

BOTTLE BIOASSAYS OF FIELD COLLECTED MOSQUITOES FOR LEVEL OF ETOFENPROX RESISTANCE IN CENTRAL MASSACHUSETTS 2019 UPDATE

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ABSTRACT

Using protocols and supplies distributed from the Centers for Disease Control and Prevention, CMMCP conducted bottle bioassays on locally collected adult mosquitoes against the active ingredient etofenprox. The overwhelming majority of these specimens were of the species *Coquilleltidia perturbans*. Resistance to etofenprox was examined because the primary adulticide product of CMMCP is Zenivex® E4, of which etofenprox is the sole active ingredient. Results from these ongoing bottle bioassays are given to the CDC in an effort to help expand the understanding of pesticide resistance in mosquitoes of the United States.

BACKGROUND

Pesticide resistance in this age of vector-borne disease can hamper the ability of public health officials to successfully control threats. Potential resistance may also lead to the reemergence of several diseases that would have been otherwise contained through control measures (Brogdon 1998). Current resistance in select mosquito populations may be the result of historical insecticide use in the agricultural and pest control industries (Rodriguez 2005). The bygone use of DDT for example, could have contributed to current resistance to synthetic pyrethroids, due to the mechanism for resistance being similar for both (Brogdon 1998; McAbee 2003). Another associated and contributing factor may be the contracting classes and options for public health insecticides as well as growing regulatory restrictions (Brogdon 1998).

Although examples of pesticide resistance have been well documented, the scope of the issue and its real world impact on public health control activities is not known. This is partially due to varying levels of resistance surveillance programs that currently exist. This factor is shown in Massachusetts where some organized mosquito control agencies conduct zero resistance surveillance, while others have limited to well-developed programs collecting data on pesticide resistance in mosquitoes. In addition to the variety of resistance surveillance programs, resistance also appears to be quite localized which further clouds the impact. One noted example involved two separate mosquito populations that not only differed in resistance levels, but also resistance mechanism, all despite being only a few miles apart (Brogdon 1998). These continuing uncertainties surrounding insecticide resistance have supported CMMCP efforts to monitor for detection

of early resistance. In the case of observed resistance, adulticide protocols could be modified to ensure continued efficacy.

The primary adulticide product used by CMMCP during the 2018 season was Zenivex® E4 (Wellmark International, Schaumburg, IL) (EPA Reg No. 2724-807), a synthetic pyrethroid that utilizes the active ingredient etofenprox. Prior to this season CMMCP had used another synthetic pyrethroid, ANVIL® 10+10 (Clarke Mosquito Control Products, Inc., Roselle, IL) (EPA Reg. No. 1021-1688-8329). Unlike Zenivex® E4, ANVIL® 10+10 uses the active ingredient Sumithrin along with the synergist Piperonyl butoxide (PBO). The absence of PBO in Zenivex® E4 is one of its advantages over ANVIL® 10+10.

MATERIALS & METHODS

The procedure used for these bottle bioassays comes from the Centers for Disease Control and Prevention (CDC 2010). Using the CDC diagnostic concentration established from naïve specimens against mosquito populations from the CMMCP service area, potential resistance can be observed. In these bottle bioassays, clean 250ml Wheaton bottles (Wheaton Science Products, Millville, NJ) were lined with the baseline etofenprox concentration of 12.5µg/ml. The solutions used in this project were created using pesticide grade acetone (Thermo Fisher Scientific, Inc., Fair Lawn, NJ) and technical grade etofenprox supplied by the CDC. In addition to the bottles coated with etofenprox, untreated bottles were created using only the pesticide grade acetone to establish a control for the bioassays.

Field collected mosquitoes were obtained by using CDC light traps (John W. Hock Co., Gainesville, FL) deployed in areas with a history of CMMCP adulticide applications. The CDC light traps used compressed carbon dioxide gas as an attractant at a release rate of 500cc/min. Once the labeled bottles were coated and sufficiently dried, approximately 10-15 adult mosquitoes were aspirated into each bottle mechanically. ABC standard collection nets (Clarke Mosquito Control Products, Inc., Roselle, IL) were used in conjunction with the CDC light traps and held the mosquitoes until introduction into the bioassay bottles.

With these local exposed mosquitoes aspirated into the bottles, specimen knockdown percentage was recorded at various intervals, up to 100% knockdown or ending at 120 minutes elapsed time. For the untreated control bottles lined with only acetone (zero etofenprox), knockdown percentage was observed at similar intervals. Potential differences between the plotted knockdown curves of the treatment mosquito populations and the established baseline group could be used to determine if resistance was forming in local mosquitoes. If test specimens survived longer than those of the baseline group, it could be an indication of resistance developing.

DISCUSSION

CMMCP used an etofenprox concentration of 12.5µg/ml for bottle bioassays, a level predetermined by the CDC, to help the public health community monitor pesticide resistance in local mosquito populations. Although the CDC suggests using lab reared mosquitoes for

these bottle bioassays, CMMCP currently uses field collected mosquitoes, which can introduce additional variables. Adult mosquitoes collected from the field may be at various ages and metabolic stages, processing exposures to synthetic pyrethroids at different rates. If resources allow, future bottle bioassays may attempt to use adult mosquitoes reared from locally collected egg rafts instead of adult mosquitoes directly obtained from the field. There are several advantages to conducting the bottle bioassays in this manner. If using adults reared from eggs rafts, the specimens will all be *Culex*, which helps data comparisons. This method would also allow for the specimens to be manipulated to have consistent age, and metabolic stage. CMMCP will continue to examine the local levels of mosquito resistance to etofenprox, as Zenivex® E4 is slated to remain the adulticide product for the 2020 season. If CMMCP changes adulticide products to one that uses a different active ingredient, the subsequent bottle bioassays will begin utilizing that particular chemical, and the corresponding CDC diagnostic concentration.

REFERENCES

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