A study to understand the behavior and biting patterns of *Aedes Albopictus* when close to a nopixgo® wristband.

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Carried out by *Ana Muñoz* and *Ignacio Orensanz* at *Quimera Biological Systems* in conjunction with the *Department of Parasitic Diseases* at the *Faculty of Veterinary Studies*, *University of Zaragoza*, Spain

Followed protocols: European Chemicals Assessment Evaluation (*Guidance on the Biocidal Products Regulation. Version 3.0. April 2018*. European chemical agency)

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1. BACKGROUND

Nopixgo® is a new and unique wearable device using biopulse technology® that helps to protect the wearer from mosquito bites. The nopixgo® wristband emits an electromagnetic signal (average 0.10 W/Kg on 10 grams of material) that inhibits the biting behavior in mosquitos.

As part of NopixGlobal's ongoing commitment to proving the effectiveness of the Nopixgo®wristband, *Quimera Biological Systems* in conjunction with the *University of Zaragoza* is appointed to conduct a series of laboratory studies to better understand the behavior and biting patterns of a variety of mosquito species, when in the vicinity of a Nopixgo® wristband.

In the first study in this series, one of the world's most aggressive mosquito species, the *Aedes albopictus* (Asian Tiger mosquito) was selected on account of its human preference and highly aggressive biting behavior.

2. METHOD

Mosquito selection

Given the invasiveness of this species, these tests were performed at the University of Zaragoza, in collaboration with the Department of Parasitic Diseases at the Faculty of Veterinary Studies.

The tests were performed under controlled laboratory conditions, at optimal humidity (60-70%) and temperature (25ºC) for the mosquitos. All of the females were of the same age and were randomly selected from a batch of 960 in the same rearing cage. The lifespan of the females was seven to ten days. They had not been fed with blood and were not deprived of sugar twelve hours prior to the start of the test. They were separated 24 hours prior to the start of the test and kept in a climatic chamber.

Each test was then carried out using 30 female *Aedes albopictus* mosquitoes from the *University of Zaragoza's Faculty of Veterinary Studies*, in a room adjacent to the colony's brood chamber, with sufficient light and under the same climatic conditions (humidity 60% and 25ºC).

Product selection

Four different nopixgo® wristbands were evaluated. Each wristband had a different variation of the proprietary software loaded.

Testing method

The female *Aedes albopictus* were artificially fed blood in a *Hemotek* device, a capsule that also comprised a metal heating plate (to replicate human body temperature) and a thin membrane covering (mimicking skin). The female mosquitoes were attracted to the heat and access the blood to feed via their sucking organs.

A Hemotek device with a niopixgo® wristband wrapped around it.

Each of the nopixgo® was tested five times, along with the control and each of the tests was carried out in a small cage with a different batch of mosquitos.

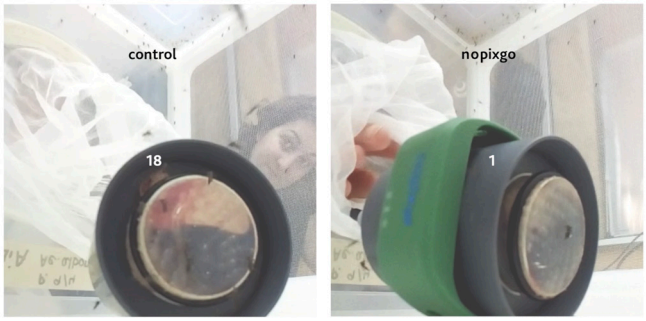
First a batch of mosquitos were exposed to just the capsule and membrane (control) for three minutes and the number of landings, probes and bites (feeds) were counted.

Following this, one of the four nopixgo® wristbands was placed on the capsule close to the feeding membrane, exposed to a new batch of mosquitos for three minutes, and the number of landings, probes and bites (feeds) were counted.

This was repeated another three times until each of the wristbands had been tested with a new batch of mosquitos. A fresh batch of mosquitos was important for each part of the test to eliminate any mosquito behavior change that occurred as a result of feeding.

The number of bites from the membrane area was recorded to evaluate the inhibitory effect of nopixgo® while the time until the first bite occurred was recorded to evaluate the protection time. Any landing or probing on the rest of the capsule has not been evaluated.

Following the five sets of three-minute tests, a further 10-minute test was carried out for each wristband to verify the three-minute observations.



Video image of control (no protection) and a nopixgo® wristband.

3. RESULTS

The results of this first study are promising, with on average 40 percent fewer bites from the mosquitos exposed to the Nopixgo® biopulse technology® compared to the control group in the three-minute test.

In the first test, three out of the four wristbands demonstrated protection of more than 80 percent, compared to the control. The tests also showed that some wristbands performed better than others. In two of the tests, it was almost the full three minutes until the first bite and on average, once all of the three-minute tests had concluded, 88 percent of the mosquitos exposed to Nopixgo® had not bitten (fed) at all. This is the version of Nopixgo® which currently is sold to consumers.

Further, at the conclusion of the ten-minute test, 68 percent of the mosquitos exposed to the Nopixgo® biopulse technology® had not bitten.

As well as a reduction in bites, the study also found a clear behaviour change in the mosquitos exposed to the Nopixgo®. The mosquitos became calmer, more passive and were noticeably trying to hide, compared to those in the control group (also positive considering the mosquitos were confined to a small area). This behavior change has never been seen before at *Department of Parasitic Diseases* at the *Faculty of Veterinary Studies*, *University of Zaragoza* and further research is planned to examine it more closely.

4. OBSERVATIONS

Conducting tests on the Nopixgo® wristband in laboratory conditions has some obvious limitations. The study protocol was based on the protocols used for chemical repellent products. This in itself has limitations given that the Nopixgo® wristband has a completely different mode of action to a chemical repellent (the biopulse technology® is designed to stop the mosquito from biting, rather than repelling the mosquito, whereas the chemical repellents are designed to repel). Therefore, in this test method the landings are counted as an unwanted effect similar to a bite, even though with the Nopixgo® wristband a landing without a bite is expected.

The protocol also meant that the environment was not optimal for the Nopixgo® wristband (the mosquitos were confined to a small cage just 30cm x 30cm in size) and we know that due to its mode of action the Nopixgo® wristband is most effective when used outdoors and away from confined spaces.

The different software and settings on each of the wristbands tested means that it is difficult to definitively statistically compare the results. However the data does show that some wristbands performed better than others. This was expected and warrants further investigation in future studies.

Finally, the *Aedes albopictus* is well recognised as one of the most aggressively biting species of mosquito – and the study results suggest that they became more aggressive throughout the day which could have an effect on the results. Based on these data we expect that with less aggressive mosquito species, the effectiveness of the Nopixgo® wristband will be even greater.

Overall, *Quimera Biological Systems* are encouraged by these results and will work with *NopixGlobal AG* and the *University of Zaragoza* to conduct further studies with the Nopixgo® wristband on other mosquito species and in different environments, such as large rooms and outdoor field tests.

We expect that in these future studies, which will more accurately reflect the real-world environment, the Nopixgo® will demonstrate much higher protection and even greater mosquito behavior change.

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