# WHO PQT/VCP Implementation Guidance – Bioassay Methods for ITNs – Wire ball Test

#### WHO Policy regarding the use of the wire ball test in studies submitted for assessment by PQT/VCP

The wire ball test has not been robustly validated and there is little supporting evidence for it's use as a standardised bioassay for ITN assessment. Therefore, studies submitted which utilize the wire ball test will not be considered by WHO in the prequalification assessment of ITNs.

However, if an applicant identified value of data generated using this method, validated the method, and justified the use of the method in the context of the product being evaluated, the prequalification assessment may consider data generated using this method.

The information provided in this document has been compiled from previous WHO publications for the purpose of providing the available information.

## Equipment and methods

The test set up comprises cubical or circular frames wrapped in ITN fabric (Figure 1). Eleven mosquitoes are introduced to the wrapped frame and held for three minutes before removal to holding cups. Knockdown at 60 minutes and 24 hour mortality is recorded. The median time to knockdown can be observed by recording the time when the sixth mosquito is knocked down. This median knockdown is stated to be an indicator of bioavailability.



Figure 1. Wire ball test arena. WHO/CDS/NTD/WHOPES/GCDPP/2006.3 via Mark Rowland

# Documentation and supporting evidence

The wire ball test is described in the 2006 WHO Guidelines for testing mosquito adulticides. In these guidelines, the test is described as a potential alternative to the cone test, but also notes that further calibration is required before it can be widely used in testing and evaluation of insecticide for treatment of bednets. No thresholds or acceptability criteria for the test are described in the guidelines.

There appears to be little evidence that this calibration has been conducted, and no information in the 2006 guidelines about the form that it might take.

### Test design and purpose

The expressed purpose of the wire ball test is to induce higher mortality than might be achieved in cone tests by creating a test arena that does not give mosquitoes a non-treated surface on which to rest, as is provided by the plastic cone in cone tests.

Academic publications that have employed the wire ball test generally report the lethality results in relation to results from the cone test, although there is the occasional publication that uses the wire ball test as a standalone method.

### Discussion

The test design may induce greater mortality than cone tests in part due to mosquitoes becoming covered in insecticide on all body parts including the thorax and abdomen, instead of only on the legs and antennae as is assumed is the case in WHO cone tests. If exposure in the wire ball assay does result in mosquitoes becoming covered in insecticide, this may be an unrealistic method of exposure as compared to the typical interaction of the mosquito and an ITN

WHO. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. WHO/CDS/NTD/WHOPES/GCDPP/2006.3 <a href="http://apps.who.int/iris/bitstream/handle/10665/69296/WHO">http://apps.who.int/iris/bitstream/handle/10665/69296/WHO</a> NTD WHOPES GCDPP 2006.3 <a href="http://apps.who.int/iris/bitstream/handle/10665/69296/WHO">http://apps.who.int/iris/bitstream/handle/10665/69296/WHO</a> NTD WHOPES GCDPP 2006.3 <a href="http://apps.who.int/iris/bitstream/handle/10665/69296/WHO">http://apps.who.int/iris/bitstream/handle/10665/69296/WHO</a> NTD WHOPES GCDPP 2006.3 <a href="http://apps.who.int/iris/bitstream/handle/10665/69296/WHO">http://apps.who.int/iris/bitstream/handle/10665/69296/WHO</a> OS NTD WHOPES GCDPP 2006.3 </a>