

US EPA ARCHIVE DOCUMENT

(Draft)

United States Environmental Protection Agency  
June 19, 2007  
Workshop for Sharing Technical Information on Updated Methods for Testing  
Efficacy of Skin-Applied Insect Repellents  
Public Meeting

Tuesday June 19, 2007  
One Potomac Yard  
2777 Crystal Drive  
Arlington, VA. 22202

Docket telephone: 703-305-5805  
Docket Number: EPA-OPP-2007-0474

Organizers

Clara Fuentes, OPP/BPPD; Kevin Sweeney, OPP/RD;  
Candace Brassard, OPP/FEAD, and Michael McDavit, OPP/BPPD

Speakers

Daniel Strickman (ARS.USDA), Ulirich Bernier (ARS.USDA),  
Kendra Lawrence (DOD), Niketas Spero (ICR), Matt Kramer (ARS.USDA),

- 9:00 a.m. Registration  
9:30 a.m. Welcome  
9:40 a.m. Introductions  
  
9:45 a.m. Purpose/Objective of the workshop  
  
10:00 a.m. Presentations  
  
10:00 a.m. *“Statistical Comparison of in Vitro Efficacy Tests”*  
by Dr. Daniel Strickman (20 minutes)  
  
10:20 a.m. Questions  
  
10:30 a.m. *“Repellent Testing at the USDA-ARS Center for Medical,  
Agricultural, and Veterinary Entomology”*  
by Dr. Ulrich Bernier (20 minutes)

Types of bioassays- invitro and invivo methods

Human systems are relevant and accurate

What causes some variation- skin effects- some of physical effects –

Next slide- some of the abiotic factors- how they reared- cages sizes and shape-

Variability- attractiveness with humans-

Lab data shows differences between species

Can we use animal host instead of humans- probably not-

Questions:

Preselection of females is a good idea- and will standardize the data

Does help minimize the variability in the data

Biting pressure- is important –

100 to 2000 mosquitoes have been used in studies

150 taking blood meal can be uncomfortable-

What size is the cage size- repellent 38X 38X 38

All different sizes- but this is the the one they use

Cage size does impact

10:50 a.m. Questions

11:00 a.m. Break

11:10 a.m. *“Military Repellent Testing”*  
by CPT Kendra Lawrence, Ph.D. (20 minutes)

Military repellent testing- primarily field testing- personal opinion-

Soldier is the typical consumer –

Relative protection is important –

Percent reduction in landings – is that military uses-

Landing with intent to bite- indigenous population- not interested in indigenous population

Field testing is compared to DEET – 95% efficacy

1 mosquito per landing – 20 minute landing testing-

1 landing per minute

Variability- person., location, weather, variable in the field

Good success so after-

Another study in September

Data transformation- standard models- standard regression analysis- doesn't work as well as they would like to see it work-

GLMM model is a way to account for person- account for time-

This field testing – has the statistical power – not perfect- but does have the power-

Number of days of participants can't be guaranteed

Issues of compensation- what type for risk to test subjects-

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Always to compare against the standard military repellent –

Going through IRB- explain the risks to all test subjects-

Malaria- dengue fever, risk communication to volunteer

Adequate monitoring and follow-up – negative control can work-

Questions;

Testing 4 products – 5 repellents- 4 candidates plus DEET- positive control  
Card and a repellent- one day 2 day 3 day

Show up for certain times- then tells use which repellent we use on that person-

Treatment on leg- separate person has a positive control  
Below the knee to the ankle- 1.5 grams of product for the area- 1 to 1.5 grams per person-

One leg untreated and one leg treated – per person-

4 or 5 people- one supervisor-  
Limited experience – they are able to aspirate for each leg- can put in each cup-

Once they collect 20 mosquitoes on their leg- their challenge is over-  
First confirmed bite- as soon as first landing=  
Legs are covered- rubbing the compound on pants???

4 compounds:  
2 ARE PICARIDIN – 2 ARE in 3535 –

SELECTED BASED ON WHAT IS AVILABLE TO THE MILITaRY –  
Same amount and crème- one to 1.5 grams –

Spray can use one gram- historically what EPA recommends

EP has not registered products themselves-

Control the legs and the repellent to see if there was an any carryover- volatile-  
would there be a way to see that in the study-  
Area repellent-

May be a risk that we are not taking into account-

,most repellents do not seem to have a long range- she doesn't think there is a difference-

ICR- just did 5% citronellas a product-  
No effect on negative control-  
Citronella did not show a difference-

Controls per product many be variability-

Tests are designed to show no effect- NOEL- would take an extreme difference to see an effect-

1.5 grams were used to allow for difference in rate misapplication- did not weight the gloves after application- she just errs on the high end-

Within tat range-

Goes with high range

Did it provide an adequate coverage- can go a long way- seems to provide adequate coverage-?

Camouflage pain- can see that you have very good coverage- with that product-

Enough repellent at lower level?? May not have been adequate- working with legs-hairs-

In Belise they do not have gender differences- it is primarily males being testing- women stay home and take care of their children- women are not allowed to be in that type of work-

Males can be large enough volunteer pool-

Rule

11:20 a.m. Questions

11: 30 a.m. “*Mosquito repellent Testing for Industry by a Contract laboratory*”  
by Niketas Spero (20 minutes)

In vitro blood filled wells- citrated bovine blood  
Maintained at human temperature to the body

Apply membrane and the mosquitoes can penetrate-

Probing rate for every 2minuytes for up to 25 minutes

**Human subjects are paid**

**ICR has been compliant with IRB since 1995- informed consent protocol- IRB is concerned with ethical treatment of our subjects-**

**In vivo lab studies- apply at 1.67 mg/cm<sup>2</sup> as recommended by EPA-**

**Monitor for bites for 5 minutes- 2 people per cage –  
25 mosquitoes to 1000 numbers of mosquitoes are disease free- and are deprived of sucrose before the study**

**Consent forms are filed out- typically evaluating compounds up to 8 hours  
Each cage will be evaluated by untreated control arms- then number of landings will be recorded on control-  
First confirmed bite and is followed by second bite within th30 minutes**

**Stable flies are also recorded – they are painful  
2 products per test subject –**

**Individual variation is true – based on their experience**

**Test subjects- suits/fully clothed- number of landings are recorded-**

**Landing rates for spatial repellency**

**Field trials after lab studies- to validate**

**More challenging – previously EPA was recommending field studies-**

**Oregon, MN, TX, FL, VA- MD, depending on species – location will be dependant on –**

**Filed sites are selected on history – like to work with mosquitoes abatement districts- can tells us in advance-**

**May need alternative site – if there is a problem- 5 inches of rain- to occur over the weekend-**

**Field trial- can be rained out- the costs of spending an extra day- is cheaper than scheduling another study-**

**How to apply in the field- syringe without the needle-**

**Control and evaluate pest pressure- number of landings per one minute for every hour-**

Mosquito abatement district indicating that there were midges- but then got there- did not find any- need to have site approval for multiple sites-

First confirmed bite- is their measurement –

Had tested in the pasted continuous exposure

Break down for 8 hours –  
Slide 26

2 controls for 5 minutes

Monitor

Draft report for sponsor-

customers- if efficacy is sufficient for registration- then they will submit to the Agency- the registrant will do that- not ICR

Evaluate 1-4 days for a particular study- for each compound- more than one day-

11:50 a.m. Questions

12:00 p.m. Lunch

1:15 a.m. Matt Kramer (20 minutes)

Matt Kramer-

First part of talk- modeling- data collected by BARC

Second part of talk – is going to be on questions – Uli brought up by correspondence  
Modeling when you know how many mosquitoes there are- Lab testing- or hut and houses – known number of mosquitoes that can bite- binomial distribution- binomial response variable-

Some proportion of that bite-

Left side of equation- type of model- on predict on how many will bite-

Frame work Matt uses- 2 types –

Fixed

Random-

Fixed- which compound the person is getting-

**Temperature-**

**Random effects are – sampling the population that is available-**

**Want to be as wide as possible- do not have time to test every person-**

**Levels are random-**

**This is important for statistical theory- so you can estimate random effects-**

**Result of experiment apply only to people**

**Block effects- each day is a sample only a couple of samples of days- need to understand –**

**Random effects- nested in volunteer**

**Error- is also a random effect-**

**Response variable-**

**Individual mosquito- or group of mosquitoes-**

**Preferred- logit transformation-p represents the true number-**

**Probit and logit are almost identical –**

**Logit can be handled by a lot of software-**

**Parameters are affected by these effects- repellents if they are going to work- will affect the binomial distribution and will affect the number of bites-**

**Steep slope- effective-**

**Shallow slope- not effective-**

**Interaction between effectiveness of treatment and concentration is critical-**

**Typical computer software –**

**If incomplete block design the you need more individuals for testing-\**

**Let say you ignored the effect of the volunteer –the result of – you get an open dispersion-**



If you don't have independence and if you say hinges are different and they are really and you just ignored effects that really do count- to make sure you do not have over dispersion-

What concentrations should be used for a dose /response- for ED50 EDA 95- need to figure out where you want to estimate things?

At least 10 – preliminary analysis

Mimic effect that is happened to that curve-

Low concentrations that have no effect-

Better study if have larger number of mosquitoes-

SAS is proc limit- ARP can also be used- can fit the same models-

Field studies-

N is number of unknown- not know hoe many mosquitoes there are-

N is large-

Random effect- generally need number of subjects: 30 people are needed to address variance issues-

Variances are much more difficult than means- to estimate –

Best design – is the use the same volunteer repeatedly- block by days-

Always need to sue concentrations where mosquitoes are biting-

Assume distribution is different- posson distribution-

X2 tests do not take into account of the p values- ignore the day to day variation- time of day and people to people variation- p values will be wrong- need to right model-

Is there a difference between the 2 compounds?

PT? FCB- need to have large number-

After dan's talk- should not test same mosquitoes- so changed his mind- different mosquitoes-

Some alternatives-

Mean of certain number of bites- maybe 5 – square root of N-

Cut the standard error in half-

Assuming mosquitoes act independently-

Power should be easy to calculate- to estimate how good your results are going to be –

Software- SAS or R estimate generalized linear mixed models- does not give you the right P;s or the right confidence intervals-

Each person should be there own control- only in the case where you do not know N-

If you do not N – then you do not know biting pressure-

Biting pressure per person-needed-

If all mosquitoes are coming from the same pool-

Difference from day 1 to day 3 there is so much variability-

100 mosquitoes to 300 mosquitoes- need to standardize things-

How do you compare those things-?

You want to make things as similar as possible going in- for active repellents- do not want to look for differences in populaitons of mosquitoes- want to assume there is no difference-

If that is true than statistics is cleaner- if not then you are adding noise-

Will have non-responders- but might have fewer percentage that is not responders-

1:35 a.m. Questions

1:45 a.m. Session is open for general discussion

