Repellent Testing at the USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology

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US EPA Repellent Efficacy Discussion – 19 June 2007
Presentation Topics

- MFRU paradigm for repellent testing
  - Spatial
  - Topical

- Further examination of topical repellent assays
  - Types of bioassays
  - Influences

- Alternatives to testing on humans?

- Future studies (Summer/Fall 2007) and Summary
Repellent bioassays

- Given *biological relevance and precision*...

- Objective is to *correlate* results from different methods to obtain an *accurate* estimate of repellency
Questions about a repellent that can be answered by biological assay...

• Is it repellent?
• How much is repellent?
• How long is it repellent?
What is a Repellent?

1. Substance that prevents or reduces the number of arthropod bites
2. Exact mode of action uncertain
3. Candidate repellents are tested to determine:
   - if repellency occurs
   - the dose required for protection
   - the duration of protection
4. Repellency is a function of:
   - arthropod biting pressure
5. Repellency is influenced by:
   - biotic and abiotic factors
   - human subject variation
   - testing methodology

Attraction responses of Aedes aegypti to eight different human subjects
Repellent testing and classification methods

- Cloth tests (class 1-5)
- Topical Hazard Evaluation Program (THEP)
- Skin tests (class 1-5)
- Advanced toxicology, part I
- Field tests (CPT ≥ deet)
- Advanced toxicology, part II
- EPA involvement
Chemical Barriers on Skin

desirable characteristics of a topical repellent

- nontoxic
- non-irritating
- broad activity spectrum
- long lasting
- economical
- pleasant to use
Spatial Repellents

- effective at a distance from the point of application
- dispensed into a 3-dimensional environment
- inhibit mosquito activation [initial response to host presence]
- inhibit ability of mosquito to locate and track a target [host]
- allethrin [ThermaCELL®]
- linilool, dehydrolinalool [Mosquito Cognito®]
Bioassay of Candidate Attraction-Inhibitors for Use in Field-Deployed Devices to Cloak Humans from Mosquitoes

[Graph showing the comparison of attraction inhibition across different treatments, with labels for Attractant Blend and Efficient Inhibition.]
Structure-Activity Studies to Develop Alternative Repellents

- **Collaboration:** Department of Chemistry at University of Florida

- **Research problem:** Characterize the molecular structural and electronic properties that:
  - correlate with repellent activity against multiple insect species

- **Key point:** Since 1942, USDA has compiled records of more than 30,000 compounds candidate repellents and insecticides.
# Model-Predicted Classification vs. Experimental Classification of Repellents

<table>
<thead>
<tr>
<th>Predicted Classes</th>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
<th>Class 4</th>
<th>Class 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>11 (50%)</td>
<td>4</td>
<td></td>
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<tr>
<td>Class 2</td>
<td></td>
<td>2 (14%)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 3</td>
<td></td>
<td></td>
<td>3 (50%)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Class 4</td>
<td></td>
<td></td>
<td></td>
<td>1 (50%)</td>
<td></td>
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<tr>
<td>Class 5</td>
<td></td>
<td></td>
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<td></td>
<td>1 (33%)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Experimental Classes</th>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
<th>Class 4</th>
<th>Class 5</th>
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</thead>
<tbody>
<tr>
<td>Class 1</td>
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<td>Class 3</td>
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<tr>
<td>Class 4</td>
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<tr>
<td>Class 5</td>
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</tbody>
</table>
Bioassay of Synthetic Piperidines as Candidate Attraction-Inhibitors (Candidates are coded P1-33)
Piperidine Candidate Topical Repellents Cloth Patch Test Assay Reciprocal Duration vs. DEET (Candidates are coded P1-33)
Structure-Activity Studies

- Cloth patch assay screening of 33 synthetic piperidines and 34 carboxamides have been completed.
- Novel piperidines provided the longest protection times on cloth.
- Additional material is being synthesized for additional tests using a larger number of volunteers.
Types of Repellent Testing [Bioassay] Methods

Lab – small cage method
ASTM E951-02, K&D module

Lab – large cage method
US EPA OPPTS

Types of repellent bioassay systems...

... *in vitro*  
  cloth  
  filter paper  
  membrane  
  olfactometer

*in vivo*...  
  animal  
  human
In vitro systems:

- Fast
- Safe
- Inexpensive
- Can test many candidates

but...

- Poor comparability among methods
- Accuracy unknown
In vivo systems:

- Slow
- Potential for toxicity
- Expensive
- Test one or a few candidates at a time
- Require review board approvals
- No correction for systems differences
  
  *but...*

- Human systems are relevant/accurate
What causes variability in a laboratory repellent bioassay?

Skin mediated effects on the repellent...

- Absorption
- Penetration
- Skin chemistry
What causes variability in a laboratory repellent bioassay?

Skin mediated effects on the repellent...


What causes variability in a laboratory repellent bioassay?

Physical loss of the repellent by...

- Evaporation
- Abrasion
- Perspiration
- Washing/rinsing
What causes variability in a laboratory repellent bioassay?

Physical loss of the repellent by...


What causes variability in a laboratory repellent bioassay?

Abiotic factors…

- Light
- Temperature
- Relative humidity
- Repellent dose
- Exposure time
- Test cage size and shape
What causes variability in a laboratory repellent bioassay?

Abiotic factors…


What causes variability in a laboratory repellent bioassay?

<table>
<thead>
<tr>
<th></th>
<th>Large Cage</th>
<th>Small Cage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>64,000 cm³</td>
<td>80 cm³</td>
</tr>
<tr>
<td>Volume per mosquito</td>
<td>320 cm³</td>
<td>80 cm³</td>
</tr>
<tr>
<td>CPT</td>
<td>7.5 ± 1.1 hours</td>
<td>9.0 ± 1.0 hours</td>
</tr>
</tbody>
</table>
What causes variability in a laboratory repellent bioassay?

Biotic factors…

- Larval nutrition
- Carbohydrate intake
- Mosquito age
- Oviparity
- Endogenous cycles
- Subject attractiveness
What causes variability in a laboratory repellent bioassay?

10% Sucrose Solution
12 hours pretest
CPT = 9.1 ± 0.9 hours

Water Only
12 hours pretest
CPT = 7.3 ± 0.8 hours
What causes variability in laboratory and field repellent bioassays?

- Innate attractancy/non-attractancy
- Nine male subjects
- Tested in olfactometer
- Attractancy: 23 to 70%
- Non-attractancy: 30 to 77%
# Comparison of Topical Repellent Products [Laboratory]

<table>
<thead>
<tr>
<th>Product name</th>
<th><em>Ae. albopictus</em></th>
<th><em>Cx. nigripalpus</em></th>
<th><em>Oc. triseriatus</em></th>
<th>Avg CPT</th>
<th>$R_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autan</td>
<td>5.7</td>
<td>8.0</td>
<td>7.8</td>
<td>7.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Bite Blocker</td>
<td>5.5</td>
<td>8.3</td>
<td>7.8</td>
<td>7.2</td>
<td>1.5</td>
</tr>
<tr>
<td>BugGuard</td>
<td>1.8</td>
<td>5.7</td>
<td>2.0</td>
<td>3.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Bygone</td>
<td>0.2</td>
<td>4.7</td>
<td>0.0</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Natrapel</td>
<td>1.3</td>
<td>5.2</td>
<td>0.5</td>
<td>2.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Offl</td>
<td>7.2</td>
<td>7.0</td>
<td>7.3</td>
<td>7.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Repel</td>
<td>7.8</td>
<td>7.3</td>
<td>7.8</td>
<td>7.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Skinsations</td>
<td>5.0</td>
<td>4.8</td>
<td>4.7</td>
<td>4.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Field test:

- compare PMD, IR3535, and KBR3023 with deet
- *Ochlerotatus taeniorhynchus*
- Everglades National Park, FL
- measure % $R$, $CPT$
- evaluate aspects of bioassay procedure
Field test

- 5 human subjects
- tests 6 hours duration
- start 0730 and 1345 h
- 4 repellent-treated subjects
- 1 negative control subject
- CRD; 5 replications
Mean percent repellency
Mean protection time (h)
Summary:

- $% R$: KBR3023 > deet > PMD > IR3535
- $CPT$: deet > KBR3023 > PMD > IR3535
Question: are mosquito biting rates on repellent-treated subjects the same as on non-treated subjects?

- \( BR \) = mosquito biting rate/min.
- \( BR_t \) = biting rate on repellent treated subjects
- \( BR_{ut} \) = biting rate on EtOH treated subjects
- Ho: \( BR_t = BR_{ut} \)
Mean $BR_t$ as a percent of $BR_{ut}$
Question: how precise are estimates of mosquito biting rate?

- coefficient of variation = 146%
- 95% CI: 21–61 bpm
- decrease CI by 50% (to 31-51 bpm), \( n = 441 \)
- decrease CI by 75% (to 36-46 bpm), \( n = 1757 \)
- salary costs increase from $6,750 to $85,050 to $338,850
- solution: (a) data transformation; (b) preselect test subjects using attractancy or repellency bioassay
What causes variability in a field repellent bioassay?

- Exogenous cycles in the environment
- Endogenous cycles in mosquitoes
- Fluctuations in mosquito biting pressure
- Positional effects
- Human subject variability
- Treatment/control subject crossover effects
Mosquito attractants

- isolate, identify skin VOCs
- find attractant; blends
- non-competitive tests
- olfactometer
- human hand 85%
- attractant blend, *Ae. aegypti*, 95%
- attractant blend, *An. albimanus*, 97%
Comparison of Reconstructed Ion Chromatograms from Humans of Markedly Different Ability to Attract Aedes aegypti

Cryofocused GC/MS, EI, 3 Glass Beads, 25 m x 0.20 mm I.d. HP-FFAP F25T column (d=0.23 μm)

Diethyl Ether Impinged Volatiles from Bovine and Chicken Blood—6 h collection
DB-WAXetr column

**Bovine**
- Methoxyflurane
- Ethylbenzene
- Limonene
- Diethyl disulfide
- Trimethylbenzene
- Trimethylcymene
- Benzaldehyde
- Naphthalene

**Chicken**
- Cyclosiloxanes
- Octanal
- Benzaldehyde

Time (min):
- Bovine: 4.00 - 19.00
- Chicken: 4.00 - 19.00
Future Experiments

- Examination of persistent effect of topical repellents from repeated exposure upon a population of mosquitoes

- Examination of bioassay methods (from screening through semi-field or field tests as discussed in paradigm) for current recommended commercial repellents.
Summary

- Laboratory assays should be used since they provide the greatest control over abiotic and biotic factors that can influence the results – how experiments are designed can have significant impact.

- Assays should be conducted *in vivo* to produce the most meaningful results
  - Screening methods usually examine “subsets,” e.g. behaviors, smaller sample sizes.
  - The human (or other animal) kairomone mimics are not representative of the true complexity of the host semichemical profile.

- The use of complete protection time statistic (in my estimation) has greatest biological relevance; however, estimation of this value may contain the largest error and require larger sample sizes for increased precision in its estimation.