Bio-efficacy and Wash-fastness of a Lamda-cyhalothrin Long-lasting Insecticide Treatment Kit (ICON® Maxx) Against Mosquitoes on Various Polymer Materials

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Research

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Abstract

Background

Long-lasting efficacy of insecticide treated nets is a balance between adhesion, retention and migration of insecticide to the surface of netting fibres. ICON® Maxx is a twin-sachet 'home-treatment kit' of pyrethroid plus binding agent, recommended by the World Health Organization for long-lasting, wash-fast treatment of polyester nets. While knitted polyester netting is widely used, fine woven polyethylene netting is increasingly available and nets made of cotton and nylon are common in Africa and Asia. It is important to investigate whether ICON Maxx is able to fulfill the WHO criteria of long-lasting treatment on a range of domestic fabrics to widen the scope for malaria protection.

Method

This study was a controlled comparison of the bio-efficacy and wash-fastness of lambda-cyhalothrin CS, with or without binder, on nets made of cotton, polyethylene, nylon, dyed and undyed polyester. Evaluation compared an array of bioassays, WHO cone and cylinder, median time to knockdown and WHO tunnel tests using *Anopheles* mosquitoes. Chemical assay revealed further insight.

Results

ICON Maxx treated polyethylene and polyester netting met the WHO cone and tunnel test bio-efficacy criteria for LLIN after 20 standardized washes. Although nylon and cotton netting failed to meet the WHO cone and cylinder criteria, both materials passed the WHO tunnel test criterion of 80% mortality after 20 washes. All materials treated with standard lambda-cyhalothrin CS without binder failed to meet any of the WHO bio-efficacy criteria within 5 washes.

Conclusion

The bio-efficacy of ICON Maxx against mosquitoes on netting washed up to 20 times demonstrated wash durability on a range of synthetic polymer and natural fibres: polyester, polyethylene, nylon and cotton. This raises the prospect of making the insecticide-binder kit into an effective approach for turning untreated nets, curtains, military clothing, blankets - and tents and tarpaulins as used in disasters and humanitarian emergencies - into effective malaria prevention products. It may provide a solution to the problem of reduced LLIN coverage between campaigns by converting commercially sourced untreated nets into LLINs through community or home treatment. It may also open the door to binding of non-pyrethroid insecticides to nets and textiles for control of pyrethroid resistant vectors.

Background

Insecticide treated mosquito nets (ITNs), developed during the 1980s proved highly effective in reducing malaria-related morbidity and mortality [1]. Operationally, however, ITNs suffered several challenges in the field; these included the logistical problem of having to retreat nets every 12 months, the recurrent cost of annual retreatment and the unavailability of insecticides in remote places [2].

The advent of long-lasting insecticidal nets (LLIN) that do not require insecticide retreatment over a 3-years' lifespan provided a technical solution to the logistical challenge of low retreatment coverage [3–6]. LLIN have since become the essential tool for vector control and malaria prevention in sub-Saharan Africa. The World Health Organization (WHO) recommends and promotes universal coverage of 1.0 LLIN for every 1.8 persons in populations at risk in malaria endemic countries [7]. The push towards this target has led to increased demand for LLIN by national malaria control programmes, international malaria control agencies and institutional buyers who have increasingly opted for LLIN as their preferred choice of malaria prevention [2, 8].

Thus far, international malaria control agencies have spent over two billion dollars on the provision of LLINs, leading to scale-up of access which currently exceeds 50% of the population of sub-Saharan Africa [7].The target of universal coverage is critical to success and while 50% is an impressive achievement, malaria elimination remains a distant prospect, and millions of African households remain unprotected particularly in the later stages, between universal coverage campaigns [9] .

LLIN are treated with insecticide during net manufacture. However, the majority of ITN that are available through the commercial retail sector are not LLIN [9] and those nets in use, sourced from retail outlets, have either never been treated or were treated only once at the time of purchase [9, 10]. Locally sourced nets which are not LLIN may lose efficacy prematurely, long before the nets physically perish from wear and tear [9, 10]. This raises a need for treatment kits that can convert these nets post-manufacture into long-lasting insecticidal nets through simple household or community dipping.

Progress has been made with long-lasting treatment kits that can transform untreated nets into long-lasting treated nets by combining a conventional insecticide with a binding agent and the simple act of immersion into aqueous solution of the mixture. With this technology the untreated or conventionally treated nets already in use may be transformed into LLINs by the community post-manufacture under field conditions.

ICON Maxx is a long-lasting insecticide formulation developed by Syngenta in kit form [11]. Thus far, ICON Maxx is the only long-lasting insecticide treatment kit that has full recommendation of the World Health Organization for use on polyester nets [12, 13]. The kit is based on a slow-release capsule suspension (CS) formulation of lambda-cyhalothrin previously evaluated by WHO and recommended for treatment of mosquito nets. ICON Maxx is presented as a twin sachet pack, containing lambda-cyhalothrin 10CS and binding agent, sufficient for the treatment of an individual mosquito net. The target dose of ICON Maxx on a family-size polyester mosquito net is 62 mg AI/m2. The actual dose received depends on the net size and can range from 50 mg AI/m² (for a large family-size net) to 83 mg AI/m² (for a single-size net). Efficacy and wash fastness of ICON Maxx has been demonstrated in several laboratory, experimental hut and field trials [14–16]. In all these studies the demonstration was made on nets made of polyester netting [14–17]. Although polyester is currently more widely used [15] it is not the only polymer used. Use of polyethylene nets is increasing, and nets of fine polyethylene weave are now available [18, 19]. Mosquito nets made traditionally from cotton are also common in countries of West Africa and South Asia. The global local retail market for cotton nets remains high. It is estimated that over 50% of nets sold in Iran and Pakistan are made of cotton. Nylon nets are used in India and Africa. There is also great diversity in the fabrics, and synthetic polymers used in curtains, blankets and other barriers to mosquitoes that are potentially treatable in the home.

The question is whether binder formulations can make these other types of polymer, aside from polyester, long-lasting? The efficacy and wash resistance of ICON Maxx needs to be confirmed on nets made of cotton, nylon, polyethylene and other synthetic materials before this product can have the widest possible application or impact on malaria.

Polyester and other netting materials come in a range of colours. There is some evidence that dye may affect the uptake and retention of conventional insecticide formulations during immersion [15]. It is important to confirm that uptake and retention of insecticide-plus-binder is not adversely affected by textile finishing.

The present study reports on the laboratory evaluation of bio-efficacy and wash-fastness of ICON Maxx on netting made of cotton, polyethylene, nylon, white and dyed polyester nets. This was done in controlled comparison with the same netting materials conventionally treated with lambda-cyhalothrin CS, a microencapsulated formulation ('Iconet', Syngenta UK) that does not include the long-lasting binder component.

Materials And Methods

Netting and treatment

Polyester white, polyester blue, polyethylene, cotton and nylon netting materials were used as substrates. Cotton nets were sourced from a manufacturer in Pakistan that supplies the national army, the polyethylene and nylon nets were sourced from manufacturers in India and the polyester nets were supplied by Vestergaard Frandsen. The absorbency of each material was determined using a test solution of ICON Maxx in de-ionised water. Solutions of ICON Maxx were specially prepared to match each material's absorbency to achieve a similar target loading dose per unit surface area. The nets were considered treated when all solution had been absorbed and all areas of the net were visibly wet without any dripping. The nets were dried horizontally in a dark room at 30°C on polythene sheeting and turned over every 10 min until completely dry. Each material sample was then cut into five 60 cm x 40 cm samples. Various positive and negative controls were introduced. Untreated samples of each material were retained as negative controls. Netting of each material treated with lambda-cyhalothrin 2.5% CS (Iconet), without binder formulation, served as positive controls for the ICON Maxx treated materials. ICON Maxx treated polyester white was used as the reference arm as it had already received recommendation by WHO [13, 18].

Washing procedure

Samples of each material were washed 0, 5, 10, 15 or 20 times. All samples were washed as 60 cm x 40 cm pieces except the polyethylene which was stiffer and harder to immerse and had to be cut into two to ensure thorough washing. The standard WHO Phase I laboratory washing procedure was adopted [14]. A soap solution of 2g/L was produced using the soap Savon de Marseille and de-ionized water. Each net was placed in a 1L bottle and immersed in 500ml of soap solution before placement in a water bath. All samples were shaken at a rate of 155 movements per minute and remained immersed at 30°C for 10 minutes. Each swatch was rinsed twice in de-ionized water under the same water bath conditions. A piece of each treated material was kept unwashed to serve as the zero-washed sample.

Mosquitoes

All mosquitoes used were insectary reared non-blood fed female pyrethroid susceptible *Anopheles gambiae* s.s (Diptera: Culicidae) mosquitoes (Kisumu strain), susceptible to all pyrethroids, reared in the National Institute for Medical Research, Ubware Centre.

Cone bioassays

To evaluate the efficacy of ICON Maxx and Iconet treated netting materials, standard WHO cone bioassays were performed, based on the WHO Phase I protocol [14] against insectary-reared pyrethroid-susceptible *An. gambiae* Kisumu. Four WHO cones were fixed to each netting sample and 5 mosquitoes aged 2-5 days old were introduced into each cone. After 3 min exposure the mosquitoes were transferred to plastic cups. Control mosquitoes were exposed to untreated netting. The 20 mosquitoes tested per replicate were provided with a pad of glucose solution for nourishment. Tests were done at 25^{o}C and 70% RH. Knock-down (KD) was recorded one-hour post-exposure and mortality 24h later. Five replicates were carried out per sample giving a total of 100 mosquitoes per treatment. If control mortality exceeded 10% on any day the results were disregarded and the test repeated; this procedure was followed in all bioassay tests described. All replicates of the various textile-wash treatments were carried out in strict rotation using Latin squares to adjust for any variation in insect batch or test conditions.

Cylinder bioassays

In preparation for this assay, treated and washed samples of each material were cut and stapled to pieces of plain paper measuring 12 cm x 15 cm before insertion inside WHO susceptibility test cylinders and securing with metal rings. Ten 2–3-days old female mosquitoes were introduced to each holding chamber and transferred into the test chamber where they were exposed for 3 minutes. After exposure, the mosquitoes were returned to the holding chamber where they were held with access to sugar solution. The number knocked down was recorded after 60 minutes and the number dead was

recorded 24 hours later. A negative control of mosquitoes exposed to untreated netting material was carried out alongside each test.

Median time to knock down (MTKD)

In the MTKD bioassay eleven mosquitoes 2-3 day old were introduced into a WHO wire ball frame covered with the treated material [14]. Knock down was defined as a mosquito lying either on its back, side or no longer able to support itself. The time taken for each mosquito to knockdown was recorded up to the median (6th) mosquito. Nine replicates were carried out for each treatment, material and wash number. Untreated net of each material was used as a negative control.

Tunnel tests

Tunnel tests were used to assess both unwashed treated netting and netting washed 20 times as a proxy for 3 years of field use, as per WHO guidelines [14]. Samples were selected at random and not according to whether they had passed or failed the cone test. The tunnels consisted of three chambers, the mosquito release chamber C1, a middle chamber C2, and the baited chamber C3 containing a caged guinea pig separated from chamber C2 by the test netting. Test netting was fitted to a rectangular frame measuring 25 cm x 25 cm which slotted across the tunnel between chambers C2 and C3. Nine holes 1 cm in diameter were cut in three rows of three through which host-seeking mosquitoes could penetrate. C1 and C2 were separated by a paper screen with a single 9 cm diameter hole through which host-seeking mosquitoes attracted by the bait odour from C3 could fly from C1 to C2 and from thence to C3 via the 9 holes. A total of 100 susceptible 5–8-day old mosquitoes were tested in two replicates of 50 mosquitoes. Mosquitoes were introduced to C1 in late afternoon and left until the following morning, 15 hours later, when the mosquitoes in each chamber were counted and their status, alive or dead and fed or unfed, were recorded. The live mosquitoes were kept in a paper cup with access to a sugar solution for a further 24 hours after which delayed mortality was scored. Two replicates of each material were carried out. A negative control test of untreated material was tested on each day; a replicate was only considered eligible if in the negative control a minimum of 50% blood-fed. Nettings were tested at random within latin squares until all replicates were completed; a negative control was included on each test day. Mortality was control-corrected.

Chemical analysis

High pressure liquid chromatography was used to determine the concentration of insecticide on each treated piece of net after washing the requisite number of times. Four pieces measuring 5 x 5 cm of each net treatment were cut and placed in a borosilicate glass vial with 1ml of acetronitrile. The vials were sonicated for 10 minutes; the solution was removed and placed in HPLC vials. The HPLC analysis was carried out at the London School of Hygiene and Tropical Medicine using a Dionex Summit range of equipment and software (Camberley, Surrey, UK). The samples were separated using an AcclaimR C18 120 (250 x 4.6 mm, Dionex, UK) column eluted with water/acetonitrile (90:10%; v/v) at a flow rate of 2 ml/min and passed through the photodiode array detector (PDA-100, Dionex) set at 27 nm. The authenticity of the detected peaks was determined by comparison of retention time, spectral extraction at 275 nm and spiking the sample with commercially available standards.

Statistical Analysis

Proportional data (bio-efficacy) were analyzed using logistic regression using STATA® 13 (Stata Corporation, Collage Station TX, USA 2005). The independent variables included treatment (ICON Maxx, Iconet), net material (5 types of polymer), number of washes (5 or 3), replications adjusting for group size, and the interactions between net type and number of washes. Multivariate analysis of variance was used to analyse the concentration of lambda-cyhalothrin in samples adjusting between netting materials, treatments and number of washes.

Ethical Clearance

Approval was obtained from the ethics committees of the London School of Hygiene & Tropical Medicine and the Tanzanian National Institute of Medical Research (Ref: NIMR/HQ/R.8a/Vol. X/86). The procedure for use of guinea pigs in tunnel tests conformed to criteria established in EC Directive 86/ 609/ECC regarding protection of animals used for experimental purposes.

Results

Chemical analysis (Figure 1)

Chemical analysis by HPLC of the Iconet treated materials (without binder) showed that cotton and undyed polyester (white) had higher affinity for lambda-cyhalothrin CS (Iconet target dose 15mg/m^2) as compared to dyed polyester (blue), nylon and polyethylene. Within 5 washes all detectable lamda-cyhalothrin was removed from the two polyester nettings and less than 1 mg/m^2 was detectable on polyethylene and nylon. More insecticide (4.8 mg/m^2) was retained in cotton fibres than in other nettings after 5 and 10 washings (fig 1a).

Chemical analysis of the ICON Maxx treated materials (with binder) showed that all materials had higher affinity for the pyrethroid with binder than for pyrethroid without binder (as in Iconet). Cotton and polyester undyed and dyed (white and blue) had the highest affinity or absorption, showing a loading dosage greater the target 50 mg/m^2, while polyethylene and nylon showed loading dosages below the target 42 and 40 mg/m^2 respectively (fig 1b). After washing 0-5 times, the two polyesters and polyethylene showed particularly high retention of insecticide; over 70% of the initial lamda-cyhalothrin content remained (fig 2), showing a non-significant decline in content after 5 washes ($p>0.05$) (fig 1b). Insecticide loss rate was greater with cotton and nylon; the decline being significant ($p<0.05$) between 0 and 5 washes (fig 1b) with neither material retaining more than 25% of loading dose (fig 2). After 20 washes it was polyethylene and polyester white which retained the most lamda-cyhalothrin (10% and 7% of initial concentration, respectively) (fig 2), and almost all lamda-cyhalothrin was removed from polyester blue, cotton and nylon (fig 1b & 2).

Cone bioassays

Iconet treated materials (Figure 3a, Table 1): The mosquito mortalities induced by Iconet treated polyester white, polyester blue and polyethylene in cone tests all exceeded 90% after loading, while cotton and nylon induced between 60% and 80% mortality after loading (Fig 3a). Comparing materials, polyethylene recorded significantly higher mortality than all other materials across each specific wash point. Polyester white induced significantly higher mortality than polyester blue after 5 washes ($p=0.001$). Mortality decreased to less than 10% after ten washes. The knockdown trend generally paralleled the mortality trend (Table 1).

ICON Maxx treated materials (Figure 3b, Table 1): The mortality induced by ICON Maxx treated polyethylene netting in cone bioassays was 100% after loading and exceeded 90% mortality after 20 washes. Mortality on polyethylene was significantly higher than all other materials across each wash point ($p<0.005$). Mortality induced by polyester white exceeded 80% over 0-15 washes decreasing to 45% only after 20 washes. Mortality induced by polyester blue was only 60% at loading decreasing to 24% at 10 and 6% at 20 washes, thus confirming the poorer adhesion and retention of the binder formulation on dyed polyester ($p=0.001$). Mortality of ICON Maxx on cotton and nylon while initially efficacious, was not sustained after 5 or 10 washes.

The knockdown trend was consistent with mortality; polyethylene recorded higher knockdown than any other material, followed by polyester white and then polyester blue. Percentage knockdown on treated cotton and nylon decreased after 10-15 washes (Fig 3b, Table 1).

Cylinder bioassays

Iconet treated materials (figure 4a, Table 2): As in cone tests, the highest mortality recorded in cylinder tests with Iconet treated netting was with polyethylene, polyester white and polyester blue (100-95%) in that order, followed by cotton (74%) and nylon (61%). Within just 5 washes polyethylene and polyester white were scoring less than 50% mortality and polyester blue, cotton and nylon were scoring less than 15% mortality. Between 5-10 washes mortality on all materials decreased to less than 10%.

With respect to knockdown, all unwashed materials recorded 100% knockdown at 60 min except nylon at 85%. However, at 5 washes, percentage knockdown of all materials except polyethylene decreased suddenly to 50% or less, and at 10 washes knockdown of all materials fell below 40% (Table 2).

ICON Maxx treated materials (figure 4b, Table 2): With all netting materials, mortality was exceptionally high (>95%) after treatment (0 washes) and also at 5 washes (except polyester blue). Polyethylene recorded significantly higher performance than all other materials with 100% mortality at 0, 5 and 10 washes and was the only material to exceed 80% mortality at 20 washes. Polyester white and cotton exceeded 80% mortality at 10 washes. Polyester blue exceeded 70% at 15 washes. Polyester white recorded significantly higher mortality than polyester blue at 0 and 20 washes (p=0.001 and 0.007 respectively), polyester white decreasing below 80% after 15 washes and blue after 10 washes. Mortalities recorded with nylon and cotton decreased below 30% after 15 washes (Fig 4b). The only materials that incurred sudden loss of activity were cotton and nylon after 10 washes and polyester blue after 15 washes.

With respect to 60-min knockdown, every material recorded between 100-97% knockdown after 0 and 5 washes. After 20 washes, only polyethylene showed 100% knockdown, and polyester white recorded 80% knockdown. Knockdown on cotton and nylon showed sudden decrease between 10-15 washes, polyester blue between 15-20 washes (Table 2).

ICON Maxx efficacy in cone and cylinder bioassays: Comparing all treated materials, higher mortality was recorded in the cylinder bioassay as compared to the cone bioassay at each wash point (Figure 5a & b). As exposure time was the same in cone and cylinder, this higher mortality was due to a higher ratio of netting covered surface to uncovered plastic surface in the cylinder as compared to the cone.

Tunnel tests (Table 3)

With the unwashed ICON Maxx materials there were differences in percentage passage inhibition between materials (Tables 3). More striking was the high level of blood-feeding inhibition compared with the untreated control: this stood at 90% with polyethylene and exceeded 90% for polyester white and blue. With these 3 materials each passed the tunnel test on the WHO feeding inhibition criterion. Percentage mortality with all four materials ranged from 91-96% and hence exceeded mortality criterion too.

After 20 washes, passage inhibition with polyethylene and polyester netting decreased by 10-30%. With polyethylene blood-feeding inhibition was 90% and hence passed the WHO criterion. With nylon, feeding inhibition was 100% at 20 washes so passed too. With the two polyesters feeding inhibition fell a few percent below the WHO threshold. Percentage mortality with polyethylene, cotton and nylon ranged between 83-88%, hence all passed the WHO mortality criterion. Mortality was significantly less with polyester white than with nylon, polyethylene and cotton (p<0.005).

Median time to knock down bioassays

Median time to knock down (MTKD) is considered to be a good indicator of surface AI. It is straight-forward to generate the median responder in an MTKD assay and to run multiple replications to generate the confidence interval around the mean of the medians.

Median time to knock down of mosquitoes with all unwashed Iconet treated materials were statistically similar with the exception of nylon which took 1.5-2 times longer to reach than with other materials. After 5 washes all treated materials

failed to reach median knockdown even after 30 min (the maximum duration of exposure observed).

With all ICON Maxx treated materials the difference in mean MTKD between unwashed and 5 times washed materials was not significantly different and thus the binder was retaining lambda-cyhalothrin on the netting surface (Table 4). With polyethylene white and polyester blue, the mean MTKDs were not significantly different between zero and 10 washes although there was some sign of MTKD becoming longer and hence losing surface AI with washes. With cotton and nylon the MTKD was not reached until after 30 min exposure on 10-times washed samples indicating low bioavailability and low activity of surface AI on this material after washing. With ICON Maxx treated polyester blue and polyethylene the MTKD rose significantly after 10 washes (Table 4).

Regression analysis showed surface content of insecticide had significant effect on MTKD; for every 1 mg decrease in insecticide content there was 3 seconds increase in MTKD ($F_{1,50} = 6.27$, $p = 0.0156$).

Discussion

The primary objective was to determine whether the pyrethroid lamda-cyhalothrin bound within a polymer resin could improve the wash fastness of the insecticide on nets made from a range of synthetic polymers, natural fibres and dye finishes. To complement the study, controlled comparison was made with a standard pyrethroid CS treatment that lacked the binder. The second objective was to take the treatments through the WHO LLIN evaluation process to determine which insecticide treated substrate would withstand 15-20 washes and potentially achieve WHO recommendation. The third objective was to introduce new types of bioassay to compare against the cone test to test their potential. The fourth was to consider some currently neglected contexts badly in need of vector borne disease control and consider whether insecticide-binder treated substrates could provide a solution.

According to WHO LLIN evaluation guides, polyethylene, cotton and nylon treated with ICON Maxx met the tunnel test criteria of >80% mortality after 20 washes in Phase I, which is a recognized surrogate for 3 years of pyrethroid durability on household LLIN [19]. Polyester white (undyed), the positive control, and polyester blue fell short of the WHO tunnel criteria in Phase I tests but elsewhere in other studies they did achieve [16, 17, 20] emphasizing the importance of multiple trials before coming to a consensus conclusion. Polyethylene also met the required criterion of >80% mortality in cone tests and was the best performing polymer of the four tested. In all bioassays except the tunnel test, cotton and nylon netting did not reach the WHO threshold. Most of the textiles performed well in one or more types of bioassay and none can be ruled out as a suitable substrate for vector control treatment. Even polyester white, the positive control, which failed to meet the required threshold in the Phase I, fared well in Phase II (experimental hut) trials and Phase III (field trials of insecticide durability) in the same locality [12, 13, 16]. Therefore, the Phase I tests reported here are best viewed comparatively, one textile versus another, rather than as pass or fail.

By contrast, none of the nettings - polymer or natural fiber - withstood more than a few washes, at best, when treated with a standard lambda-cyhalothrin CS formulation. There is no question of the superiority of the binder formulation which is a genuine technical advance for a variety of potential malaria control substrates or contexts [12].

Nylon

Nylon showed poorer adhesion of ICON Maxx initially and poorest wash resilience of all the materials, losing 84% of insecticide content within 5 washes and 98% within 20 washes. In cone bioassay, mortality decreased by 92% within 5 washes and yet in cylinder bioassay where most of the interior was netting-covered mortality stood at 97% after 5 washes and only decreased to low level after 10-15 washes. In tunnel test, ICON Maxx treated nylon passed the WHO criterion of >80% mortality at 20 washes. Of all the materials tested, nylon was the most unpredictable. While its efficacy in tunnel was encouraging nylon failed as a substrate of preferred choice for ICON Maxx treatment due to the poorer absorption, adhesion and wash-resilience. If there is a choice, the better option would be substitution of nylon with a better adhering or

wash-tolerant polymer. On ICON Maxx treated nylon net curtains, as a barrier to Aedes and prevention of Aedes borne arboviruses, it may have potential and further studies are warranted.

Cotton

Owing to the high absorptive property of cotton, the cotton samples contained the highest loading dose of ICON Maxx initially. However, as on nylon, adhesion and retention of the insecticide was poor, content decreasing by 75% after 5 washes and by 96% after 15 washes. Cone bioassay recorded only 40% mortality at zero and 4% at 20 washes but, as was the case with nylon, mortality in cylinders was high between zero and 10 washes and only decreased sharply at 15 washes. As with nylon, cotton passed the tunnel test criterion for LLIN at 20 washes. The presence of a high dose of lambda-cyhalothrin together with a low insecticidal activity suggests that bioavailability on the surface of cotton netting fibers is low, that is, most of the insecticide remained locked within the cotton fibers and failed to make contact with mosquito tarsi. This is not the case with synthetic fabrics such as polyester and polyethylene on which the insecticide is readily bio-available on the surface of fibres. Other studies have also reported the low insecticidal property of pyrethroids on cotton as compared to other fabrics [21, 22]. However, with the tunnel test, results with cotton netting exceeded 80% mortality after twenty washes, so bringing cotton into line with WHO criteria for recommendation [14].

Polyethylene

Like nylon, polyethylene treatment demonstrated a relatively low loading dosage (40 mg/m^2) but in contrast to nylon and cotton, polyethylene showed better retention at 5 washes and a more regular loss rate over the course of 0 to 20 washes. Mortality in cone and cylinder bioassay was consistently high (~95%) over the course of 20 washes, and thus a completely different trajectory compared with nylon and cotton. ICON Maxx seemed to stay bound to the polyethylene which remained fully toxic whereas the binder seemed lost from nylon and cotton during washing. As with nylon and cotton, polyethylene exceeded the tunnel test criteria at 20 washes.

Polyethylene seems an ideal substrate for ICON Maxx. In some studies, polyethylene netting materials were shown to be strong and able to tolerate five years of field use [3, 23]. More recently in larger scale surveys polyethylene has shown poor durability [24], somewhat improved by changing the knitting weave [24].

Polyester

The superiority of ICON Maxx on polyethylene compared to undyed polyester white was a surprise since the latter was the positive control and the polymer netting that ICON Maxx was designed for originally. While both polyester white and blue fell consistently short of polyethylene in an array of bioassay tests, ICON Maxx did attain WHO recommendation for use on polyester over 15-20 washes which is a significant increase in wash-tolerance compared to the standard formulation tested in this paper. After Phase II (experimental hut) trials and Phase III (three-year field trials of insecticide durability) ICON Maxx did attain WHO recommendation as a polyester long-lasting treatment (13). Comparing undyed and dyed polyester netting, chemical analysis indicated similar loading dosages, implying that the binder in ICON Maxx had largely overcome the poor adherence induced by the dye of earlier formulations on polyester (18). At most wash points the rate of loss of insecticide was similar between polyester blue and polyester white treated with ICON Maxx. While polyester white tended to record greater mortality than polyester blue, the differences were marginal and not consistent between all types of bioassay.

Comparison of ICON Maxx with KO-Tab 123

ICON Maxx is not the first wash-resilient formulation to be developed [12]. K-O Tab 123 was a wash-resilient formulation of deltamethrin (25mg/m^2) and binder rather than lamba-cyhalothrin (55mg/m^2) and binder in ICON Maxx [25]. Its development coincided with the development of factory produced LLIN and it was not taken forward to Phase III. Had it

done so, it might have proven as effective as ICON Maxx, which did go on to Phase III evaluation and obtained WHO full recommendation for 2.5-3 years of effective field use [12]. When compared with ICON Maxx on the same materials as tested in the present paper, it showed similarity in characteristics over 20 washes: high insecticide retention and bio-efficacy on undyed polyester and polyethylene and poorer retention and bio-efficacy on cotton and nylon [26].

Testing procedure

Despite having the same 3-minute exposure, the mortality/knockdown responses differed considerably between cone and cylinder tests. The purpose of the comparison was to identify whether the cylinder should supplant the cone as the primary WHO insecticide bioassay. Both are WHO bioassays. The cone bioassay was initially designed for assessment of IRS bio-efficacy and residual activity on walls and ceilings of sprayed houses. Only later was it co-opted for use as an ITN/LLIN bioassay. The IRS bioassay exposes mosquitoes for 30 min; this gives a mortality similar to that of free-flying mosquitoes entering and exiting IRS sprayed experimental huts [27] and is therefore appropriate as an exposure time. For ITN exposures the cone has limitations: contact time is short and it is difficult to 'settle' the mosquitoes on cone netting for the prescribed 3 minutes. If the purpose of a residual bioassay is to manage undesirable variables, then control of exposure time is essential in a short exposure assay. In this respect, the cylinder is an improvement; when cylinder and cone mortality are compared, mortality is higher and less variable in the cylinder than in the cone due to low ratio of plastic to netting. But if the aim is to simulate natural host-seeking behaviour in and around the net then the overnight tunnel test is the more realistic bioassay. It may be time to seriously consider switching from cone to cylinder. It would not negate the tunnel test but for laboratories that cannot access animals for bioassay it would be a move towards reducing animal use for this purpose. The tunnel test might not be needed for most types of bioassay testing except for night-time mitochondrial-acting insecticides such as chlorfenapyr [28].

Future uses

The obvious use for ICON Maxx and other treat-it-yourself long lasting pyrethroid kits is to bundle the sachets with the hundreds of thousands of untreated nets that continue to be sold in retail markets, rural and urban. Conical nets, at the 'luxury' end of the market are rarely bundled with kits, and the wholesalers and retailers of untreated conical and rectangular nets need a regular supply of kits.

This is a timely reminder for beneficiaries of free distributions of LLIN, who may know little or nothing about LLIN production, that LLIN are special because of the insecticide, and they need to treated with care and respect. Older nets can be made more protective with a top-up of insecticide, especially if the next universal coverage campaign is delayed, giving older nets a further 2-3 years of protective use. Universal campaigns are often supplemented with top-ups of new LLINs in the interval between campaigns, and if LLIN numbers are in short supply, untreated nets and older LLINs that are still serviceable would continue to provide benefit if re-treated.

Mosquito nets are not the only household product which might provide benefit from long-lasting insecticide treatment. Curtains made of nylon, polyethylene or cotton could provide family protection from *Anopheles* vectors of malaria and *Aedes* vectors of dengue, chikungunya and yellow fever. These could be immersed in ICON Maxx solution like the netting described in this article, or sprayed with deltamethrin 62 SC-PE (polymer-enhanced suspension concentrate formulation), a product specially derived from KO-Tab-123 technology as an aqueous spray formulation (K-Othrine Polyzone, Bayer Crop Sciences, Germany) [29].

Armed services have favored the use of permethrin on combat clothing for personal protection owing to its high repellence [30, 31]. Alphacyano-pyrethroids such as ICON Maxx might be preferred in certain locations due to its higher toxicity compared to permethrin. To prevent skin irritation the treated material might be separated by a non-treated inner layer [32].

Disasters and humanitarian emergencies

The same arguments apply to civilian bedding and to top-sheets and blankets treated and distributed in epidemics, disasters or emergencies [33, 34]. Standard issue in humanitarian emergencies are blankets, tents and polyethylene tarpaulins [32, 34, 35] particularly for refugee populations on the move, i.e. situations where nets are dysfunctional or where sprayable housing is absent. Acute phase emergencies are a niche which has proven difficult to supply with adequate vector control protection. The problem is compounded by sectorial nature of international aid. Blankets and tents in emergencies are administered by the shelter sector, vector control is administered by the health sector. Blankets, sheets and shelters are location-specific, and utility will depend on climate and temperature. The solution might be to coordinate the shelter and public health sectors to treat whatever shelter is provided on-site with a long-lasting insecticide or repellent formulation mixed with binder formulation and UV protectant, applied by immersion, spray pump, or treated at source during manufacture. Bespoke factory manufactured products may not justify the investment, bespoke long-lasting formulations to treat a variety of products would be.

The treatment of polyethylene tarpaulins or shade cloth with pyrethroid plus binder as used in emergency shelter has formed the basis of the insecticide treated wall liner concept of protection in the home [36].

Dual-AI LLIN and non-pyrethroid long-lasting treatment kits

The first Dual Active Ingredient LLINs were the PBO-synergist nets PermaNet 3.0 [37] and Olyset Plus [38]. While the pyrethroid in all WHO recommended LLINs should remain effective for 3 years, WHO is now referring to Dual-AI nets as ITNs because it is not clear whether the PBO component will last a full 3 years of field use [39]. While Olyset Plus, the first in class pyrethroid–PBO net, has demonstrated effectiveness for two years, it is not yet clear in the ongoing cluster randomized trial whether the PBO will remain effective for the full 3 years. If it falls short of 3 years, there is an opportunity here to apply PBO via a PBO-binder long-lasting kit after 2 years to take it through the third year. Similarly, there is an opportunity for a PBO-binder long-lasting kit to be applied to any pyrethroid LLIN to convert those to pyrethroid-PBO LLIN. This would apply equally to other partner AI, such as pyriproxifen or chlorfenapyr which are being used with pyrethroid in other types of Dual-AI LLIN should these fall short of 3 years' effectiveness [40]. In environments with high pyrethroid resistance, it would be a mistake to allow Dual AI nets to revert to a pyrethroid-only LLIN in their third year as users would be only be part-protected.

Conclusion

In all tests performed, ICON Maxx treated polyethylene recorded greater performance than the positive control (polyester white) and other netting materials tested. Although the efficacy of ICON Maxx on cotton and nylon netting were low compared to other materials, they still met WHO criteria for LLIN. All ICON Maxx treated materials demonstrated insecticidal efficacy after twenty washes and met WHO criteria for long-lasting insecticidal treatment in one or more bioassays. Chemical analysis confirmed that lambda-cyhalothrin was more strongly retained in the ICON Maxx-treated than in Iconet treated materials. The high efficacy, wash-fastness and versatility of ICON Maxx raises the prospect of it becoming an all-purpose formulation for such purposes as military clothing, civilian bed covers and curtains, or for blankets, tarpaulins and tents distributed in epidemics, disasters or humanitarian emergencies, rather than pursue bespoke long-lasting insecticidal products for niche markets that may not be viable investment for manufacturers. ICON Maxx may provide an answer to the problem of reduced LLIN coverage between distribution campaign though turning commercial retail-sourced untreated nets into LLINs through simple home or community treatment.

Declarations

Ethics, consent, permission and consent to participate

Ethical clearance was obtained from the ethics committees of NIMR Tanzania (Ref: NIMR/HQ/R.8a/Vol X/86) and LSHTM. This paper is published with permission from the Director General, National Institute for Medical Research Tanzania.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests. The manufacturer Syngenta had no say in the content of the paper or interpretation of the data.

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Authors' contributions

PKT managed the trial, supervised the study implementation in the field and the laboratory, undertook the data collection and analysis, interpreted the data and drafted the manuscript. WS conducted the laboratory bioassays. HK supervised chemical analysis of net samples.SM contributed to the study implementation and revised the manuscript. MR conceptualized the study secured the funding, assisted with data interpretation, and revised the manuscript. All authors have read and approved the final manuscript.

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Tables

Due to technical limitations, tables ppt is only available as a download in the Supplemental Files section.

Figures

Figure 1a.

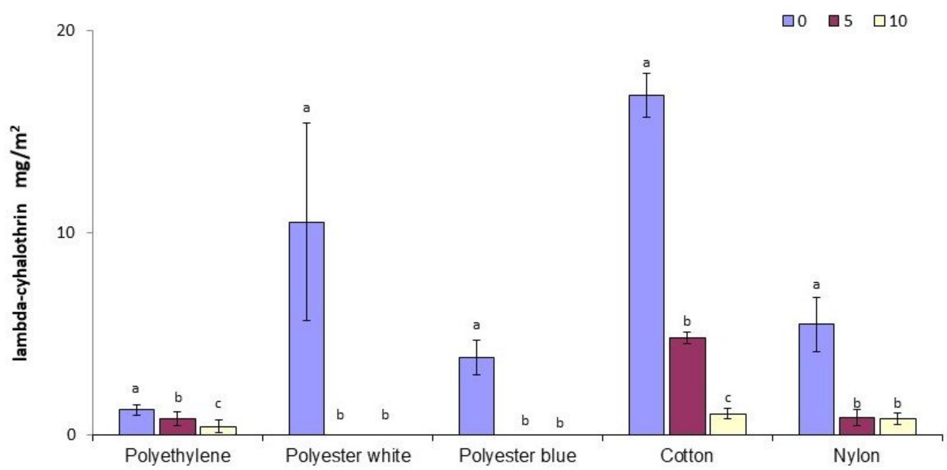

Figure 1b.

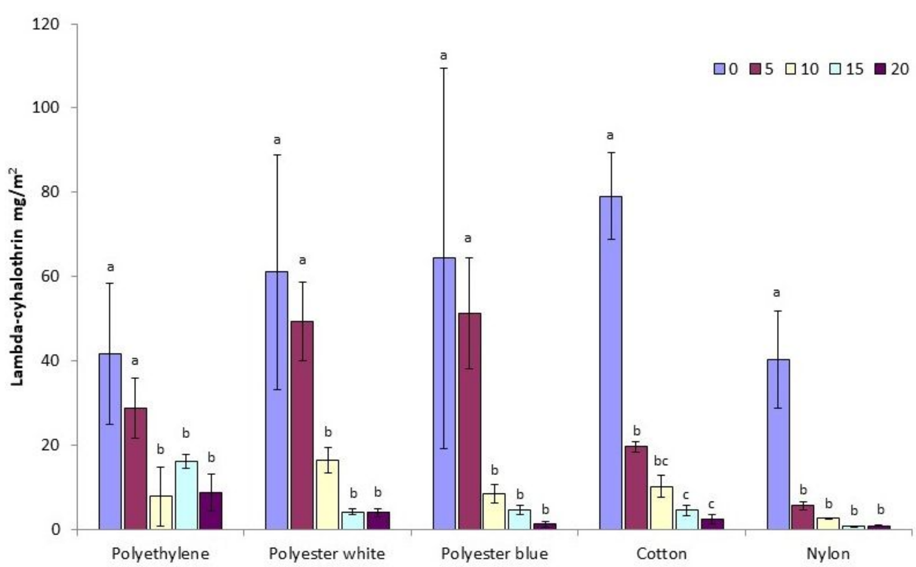

Figure 1

1a. Mean lamda-cyhalothrin content (± 95% CI) for netting materials treated with Iconet and washed up to 10 times. Note: For each material, bars sharing same superscripts do not differ statistically (p>0.05) 1b. Mean lamda-cyhalothrin content

(± 95% CI) for netting materials treated with ICON Maxx and washed up to 20 times. Note: For each material, bars sharing same superscripts do not differ statistically ($p>0.05$)

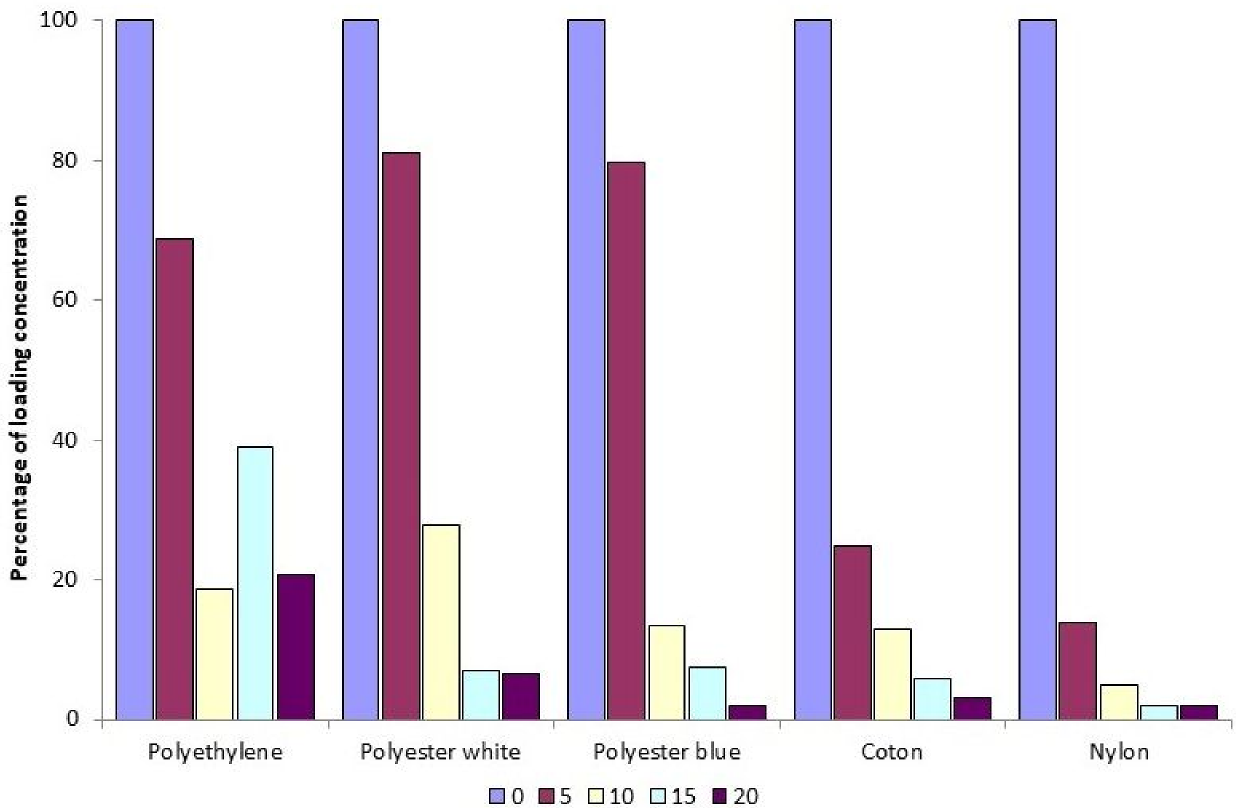

Figure 2

Content of lamda-cyhalothrin as a percentage of the ICON Maxx loading dose over 20 washes

Figure 3a.

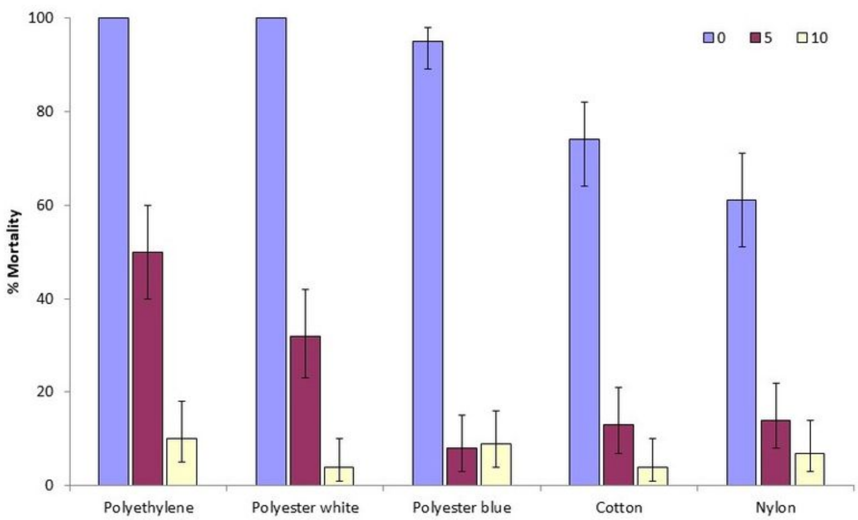

Figure 3b:

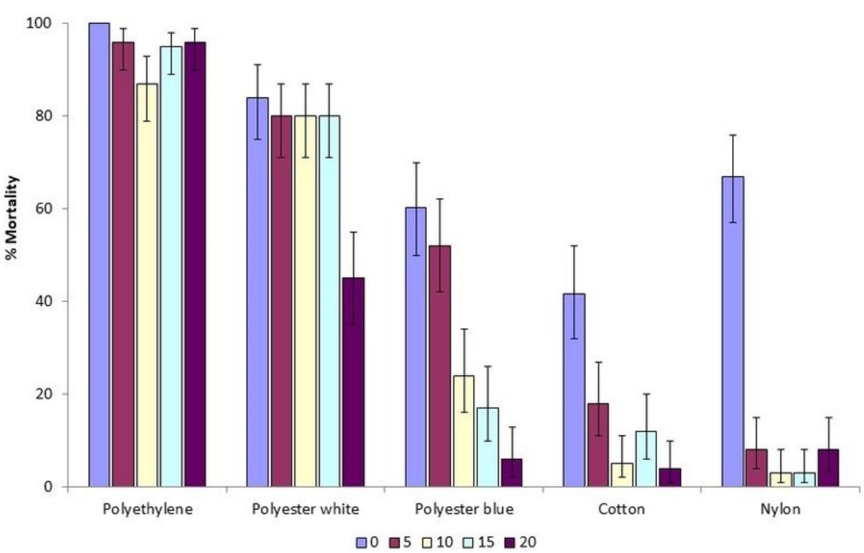

Figure 3

3a. Cone bioassay: Percentage mortality (± 95% CI) at 24 hours after exposure for netting materials treated with Iconet and washed up to 10 times. 3b: Cone bioassay: Percentage mortality (± 95% CI) at 24 hours after exposure for netting materials treated with ICON Maxx and washed up to 20 times.

Figure 4a:

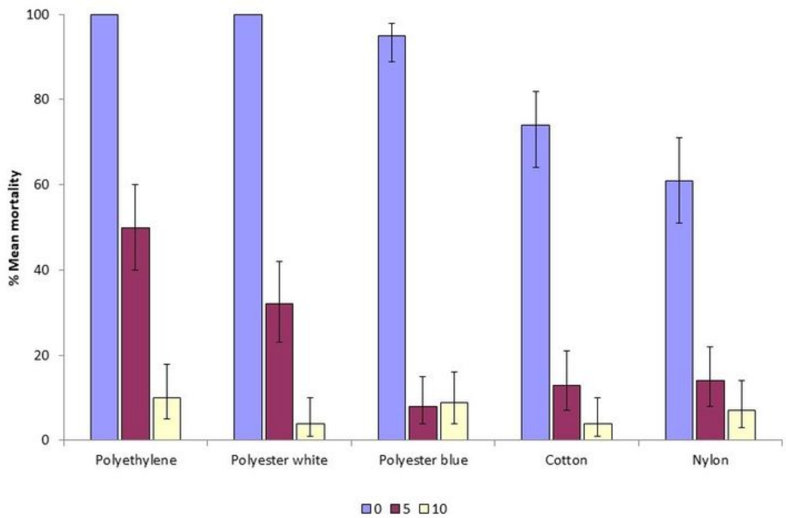

Figure 4b:

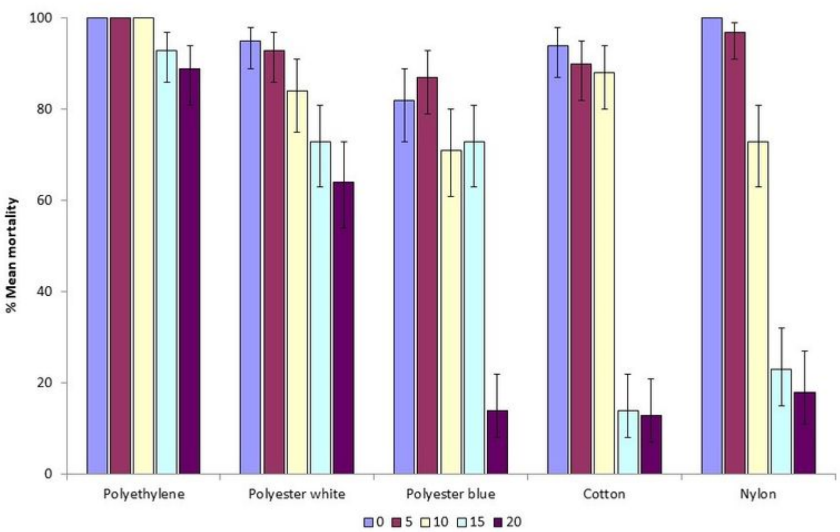

Figure 4

4a: Cylinder bioassay: Percentage mortality (± 95% CI) at 24 hours after exposure for netting materials treated with Iconet and washed up to 10 times. 4b: Cylinder bioassay: Percentage mortality (± 95% CI) at 24 hours after exposure for netting materials treated with ICON Maxx and washed up to 20 times.

Figure 5a:

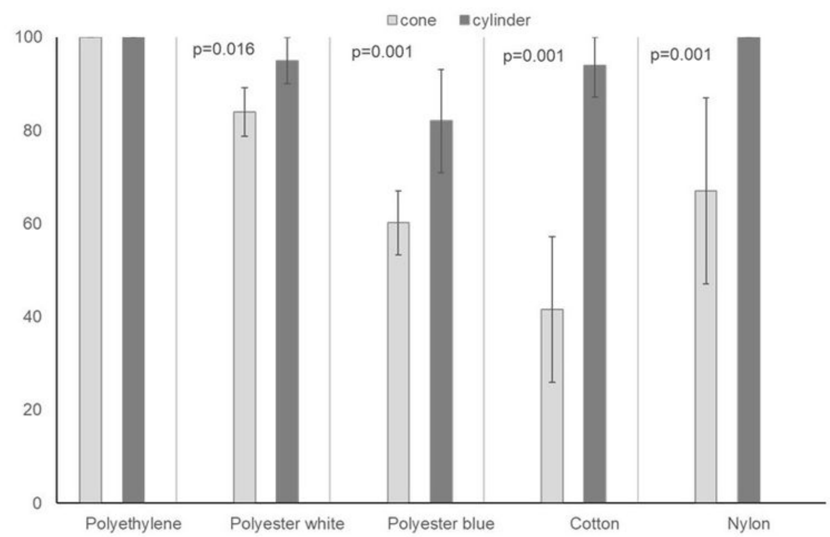

Figure 5b:

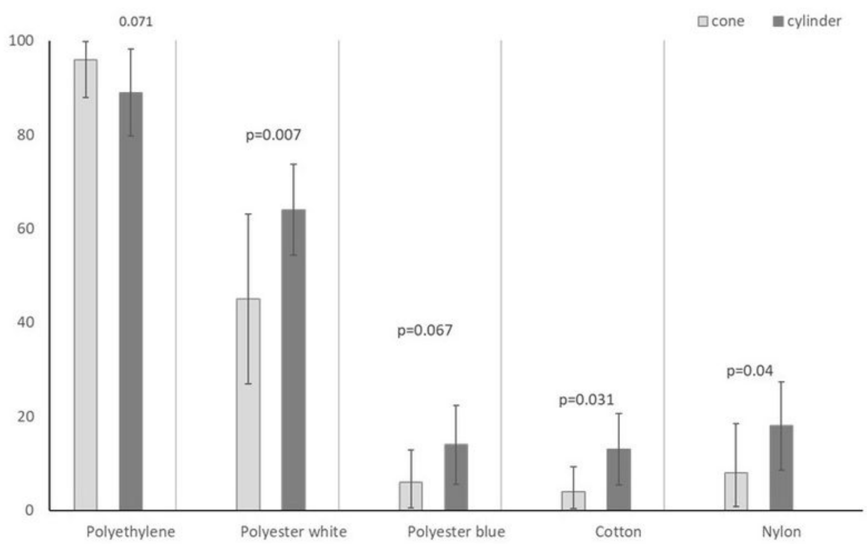

Figure 5

5a: Cone and cylinder bioassay comparison: percentage mortality (± 95% CI) at 24 hours after exposure for polyester white nets treated with ICON Maxx at 0 wash point 5b: Cone and cylinder bioassays comparison: % mean mortality at 24 hours post exposure (± 95% confidence intervals) for polyester white nets treated with ICON Maxx at 20 wash point

Supplementary Files

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