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

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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

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Repellent bioassays

- Given *biological relevance* and *precision*…
- Objective is to *correlate* results from different methods to obtain an *accurate* estimate of repellency

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Questions about a repellent that can be answered by biological assay…

- Is it repellent?
- How much is repellent?
- How long is it repellent?

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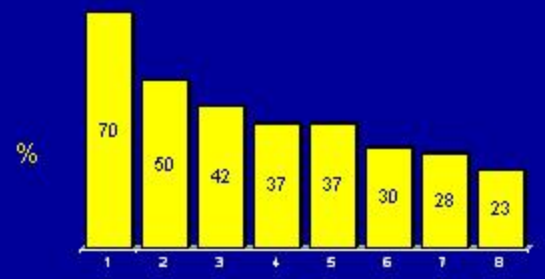
What is a Repellent?

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

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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

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Chemical Barriers on Skin

desirable characteristics of a topical repellent

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

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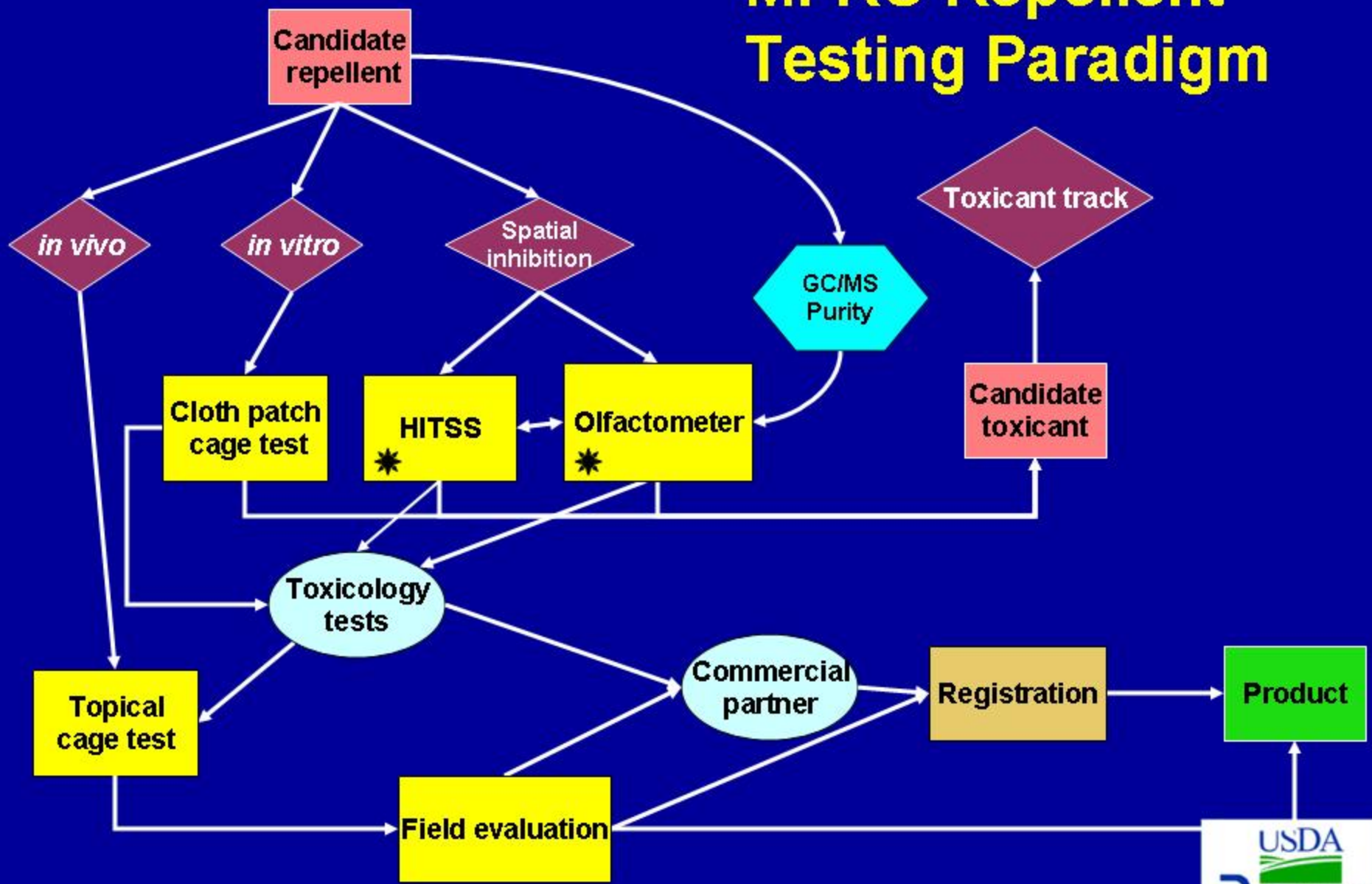
MFRU Repellent Testing Paradigm



✷ Also tested with infected mosquitoes

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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®]

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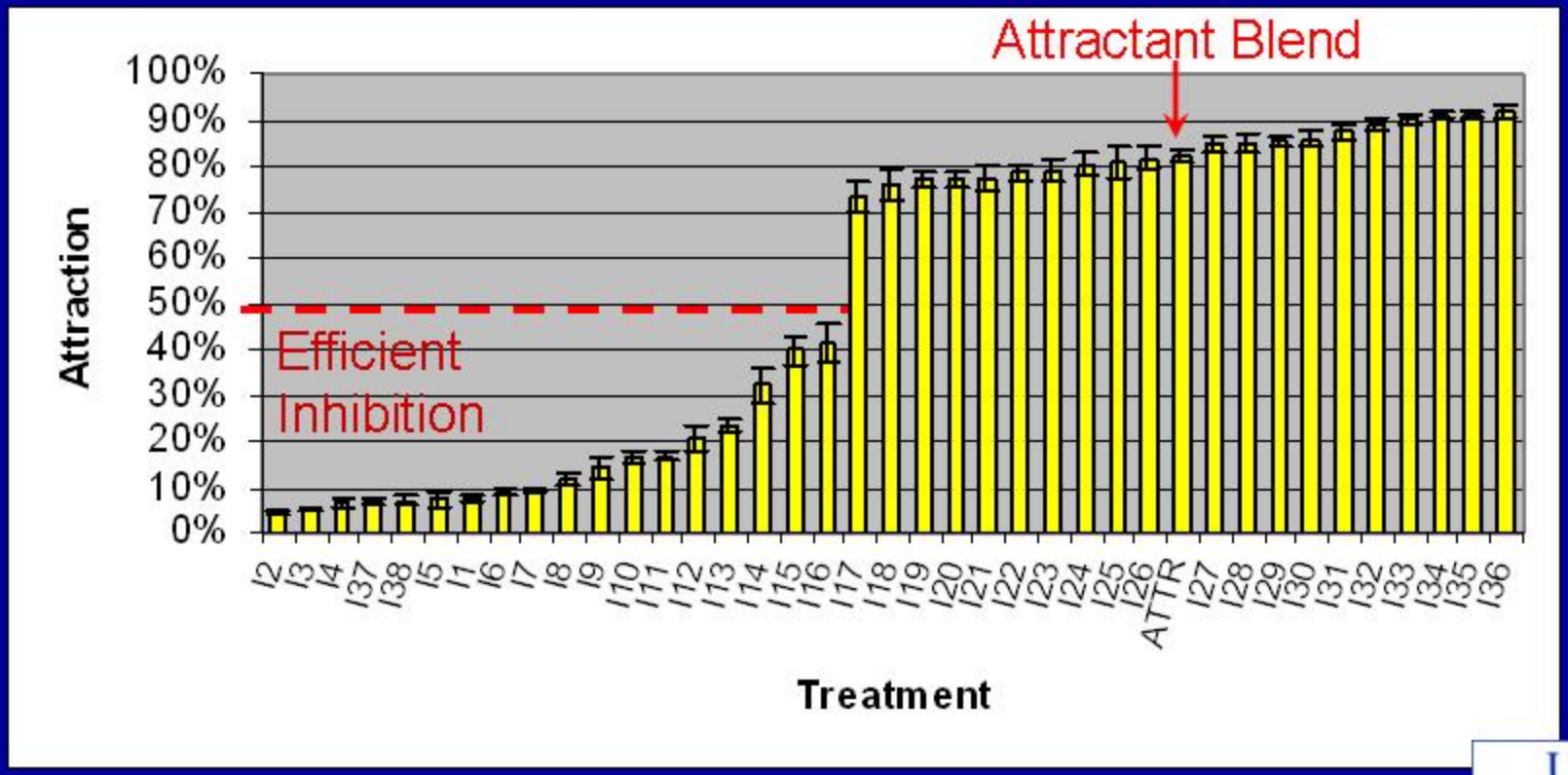
Bioassay of Candidate Attraction-Inhibitors for Use in Field-Deployed Devices to Cloak Humans from Mosquitoes



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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.

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Model-Predicted Classification vs. Experimental Classification of Repellents

Predicted Classes	Experimental Classes: Class 1	Class 2	Class 3	Class 4	Class 5
Class 1	11 (50%)	4			
Class 2	4	2 (14%)	1		
Class 3	7	7	3 (50%)	1	2
Class 4		1	2	1 (50%)	
Class 5					1 (33%)

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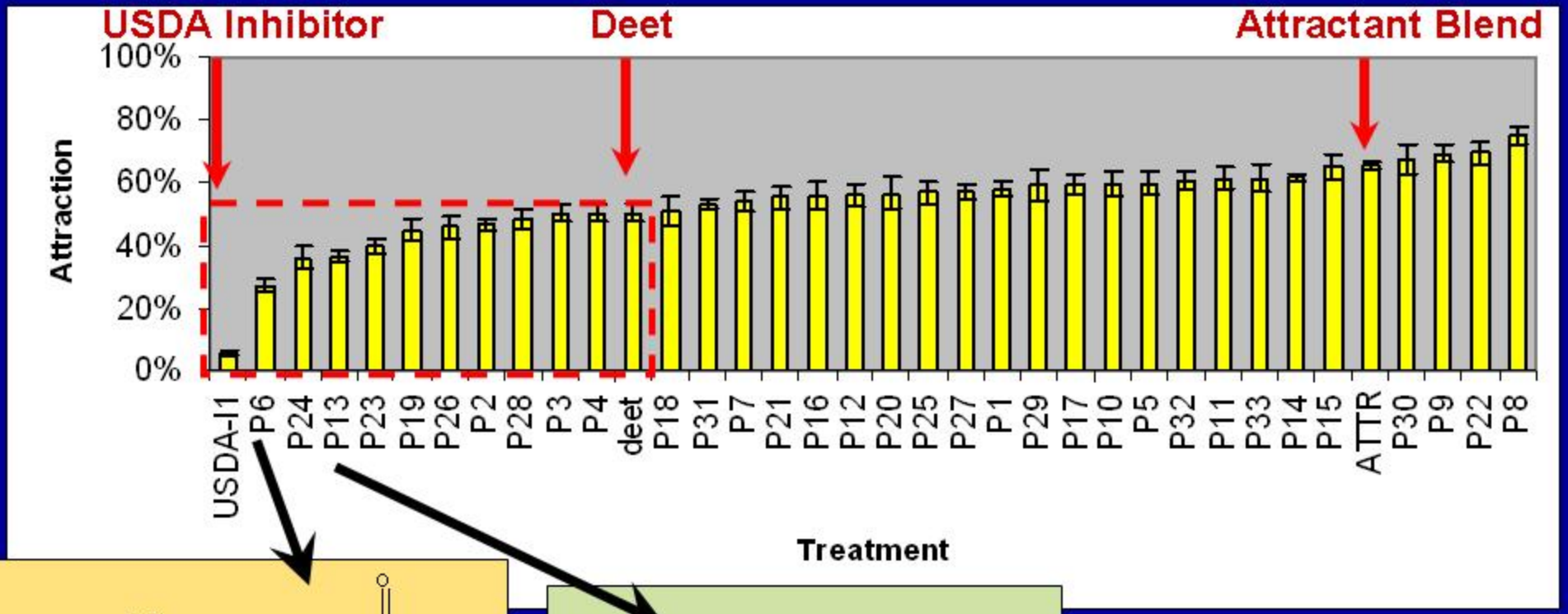
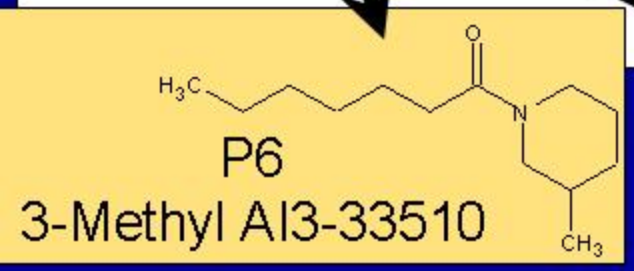
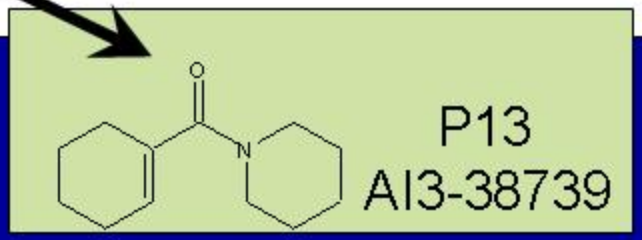
Bioassay of Synthetic Piperidines as Candidate Attraction-Inhibitors (Candidates are coded P1-33)







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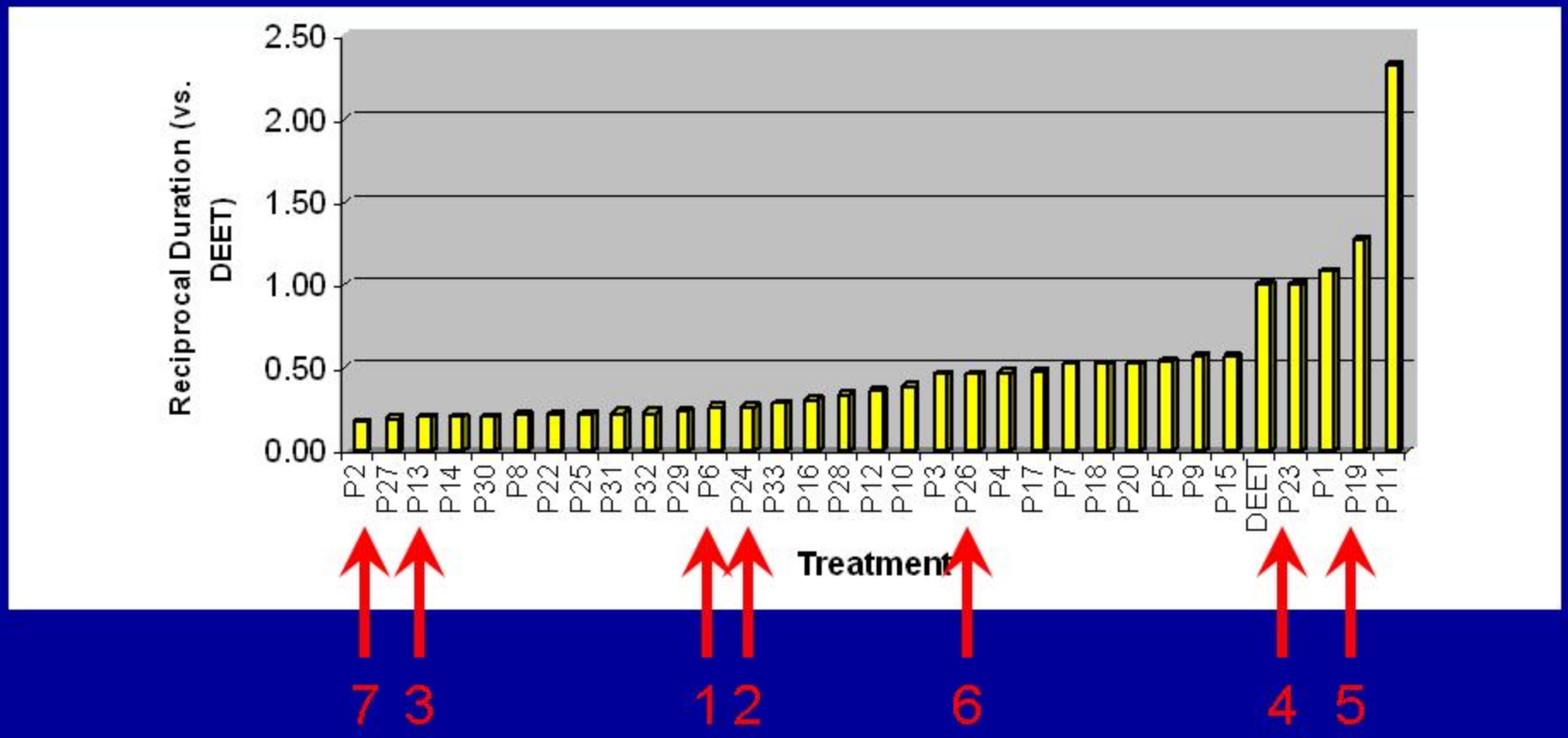
Piperidine Candidate Topical Repellents
Cloth Patch Test Assay
Reciprocal Duration vs. DEET
(Candidates are coded P1-33)



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.

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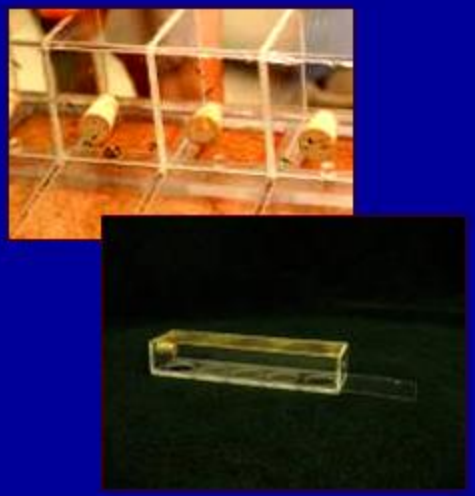
Types of Repellent Testing [Bioassay] Methods



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

Lab – large cage method
US EPA OPPTS

Field – ASTM E939-94,
US EPA, WHO

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Types of repellent bioassay systems…

…in vitro	*in vivo…*
cloth	animal
filter paper	human
membrane	
olfactometer	

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In vitro systems:

- Fast
- Safe
- Inexpensive
- Can test many candidates

but…

- Poor comparability among methods
- Accuracy unknown

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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

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What causes variability in a laboratory repellent bioassay?

Skin mediated effects on the repellent…

- Absorption
- Penetration
- Skin chemistry

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What causes variability in a laboratory repellent bioassay?

Skin mediated effects on the repellent…

- Selim, S. *et al.* 1995. Absorption, metabolism, and excretion of deet following dermal application to human volunteers. *Fundamen. Appl. Toxicol. 25: 95-100.*
- Reifenrath, W. G., and T. S. Spencer. 1989. Evaporation and penetration from the skin, pp. 313-334. *In:* R. L. Bronaugh and H. I. Maibach (eds.). *Percutaneous Absorption: Mechanisms – Methods – Drug Delivery*, 2nd ed. Marcel Dekker, NY.

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What causes variability in a laboratory repellent bioassay?

Physical loss of the repellent by…

- Evaporation
- Abrasion
- Perspiration
- Washing/rinsing

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What causes variability in a laboratory repellent bioassay?

Physical loss of the repellent by…

- Gabel, M. L. *et al.* 1976. Evaporation rates and protection times of repellents. *Mosq. News.* 36: 141-146.
- Rueda, L. M. *et al.* 1998. Effects of skin abrasions on the efficacy of the repellent deet against *Aedes aegypti*. *J. Amer. Mosq. Control Assoc.* 14: 178-182

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What causes variability in a laboratory repellent bioassay?

Abiotic factors…

- Light
- Temperature
- Relative humidity
- Repellent dose
- Exposure time
- Test cage size and shape

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What causes variability in a laboratory repellent bioassay?

Abiotic factors…

- L. C. Rutledge *et al.* 1985. Mathematical models of the effectiveness and persistence of mosquito repellents. *J. Amer. Control Assoc.* 1: 56-62.
- Gupta, R., and L. C. Rutledge. 1989. Laboratory evaluation of controlled release repellent formulations on human volunteers under three climatic regimens. *J. Amer. Control Assoc.* 5: 52-56.
- Barnard, D. R. *et al.* 1998. Mosqito density, biting rate, and cage size effects on repellents tests. *Med. Vet. Entomol.* 12: 39-45.

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What causes variability in a laboratory repellent bioassay?

Large Cage	*Small Cage*
64,000 cm^3	80 cm^3
320 cm^3 per mosquito	80 cm^3 per mosquito
CPT = 7.5 $\pm$ 1.1 hours	CPT = 9.0 $\pm$ 1.0 hours

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What causes variability in a laboratory repellent bioassay?

Biotic factors…

- Larval nutrition
- Carbohydrate intake
- Mosquito age
- Oviparity
- Endogenous cycles
- Subject attractiveness

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What causes variability in a laboratory repellent bioassay?

10% Sucrose Solution	*Water Only*
12 hours pretest	12 hours pretest
CPT = 9.1 ± 0.9 hours	CPT = 7.3 ± 0.8 hours

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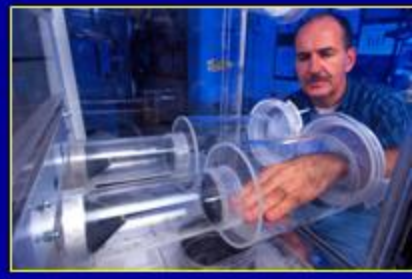
What causes variability in laboratory and field repellent bioassays?

- Innate attractancy/non-attractancy
- Nine male subjects
- Tested in olfactometer
- Attractancy: 23 to 70%
- Non-attractancy: 30 to 77%



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Comparison of Topical Repellent Products [Laboratory]

	Estimated CPT [hr]				
Product name	*Ae. albopictus*	*Cx. nigripalpus*	*Oc. triseriatus*	Avg CPT	R_i
Autan	5.7	8.0	7.8	7.2	**1.5**
Bite Blocker	5.5	8.3	7.8	7.2	**1.5**
BugGuard	1.8	5.7	2.0	3.2	**0.8**
Bygone	0.2	4.7	0.0	1.5	**0.3**
Natrapel	1.3	5.2	0.5	2.3	**0.5**
Off!	7.2	7.0	7.3	7.2	**1.5**
Repel	7.8	7.3	7.8	7.6	**1.7**
Skinsations	5.0	4.8	4.7	4.8	**1.0**

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Field test:

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

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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

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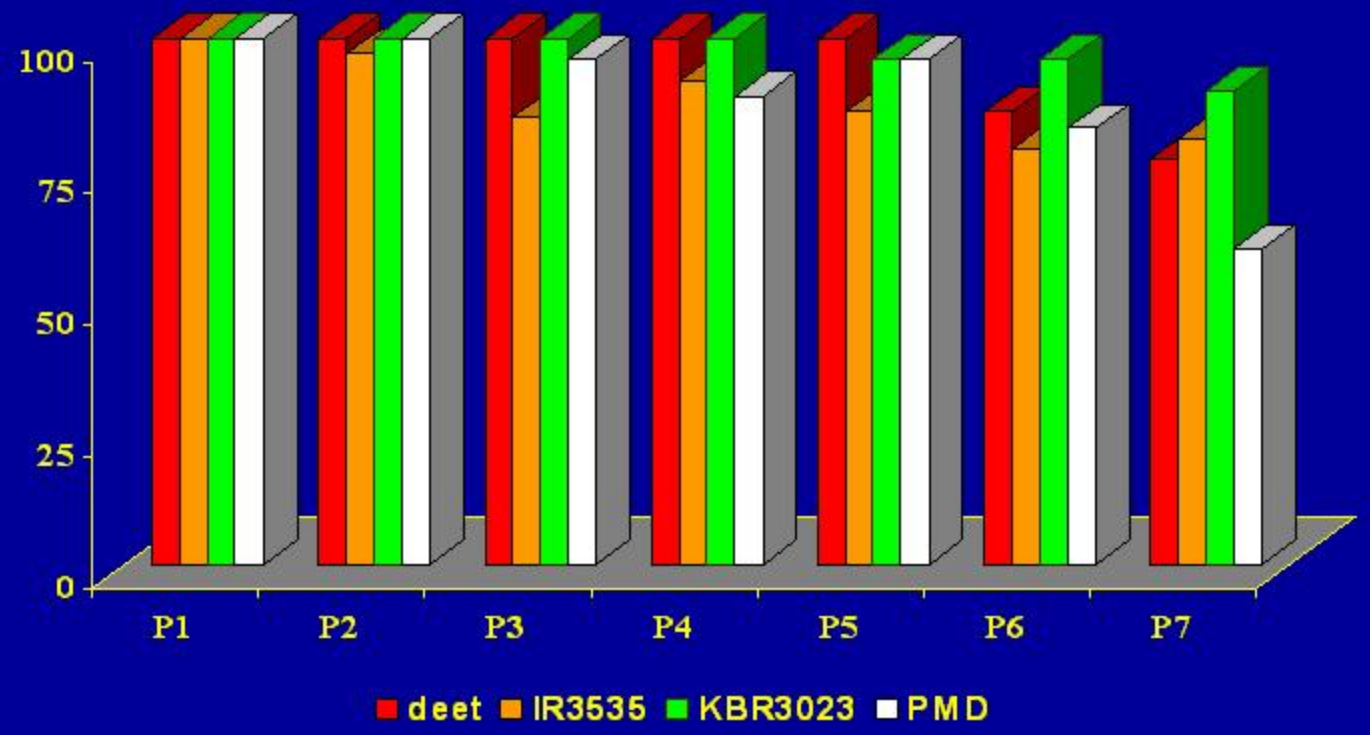
Mean percent repellency



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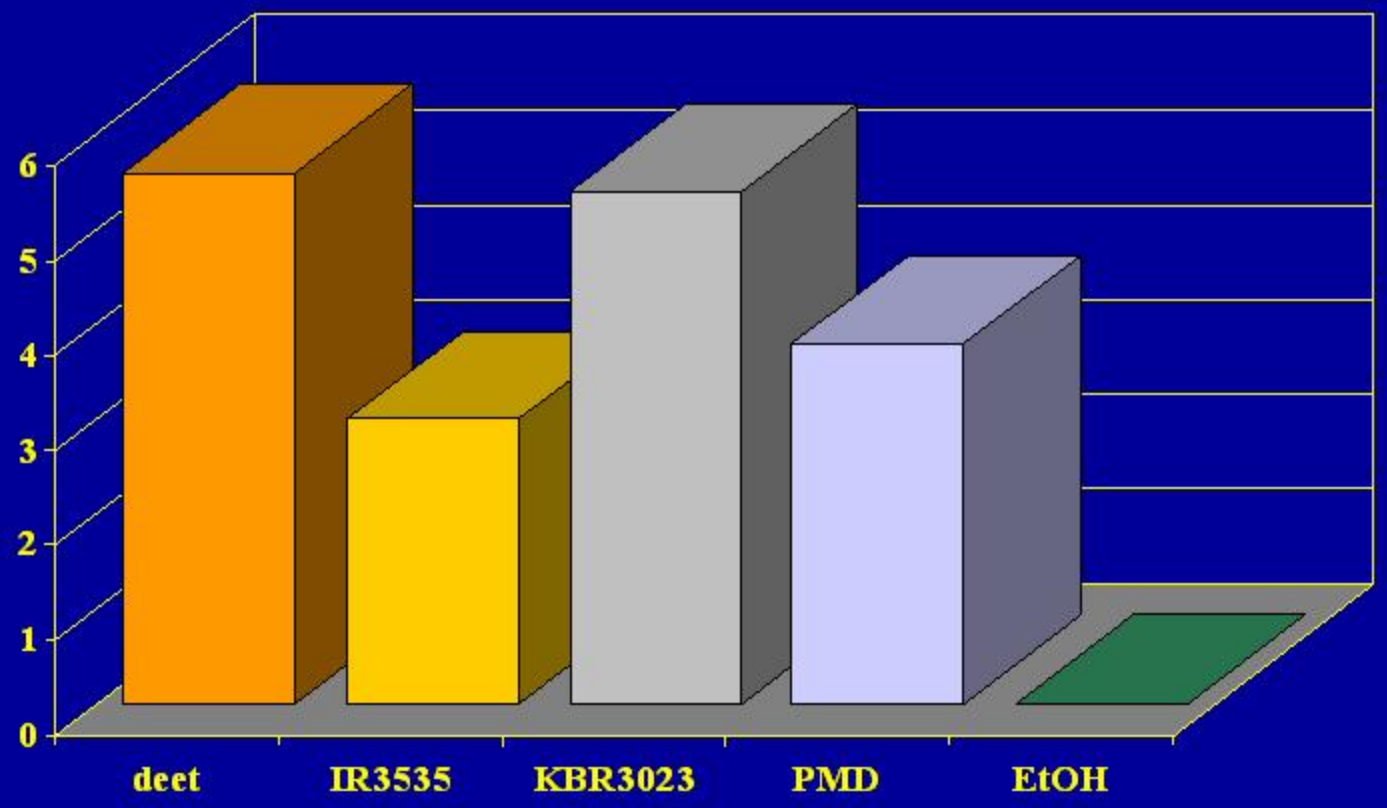
Mean protection time (h)



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Summary:

- *% R*: KBR3023 >deet >PMD >IR3535
- *CPT:* deet >KBR3023 >PMD >IR3535

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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:*** $BR_t = BR_{ut}$

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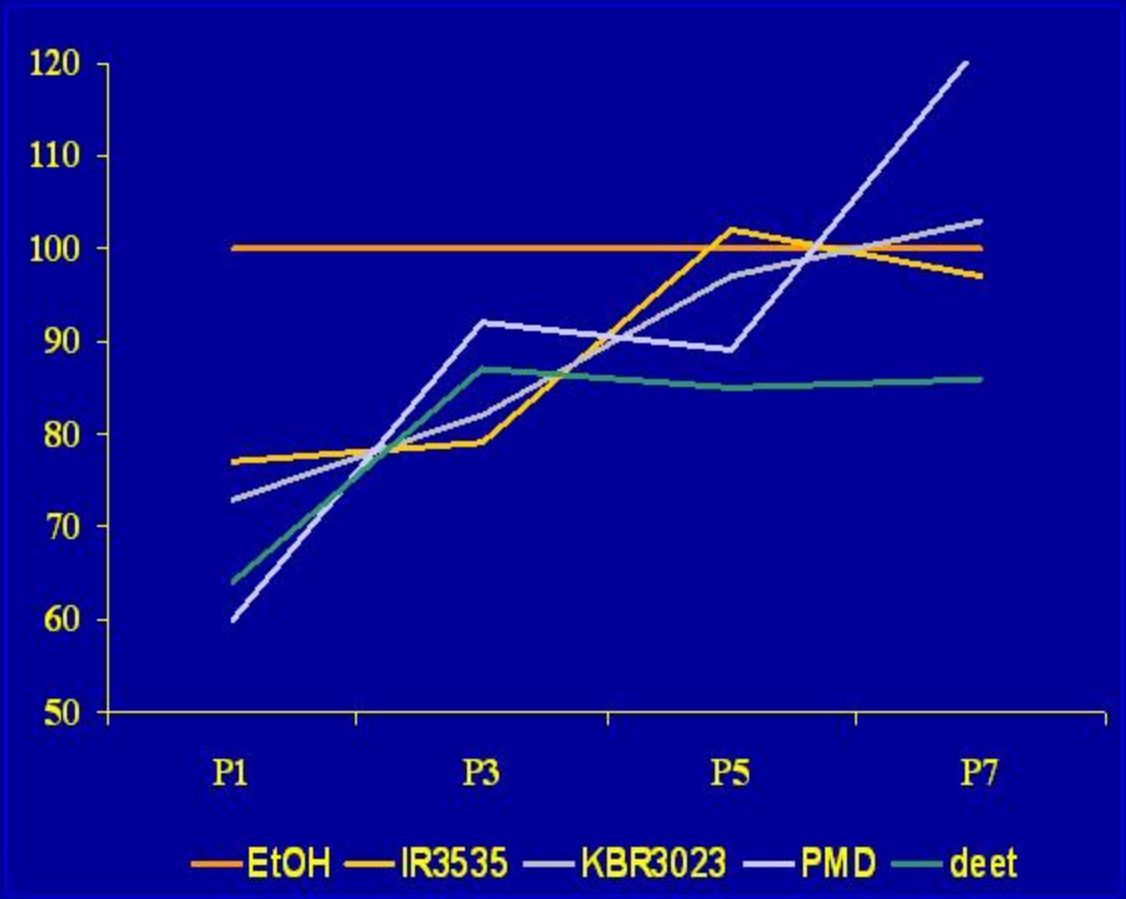
Mean BR_t as a percent of BR_{ut}



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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

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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

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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%

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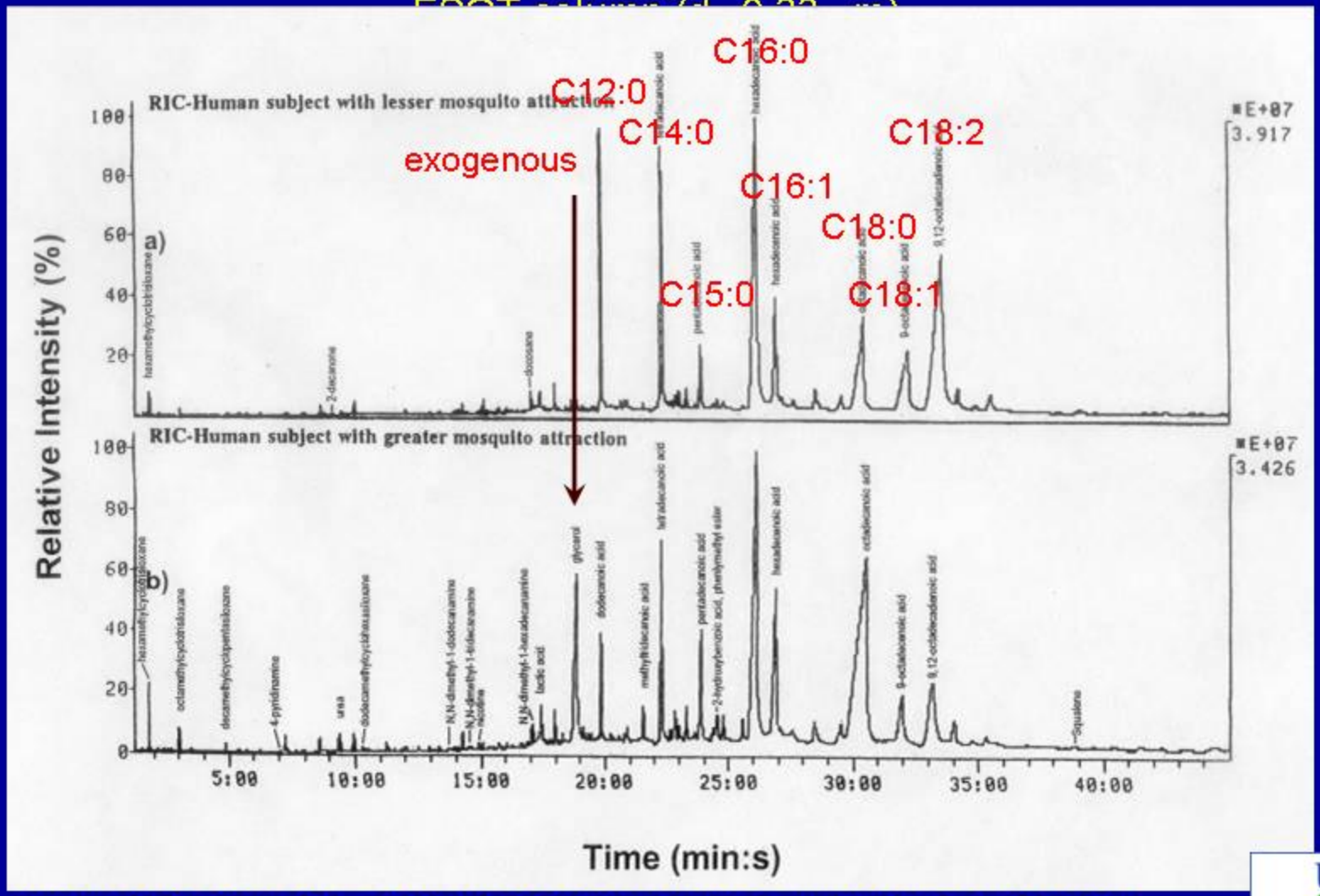
Comparison of Reconstructed Ion Chromatograms from Humans of Markedly Different Ability to Attract *Aedes aegypti*

Cryofocused GC/MS, EI, 3 Glass Beads, 25 m x 0.20 mm I.d. HP-FFAP



Bernier, U.R., Kline, D.L., Barnard, D.R., Schreck, C.E., and Yost, R.A., Chemical Analysis of Human Skin Emanations: Comparison of Volatiles from Humans that Differ in Attraction of Aedes aegypti (Diptera: Culicidae), *J. Am. Mosquito Control Assoc.*, **2002**, *18(3)*:186-195.

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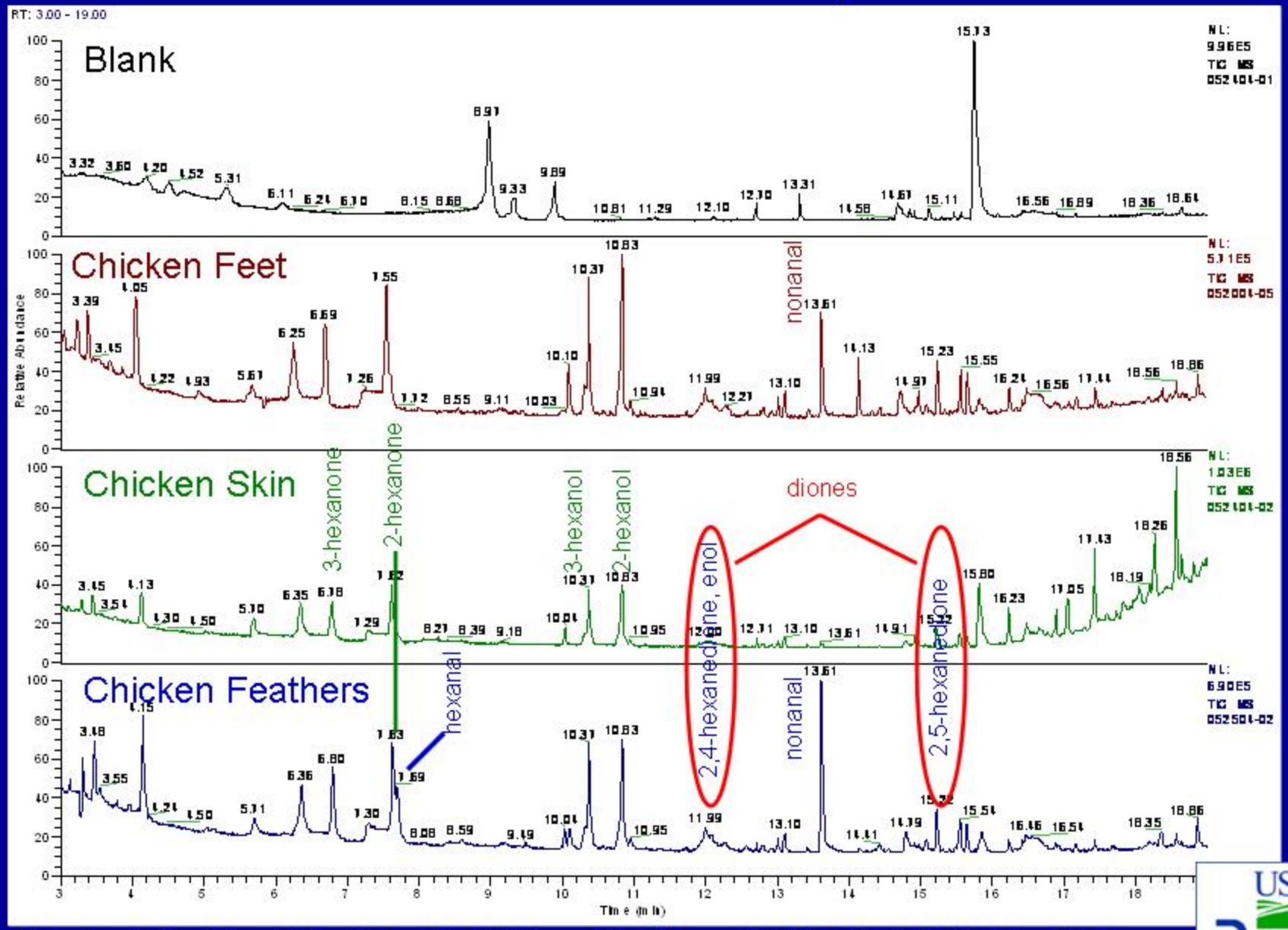
Hexane Extracts of Chicken Emanations, DB-WAXetr Column



Bernier, U.R., Allan, S.A., Quinn, B.P, Kline, D.L., Barnard, D.R., Clark, G.G. Identification of Compounds in Solvent Extracts from a White Leghorn Chicken (*Gallus gallus domesticus* L.) as Candidate Attractants of Ornithophilic Mosquito Species, *J. Sep. Sci.*, **2007**, *submitted*.

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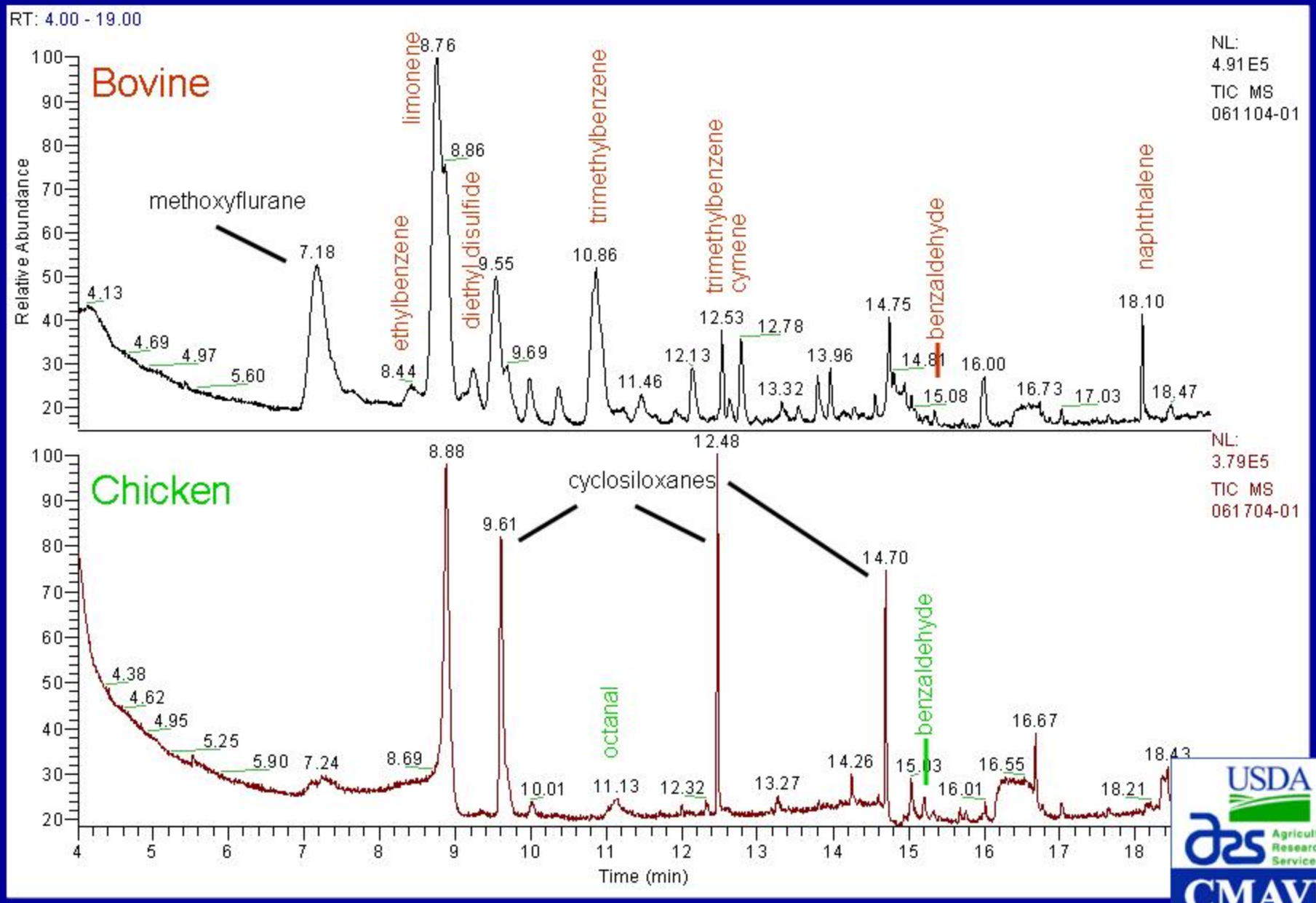
Diethyl Ether Impinged Volatiles from Bovine and Chicken Blood--6 h collection
DB-WAXetr column



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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.

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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.

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