Statistical Comparison of Laboratory Tests

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What is a “Repellent”

- The mode of action of various active ingredients is not the same
- Considering all active ingredients that stop biting as “repellents” has confused nomenclature and research
- Active ingredient that alters biting behavior might better be called a “phagomone”
- Product used by public to stop biting better called a “repellent product”

Mosquito Preparation

- Some species adapt to host animal in a few generations
- Provide sugar or not
  - Probably species dependent
  - Best to follow biology (e.g., starve *Ae. aegypti*, not *Culex quinquefasciatus*)
- Do not withhold water
- Almost never previously blooded
- Larval nutrition and density have big effects on avidity
- Age affects avidity
- Number of mosquitoes and density in cage affect avidity
- Diel patterns to feeding, even in lab
- Preselection for avidity
- Single exposure to AI can alter behavior for long period

Host Models

- Human models
  - Common to have 4x variation in subjects
  - Systematic gender differences documented
  - Systematic racial differences not known
  - Many test systems:
    - External cage (cage on arm or leg)
    - Arm in cage
- Animal models
  - Not currently popular
  - Limited studies showing correlation with human data, but overall encouraging
  - Duration correlated to humans, but not the same
  - Avidity of colony to animal species

Two Kinds of Measurements

- Inherent repellency
  - The minimum mass or molar concentration per surface area that has the desired effect
  - Does not necessarily predict duration because of loss from volatility, absorption, or ablation
- Duration of effectiveness
  - The time between activation and decrease in effect below minimum standard
  - Activation not always same as application

Inherent Repellency

- Compares biting inhibition at various dosages
- Assumes no loss of AI following application, therefore conducted soon after application
- AI usually not formulated but solvents vary
- Only realistic method for *in vitro* tests because no way to model loss of AI from surface
- Based on experience can set threshold at $ED_{90} \leq 0.01$ mg/cm^2
- Useful for prioritizing candidate compounds

Duration of Effectiveness

- Field testing and consumer experience are only realistic measures of individual product, but nearly impossible to compare studies for ranking of products
- Mosquito avidity varies over time, much better to apply at intervals, test simultaneously
- Mosquitoes per cage and density makes a big difference in avidity
- Challenge is to reduce experimental error by decreasing variation in mosquito or tick source and in procedure

Application Rate

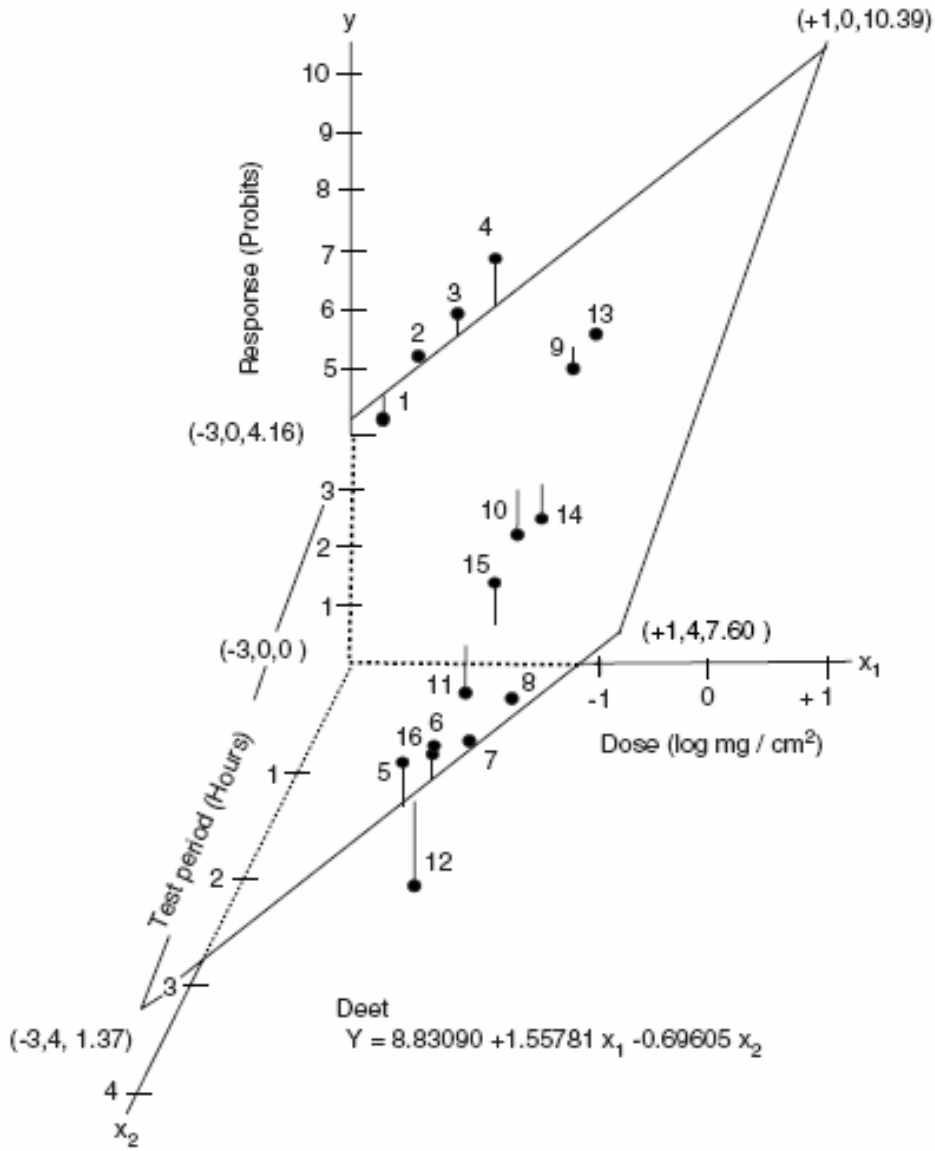
- Must be on per area basis
  - Can translate old data based on assumptions
  - Advantage of external cage models, but edge effects
- Moles logical chemically, but complicate translation to practical application
- Product formulation influences likely application rate

Percentage Efficacy Observed

- Highly dependent on number of potential biting arthropods in test
- If only 10 in a cage, then first bite lowers efficacy from 100% to 90%
- If 200 in cage, then first bite lowers efficacy from 100% to 99.5%
- If number of potential biting arthropods not reported, then assume 95% at first bite

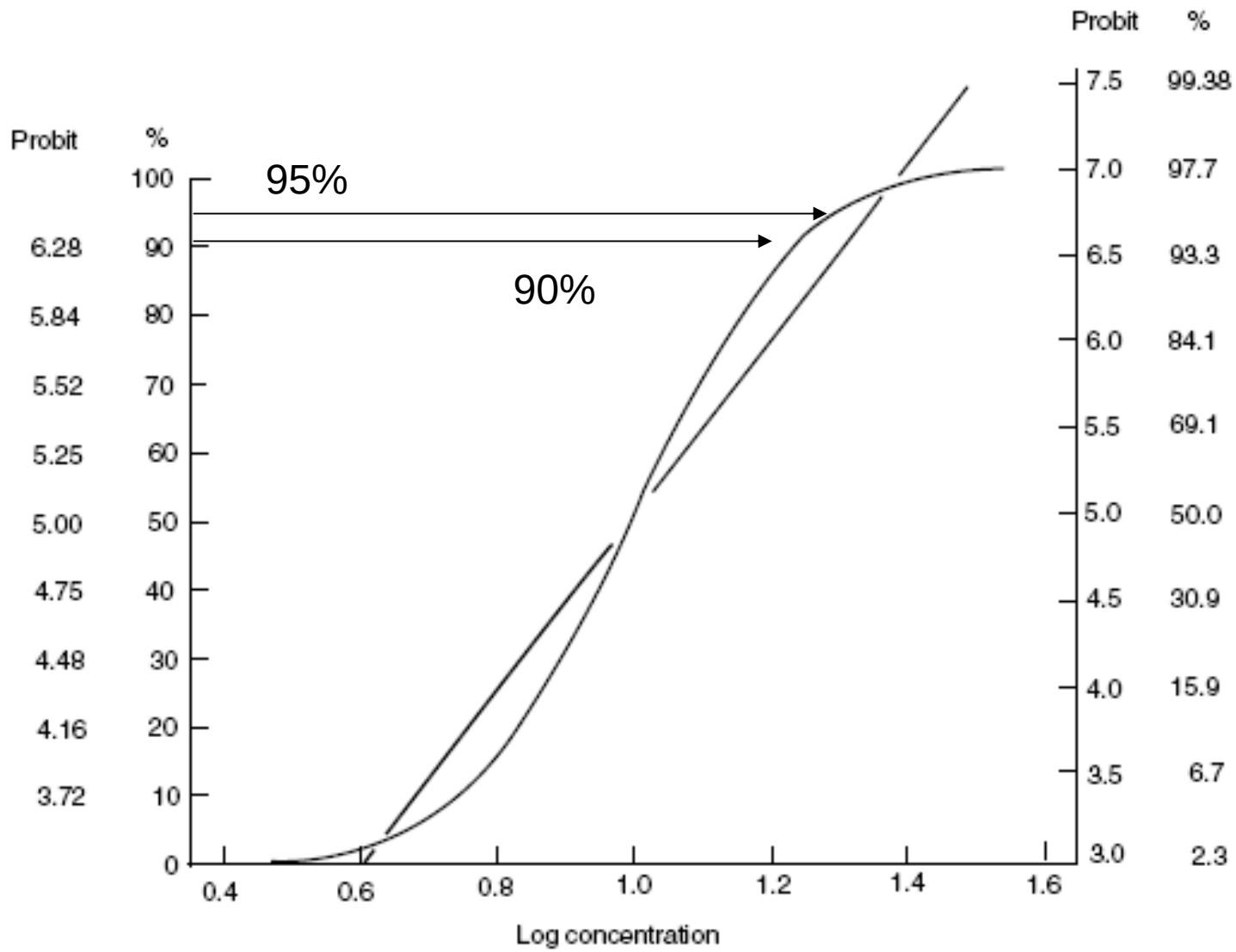
Probit Regression

- Probit defined: “inverse cumulative distribution function of the normal distribution”
- If test stopped at first bite, then impossible to do probit regression.
- If test stopped at first bite, translate percentage protection at first bite to standard percentage protection by probit extrapolation
- If test proceeded to greater failure rate (ideally at least 50%), then possible to perform proper regression with error rate


y
(+1,0,10.39)
10
9
8
7
6
5
Response (Probits)
4
3
2
1
13
9
(-3,0,4.16)
3
2
1
10
14
15
(+1,4,7.60)
(-3,0,0)
11
8
-1
0
+ 1
x_1
Dose (log mg / cm^2)
16
6
7
5
1
2
12
Test period (Hours)
3
(-3,4, 1.37)
4
x_2
Deet
Y = 8.83090 +1.55781 x_1 -0.69605 x_2

What is “Best” Percentage Protection

- Inherent statistical accuracy of probit curve is optimum at ET_{50}
- ET_{95} gets into top, curvy part of sigmoid curve
- ET_{90} is closer to linear portion of sigmoid curve
- ET_{90} is more realistic appraisal of efficacy


Probit
%
100
90
80
70
60
50
40
30
20
10
0
6.28
5.84
5.52
5.25
5.00
4.75
4.48
4.16
3.72
95%
90%
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Log concentration
Probit
%
7.5
99.38
7.0
97.7
6.5
93.3
6.0
84.1
5.5
69.1
5.0
50.0
4.5
30.9
4.0
15.9
3.5
6.7
3.0
2.3

Comparison of 10% and 30% AI Formulations

- Standardizes comparisons at typical low dose and high dose formulations
- Compare 10% against threshold of one-hour of protection
- Compare 30% against threshold of two-hours of protection
- Compares active ingredients, not products

Net Result

Study's % translated to ET_{90} by probit ratio; translated to standard AI % by log ratio

TABLE 22.1

Efficacy of Dimethyl Phthalate (DMP) Measured by Duration of Protection Against a Variety of Biting Arthropods

Lab	Field	Species	Method	Control	Dose	Result	10% Calc.	30% Calc.
	x	*Haemaphysa zeylandica*	Human	14–22/h	0.25	1.5	1.2	2.8
	x	*Haemaphysa zeylandica*	Human	14–22/h	0.5	2.5	1	2.4
x		*Panstrongylus megistus*	Rabbit, ec	10	1.54	2.2	0.3	1.2
x		*Aedes aegypti*	Arm in cage	100	0.385	0.7	0.4	1
x		*Aedes aegypti*	Arm in cage	NS	0.25	1.1	0.8	2.1

Summary Recommendations

- Ignore inherent repellency, use duration
- Quantify application
  - Preliminary study of actual application rate
  - Standardize tests at mean application rate (consider mean minus 10%)
- Standardize strain, rearing, and preparation of test arthropods; preselect for avidity; do not reuse specimens
- Use 50/50 gender ratio of subjects; stagger application times and test simultaneously
- Replicate with same subjects on different days
- Arm in cage, 100 mosquitoes per cage
- Sixteen tests: 30 minute intervals for 8 hours
- For new study, calculate ET_{90}
- For existing study, calculate ET_{90} from reported percentage protection using probit ratio