutions based on the various water qualities with the identical amount of detergent Savon de Marseille (2 g/liter) And compared them with the bio-efficacy datas of the nets from Figure 4 after 20 washings.

As shown in Figure 4, there was only a slight difference in pH of the washing solution based on deionized water (pH 10.6) and waters containing between 62.5 ppm and 250 ppm (pH 10.4 and pH 10.2, respectively). This does not reflect that washing in the solution based on deionized water has such negative impact on the bio-efficacy of the nets after washing, whereas this is not the case after washing with waters that contain any amount of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions. As a second finding there was a drop in pH between washing solutions based on 250 ppm water (pH 10.2) and 342 ppm water (pH 8.7) and 500 ppm (pH 8.2), respectively, which in return is not reflected by a different impact of the washing solutions on the bio-efficacy of nets after washing.

## CONCLUSIONS

Washing of Bayer Deltamethrin binder-coated LN in deionized water diminishes significantly the bio-efficacy after washings as compared to water containing any amounts of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions, more or less independent of the concentration (Figure 3).

The reduced bio-efficacy of nets washed in deionized water is not a function of a stronger wash loss of deltamethrin after washing, because there is no significant difference of the remaining deltamethrin content between nets washed with deionized water or  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  containing water (Figure 1). In contrary, industrial nets retain more active ingredient after washing with deionized water (Figure 2). An influence of the pH can also be excluded because pH is very similar for washing solutions based on water with 0 ppm (deionized water) or 62.5 ppm, 125 ppm and 250 ppm  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  (Figure 4).

The findings suggest that water depleted of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions may have a detrimental impact on the physical-chemical properties of the Bayer net coating technology.

The reason for this is not fully understood, but may be explained by the following: The deionized water is entirely depleted of ions, and due to the osmotic pressure it has the tendency to receive ions by “diluting” the polymer binder by which the net fibers are covered. This may cause the layer of polymer binder to soak up water. The swollen layer of polymer binder may become more vulnerable to chemicals such as washing detergent and more prone to mechanical abrasion during washing, and handling.

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