Insecticide Susceptibility Testing of *Culex* and *Aedes* Mosquitoes in the United States

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Background

- Numerous insecticides on the market to combat mosquito populations.
- Exposure to sub-lethal doses of active ingredients (AI) may cause resistance.
- Mosquito populations should be routinely monitored for signs of resistance/susceptibility by mosquito control programs.

Purpose

- To determine the resistance of several *Aedes* and *Culex* populations of public health importance.
- Assess the extent to which resistance differs between mosquito collection location, AI, mosquito species and in (in some cases) between years.

Significance

- Findings in this study provide information on how sub-lethal doses may affect resistance in different populations of mosquitoes.
- To protect public health and reduce costs, the most efficacious products should be used.

Methods

Mosquito Collections

- Eggs from 17 mosquito populations obtained from 4 regions:
- West: California, Utah;
- South: North Carolina, South Carolina, Florida, Louisiana, Georgia, Texas
- Midwest: Minnesota,
- Northeast: Pennsylvania
- 6 Species or hybrid species:
- *Aedes albopictus, Ae. aegypti, Culex pipiens, Cx. quinquefasciatus, Cx. nigripalpus, Cx. pipiens/quinquefasciatus*

Figure 1. Female *Culex* mosquito

Preparation of Active Ingredients

- Six AIs tested (technical grade).
- AI standards prepared in acetone.
- Concentrations verified every 2 weeks to test for degradation of AIs.
- Analyzed 3 - 4 replicate samples (1 µL) per stock solution.
- Capillary gas chromatograph with flame ionization detector.

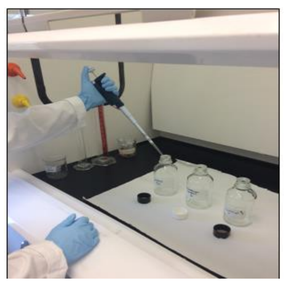

Figure 2. Coating bottles for CDC Bottle Bioassay.

AIs Tested
- Bifenthrin
- Deltamethrin
- Permethrin
- Phenothrin
- Etofenprox
- Malathion

World Health Organization guidelines:

-Susceptible: ≥ 98% mortality at diagnostic time

-Possible resistance: 80-97% mortality

-Resistance: < 80% mortality

Methods, continued

CDC Bottle Bioassay Procedure

- Three to four 250 mL glass Wheaton bottles coated with 1 mL of each AI stock solution or 1 mL of acetone as a control.
- Bottles uncapped and placed on a roller at 20 revolutions/minute for 3-4 minutes until dry.
- Bottles stored in a drawer away from light ≤ 24 h prior to bottle assays.
- Four to ten day old female mosquitoes introduced into bottles and mortality recorded at 10 time points during a two hour period (following CDC bottle bioassay guidelines).

Results

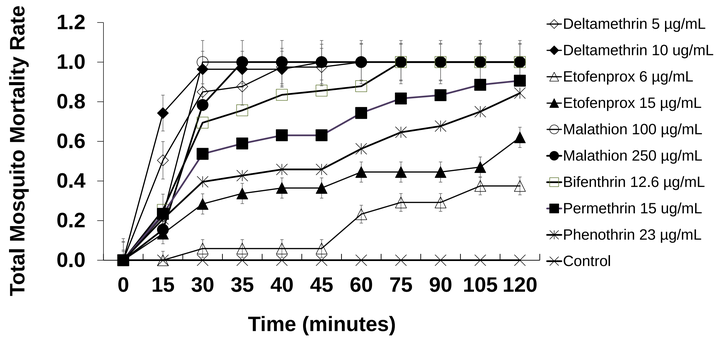

Figure 3. Insecticide resistance test for *Ae. aegypti* (F_0) from Dallas, TX.

- Resistant or possibly resistant to all tested AIs except for malathion

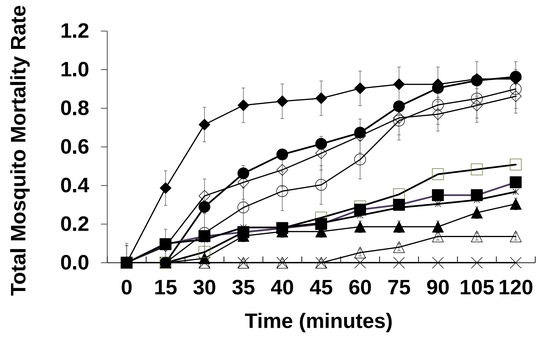

Figure 4. Insecticide resistance test for *Culex quinquefasciatus* (F_0) from Dallas, TX.

- Resistant to all tested AIs.

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Figure 5. Bottle preparation.

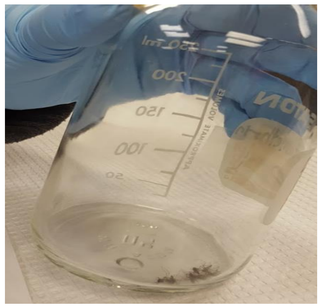

Figure 6. Dead mosquitoes in bottle.

Key Points

- Some mosquito populations were highly resistant (never achieved 80% mortality for duration of experiment).
- No *Culex* species classified as "susceptible" for malathion, etofenprox, bifenthrin, or permethrin.

Implications

Continued exposure to sub-lethal doses of insecticides could lead to resistance in mosquito populations, with the possibility of critical public health consequences.

Discussion

- Other sources of insecticide pressure
  – Agricultural and homeowner applications
- This study evaluated technical grade active ingredients and not formulated products.
- Only the most effective insecticides should be used for targeted control.
- Routine surveillance of insecticide resistance enhances the ability of control programs to protect public health.
- We expect variation in susceptibility and/or resistance of other mosquito species from different regions, for other AIs, and for these populations from year to year.

Acknowledgments

This study was funded by Bayer Crop Science. Special thanks to mosquito control districts that sent us mosquitoes and students who assisted in mosquito assays.