Insecticide Resistance in Mosquitoes

Stephanie Richards¹, Jo Anne Balanay¹, Avian V. White¹, Joe Hope², Kurt Vandock²

¹Environmental Health Science Program
  Department of Health Education and Promotion
  East Carolina University

²Bayer Crop Science
  Environmental Science, Vector Control and Pest Management
Introduction

• Routine mosquito susceptibility/resistance monitoring is an important part of a successful mosquito control program.
  o Informs management decisions by informing selection of the most effective active ingredients used in control measures.
Study Objectives

• Determine susceptibility/resistance for several US mosquito populations against six active ingredients commonly used in mosquito control.

• Investigate the extent to which susceptibility/resistance differs between active ingredients, mosquito collection locations, and (for some populations) years.
Study Design

- 17 mosquito populations were collected by abatement districts, control programs, and/or universities from 4 US regions and eggs were mailed to investigators.
- Approximately 5 more populations still to be tested.
Study Design

• Eggs of *Aedes albopictus*, *Ae. aegypti*, *Culex nigripalpus*, *Cx. pipiens*, *Cx. pipiens/quinquefasciatus*, and *Cx. quinquefasciatus* were reared to adults.

• Generation $F_0$ utilized when possible.

• If necessary, mosquitoes were blood fed and additional generations were propagated to increase sample size.
Study Design

- Six active ingredients tested (technical grade).
- CDC bottle bioassays.
- Doses standardized across mosquito populations for comparison.
- 30 min diagnostic time used for all except malathion (60 min)
  - Malathion (100 µg/mL* and 250 µg/mL**)
  - Etofenprox (6 µg/mL* and 15 µg/mL**)
  - Permethrin (15 µg/mL**)
  - Bifenthrin (12.6 µg/mL**)
  - Deltamethrin (5 µg/mL** and 10 µg/mL*)
  - Phenothrin (23 µg/mL**)

* doses used in 2015 and based on dose-response curves for susceptible Aedes albopictus colony in 2015
** doses based on dose-response curves for susceptible colonies (Culex quinquefasciatus and Aedes albopictus) conducted in 2016
Study Design

• Active ingredient standards prepared in acetone.
• Solutions stored at 4°C for duration of experiment.
• Concentrations verified every 2 weeks to test for degradation of active ingredients.
  – Analyzed 3 - 4 replicate samples (1 µL) / stock solution.
  – Capillary gas chromatograph with flame ionization detector.
  – Calibration curves generated for quantification.
Methods

- 1 mL of each active ingredient stock/bottle.
- Inside of bottles coated.
- At least 3 control bottles (1 mL acetone) used in each assay.
• Caps removed and bottles placed on bottle roller until contents evaporated (1 - 2 minutes)

• Uncapped bottles placed into dark drawer and used within 24 h.

• Mosquitoes transferred to bottles with mechanical aspirator.

• CDC bottle bioassays conducted for all active ingredients.
Mosquito mortality recorded at 11 time points using the “CDC bottle bioassay data recording form”.

![Appendix 3. CDC bottle bioassay data recording form]

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Bottle 1</th>
<th>Bottle 2</th>
<th>Bottle 3</th>
<th>Bottle 4</th>
<th>All test bottles</th>
<th>Control</th>
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<tbody>
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<td>Total in bottle</td>
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</table>
Data Analyses

- Ordinal logistic regression ($P < 0.05$) used to show differences in susceptibility, possible resistance, and resistance, if any, between active ingredients.

- World Health Organization guidelines:
  - Susceptible: $\geq 98\%$ mortality at the diagnostic time
  - Possible resistance: 80-97% mortality
  - Resistance: $< 80\%$ mortality

- Odds ratios computed to illustrate differences between active ingredients.
Examples of Results – South

- Differences observed in resistance profiles between genera.
- *Aedes* spp. generally more susceptible than *Culex*.
- However, in this case, we observed resistance in *Aedes* for several active ingredients.
Examples of Results – Comparing One Collection Site Between 2015 and 2016

- Retention pond collection site.
- Resistance profiles changed from year to year.
Results Summary

- Highest to lowest average (%) mortality at diagnostic time
  - Purple = susceptible
  - Orange = possible resistance
  - Black = resistant

**Ae. albopictus (N = 4 populations)**

Permethrin = Phenothrin ≈ Deltamethrin = Etofenprox > Deltamethrin > Bifenthrin > Malathion > Malathion > Etofenprox

- 100
- 100
- 98(10 µg/mL)
- 98(15 µg/mL)
- 95(5 µg/mL)
- 92
- 89(250µg/mL)
- 67(100µg/mL)
- 52(6µg/mL)

**Ae. aegypti (N = 1 population)**

Malathion = Malathion > Deltamethrin ≈ Deltamethrin > Bifenthrin ≈ Permethrin ≈ Phenothrin ≈ Etofenprox ≈ Etofenprox

- 100(250µg/mL)
- 100(100µg/mL)
- 96(10 µg/mL)
- 95(5 µg/mL)
- 69
- 54
- 40
- 28(15 µg/mL)
- 6(6µg/mL)

**Cx. nigripalpus (N = 1)**

Deltamethrin > Permethrin ≈ Deltamethrin > Phenothrin = Bifenthrin > Etofenprox > Malathion > Etofenprox > Malathion

- 75(10 µg/mL)
- 61
- 59(5 µg/mL)
- 37
- 37
- 31(15 µg/mL)
- 13(250 µg/mL)
- 4(6 µg/mL)
- 0(100 µg/mL)

**Cx. pipiens (N = 3)**

Deltamethrin > Deltamethrin > Malathion ≈ Phenothrin = Bifenthrin ≈ Malathion > Permethrin > Etofenprox > Etofenprox

- 99(10 µg/mL)
- 90(5 µg/mL)
- 72(250 µg/mL)
- 71
- 62
- 61(100 µg/mL)
- 45
- 27(15 µg/mL)
- 0(6 µg/mL)

**Cx. quinquefasciatus (N = 6)**

Deltamethrin > Malathion > Deltamethrin > Malathion > Bifenthrin ≈ Phenothrin ≈ Permethrin > Etofenprox > Etofenprox

- 63(10 µg/mL)
- 37(250 µg/mL)
- 34(5 µg/mL)
- 24(250 µg/mL)
- 17
- 16
- 14
- 8(15 µg/mL)
- 2(6 µg/mL)

**Cx. pipiens/quinquefasciatus (N = 2)**

Malathion > Deltamethrin > Deltamethrin > Phenothrin > Etofenprox ≈ Etofenprox ≈ Bifenthrin > Malathion ≈ Permethrin

- 75(250 µg/mL)
- 72(10 µg/mL)
- 57(5 µg/mL)
- 44
- 39(15 µg/mL)
- 37(6 µg/mL)
- 36
- 26(100 µg/mL)
- 24
General Observations

• *Aedes* spp. and *Culex* spp. exhibited variation in resistance/susceptibility.

• Resistance to active ingredients was 15 times higher in *Culex* compared to *Aedes*. 
General Observations

• Some mosquito populations were highly resistant (never achieved 80% mortality for duration of experiment).

• No *Culex* species were classified as “susceptible” for malathion (100 or 250 µg/mL), etofenprox (6 or 15 µg/mL), bifenthrin, or permethrin.

• No *Aedes* species were classified as “susceptible” for etofenprox (6 µg/mL).

• Resistance profiles varied between years.
Discussion

• Mosquito control programs may enact different insecticide pressures on *Culex* (ULV applications at dusk/dawn) compared to *Aedes* (residual barrier sprays).

• Other sources of insecticide pressure
  – e.g., agricultural, homeowner applications

• This study evaluated technical grade active ingredients and not formulated products that may contain synergists and/or other ingredients.
Discussion

• We expect variation in susceptibility and/or resistance of other mosquito species from different regions, for other active ingredients, and for these populations from year to year.

• Routine surveillance of insecticide resistance is important and enhances the ability of control programs to protect public health.

• Only the most effective insecticides should be used for targeted control.
Acknowledgements

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• We thank the many mosquito control programs and universities that contributed mosquitoes used in the study.