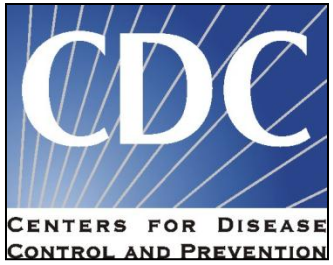
Tactical Insecticide Resistance Surveillance with the Bottle Bioassay

Bill Brogdon, Ph.D.
and
James Dunford, Ph.D.

Centers for Disease Control and Prevention



Insecticide Resistance

The primary goal of resistance surveillance is the measurement of resistance:

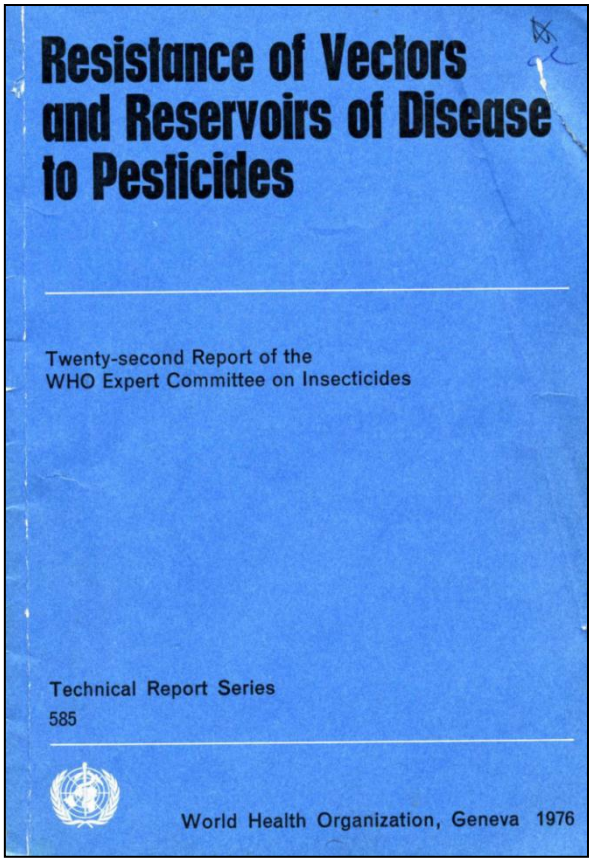
As it exists…

At a particular place…

At a particular time.

Insecticide Resistance

- Over thirty years ago, WHO recognized insecticide resistance as “the greatest single obstacle in the struggle against vector-borne disease and is mainly responsible for preventing successful vector control in many countries”.

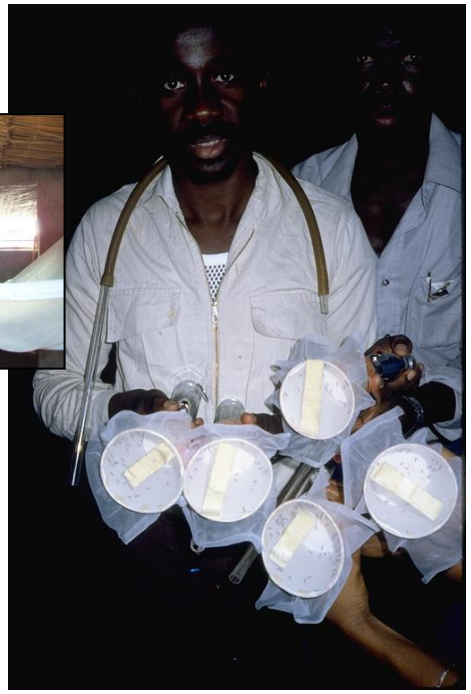


Insecticide Resistance

- Resistance is not a new phenomenon.
- By 1954, malaria vector resistance was known to be a problem world-wide.
- WHO world-wide malaria eradication program began in 1955, resistance was recognized by some to be a fatal obstacle to eradication by 1958.
- “Eradication” became “control” in the late 1960s.
- Resistance has never been more important in mosquito vector control than it is today and it will only be more important tomorrow.
- The success of programs to control malaria and dengue are absolutely dependent upon effective vector control, to include resistance monitoring.

Our resistance surveillance data are incomplete. Why is this so?

Collecting these data is a lot of work…



Insecticide Resistance

- Species diversity presents challenges in resistance detection and assessment.

For example:

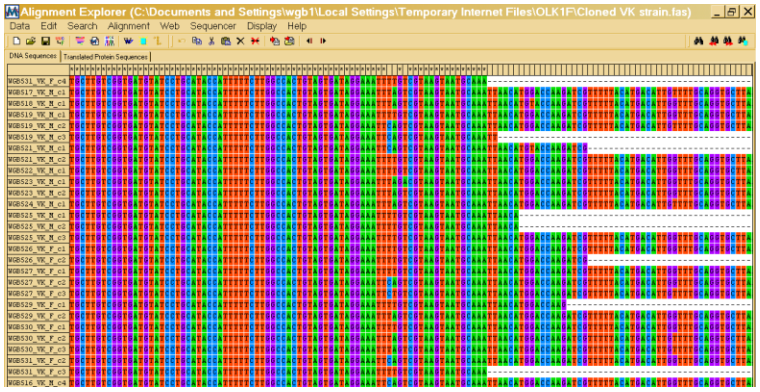
- Over 400 *Anopheles* species (100 potential malaria vectors)
  - *Anopheles funestus* complex: 11 Species
  - *Anopheles gambiae* complex: 8(?) species, 6 chromosomal types

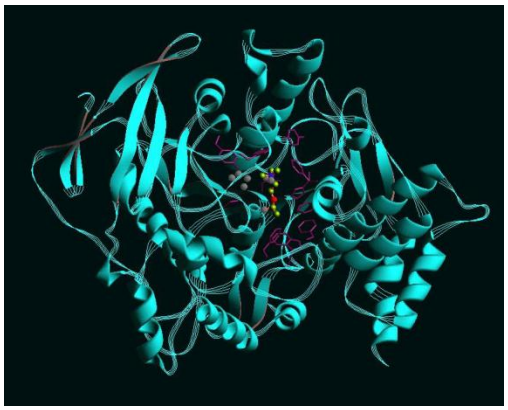
Insecticide Resistance

- Poor spray techniques
- Insecticide or net quality
- pH and hard water mixed with insecticides-deactivate insecticides (spray and wash water)

Insecticide Resistance

- Three types of resistance genes in vectors:
  - Multiple copy genes (esterases, oxidases)
  - Upregulated genes (oxidases,GSTs)
  - Point mutations (kdr, insensitive ACHE)





Insecticide Resistance

Pyrethroids

- **Kdr**
- Glutathione s-transferase
- **Esterases**
- **Oxidases**
- Insensitive acetylcholinesterase

Insecticide Resistance

Organophosphates

- Kdr
- Glutathione s-transferase
- **Esterases**
- Oxidases
- **Insensitive acetylcholinesterase**

Insecticide Resistance

Carbamates

- Kdr
- Glutathione s-transferase
- Esterases
- **Oxidases**
- **Insensitive acetylcholinesterase**

Insecticide Resistance

- What is the most appropriate technology for detecting resistance?

Primary tactical question(s):

- Will this formulation of this insecticide (or this bed net) control this vector at this location at this time?
- If not, what do I do now?

Bioassays

Bioassays are the Gold Standard

- SIMPLE
- PRACTICAL
- CHEAP

WHO Bioassay

CDC Bottle Bioassay

Bioassays

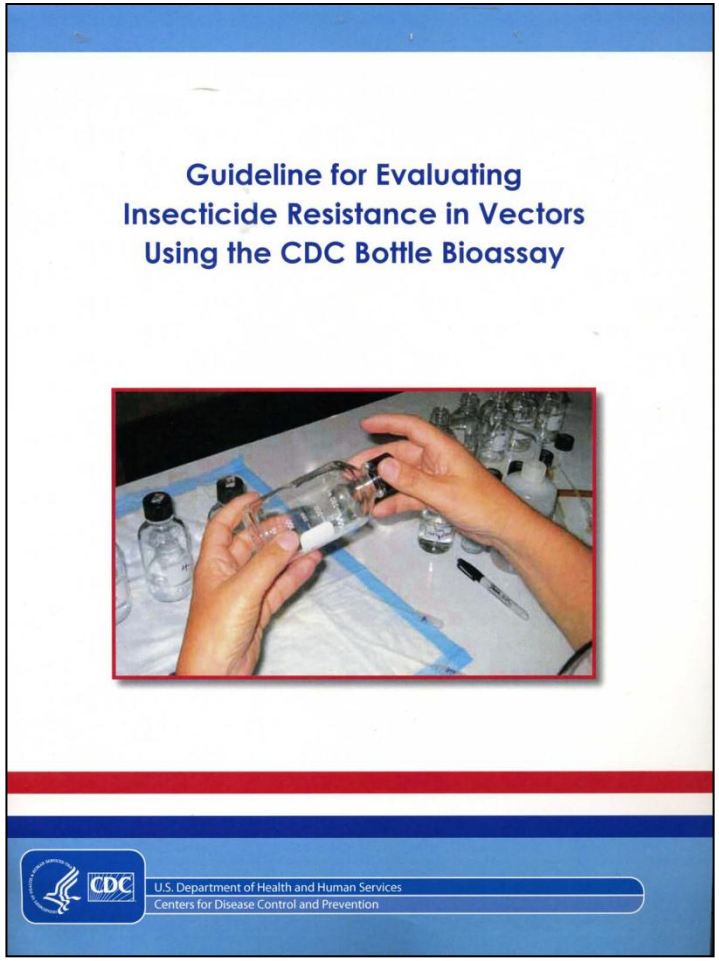
- WHO assays seek to reproduce an insecticide exposure in the field where mosquitoes rest on a surface for a brief period of time.

- With CDC technique, only the toxicological response is measured – insecticide time to reach its target and render the mosquito unable to stand.

2009. Malaria Journal. **Adaptation and evaluation of the bottle assay for monitoring insecticide resistance in disease vector mosquitoes in the Peruvian Amazon.** Elvira Zamora Perea, Rosario Balta León, Miriam Palomino Salcedo, William G Brogdon and Gregor J Devine.

CDC Bottle Bioassay

- Directly measuring a single critical toxicological parameter:
  - The time required for an insecticide to reach and interact with the target, disabling the insect.
  - This time increases in the presence of resistance mechanism(s).



CDC Bottle Bioassay

Surveillance-Response Tactics

- Establish baselines
- Periodic testing of vector populations
- Identify resistance mechanism(s)
- Correlate changes with control efficacy
- Change control strategy when data indicate
- First step in standardizing CDC bottle bioassay is determining diagnostic times and doses for regional susceptible populations

CDC Bottle Bioassay

Established diagnostic doses and times

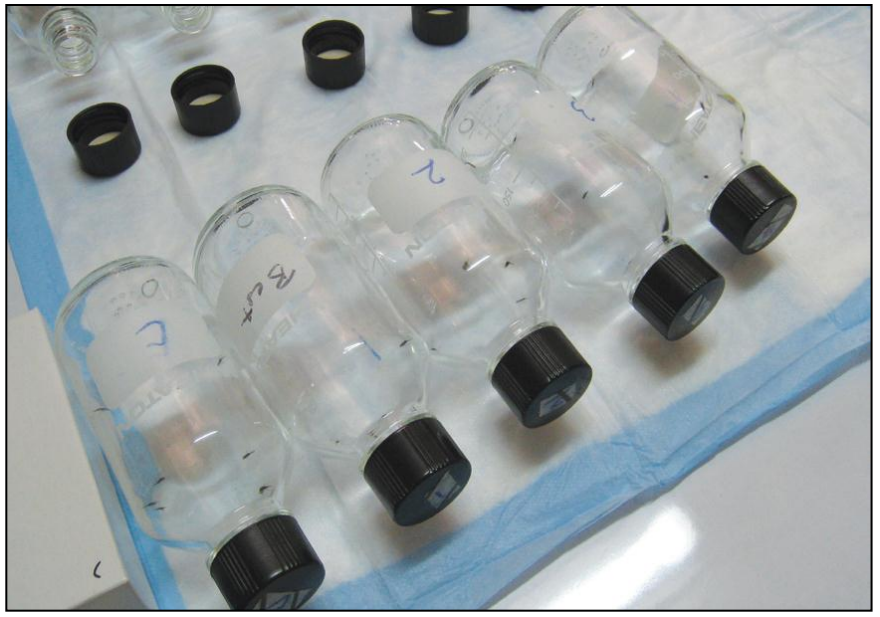
Table 1: Sample diagnostic doses and diagnostic times for *Anopheles* and *Aedes* mosquitoes.

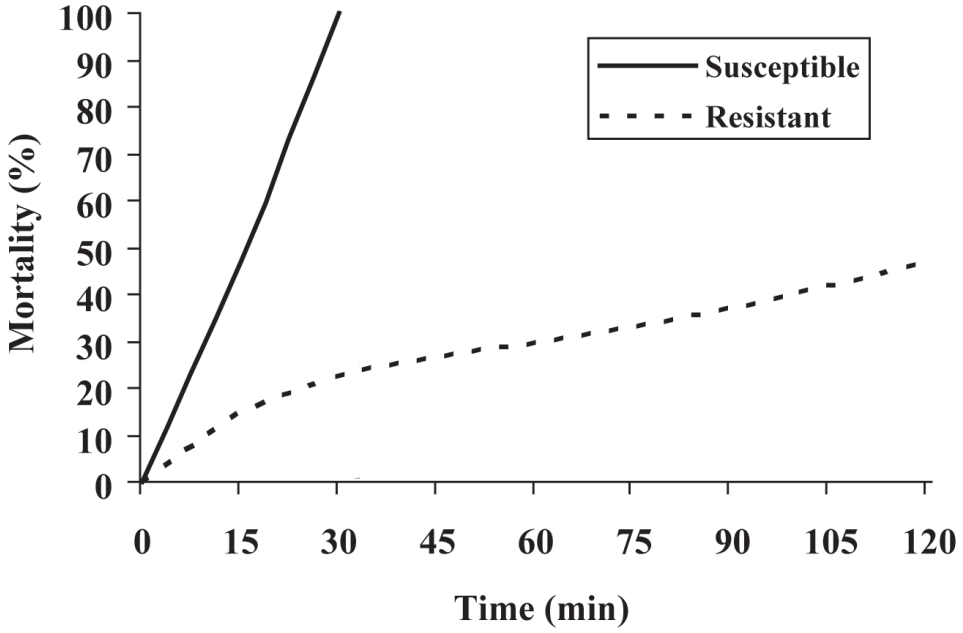
Insecticide	Insecticide concentration per species (µg/bottle)		Diagnostic time (minutes)
	Anopheles	*Aedes*	
Bendiocarb	12.5	12.5	30
Cyfluthrin	12.5	10	30
Cypermethrin	12.5	10	30
DDT	100	75	45
Deltamethrin	12.5	10	30
Fenitrothion	50	50	30
Lambdcyhalothrin	12.5	10	30
Malathion	50	50	30
Permethrin	21.5	15	30
Pirimiphos-methyl	20	—	30


ETHYL
ALCOHOL

CDC Bottle Bioassay

- Stock Solution:
  - 12.5 milligrams in 1 liter acetone or ethanol
  - For each bottle: 1 milliliter stock = 12.5 ug
  - Does 1000 bottles
- For formulations, calculate amount based upon active ingredient
  - For example:
    - Reagent grade (100 % active): use 12.5 milligrams
    - 10% formulation: use 125 milligrams
  - Milligrams reagent grade divided by % in formulation
- Reasons for using formulations :
  - Access and cost
  - Acceptability of results
  - Allows evaluation of adjuvants




100
90
80
70
60
50
40
30
20
10
0
Mortality (%)
Susceptible
Resistant
0
15
30
45
60
75
90
105
120
Time (min)

CDC Bottle Bioassay

Box 5: Interpretation of data for management purposes.

WHO recommendations for assessing the significance of detected resistance:

- 98%–100% mortality at the recommended diagnostic time indicates susceptibility;
- 80%–97% mortality at the recommended diagnostic time suggests the possibility of resistance that needs to be confirmed;
- <80% mortality at the recommended diagnostic time suggests resistance.

Note: Where <95% mortality occurs at the diagnostic time in bioassays that have been conducted under optimum conditions and with a sample size of >100 mosquitoes, then resistance can be strongly suspected.

- If resistance is detected, what mechanisms are causing resistance?
  - Biochemical/molecular analyses (microplate assays, PCR etc.)
  - Synergist assays: synergists act to abolish apparent resistance by working on specific resistance enabling enzymes (e.g., PBO inhibits oxidase activity)

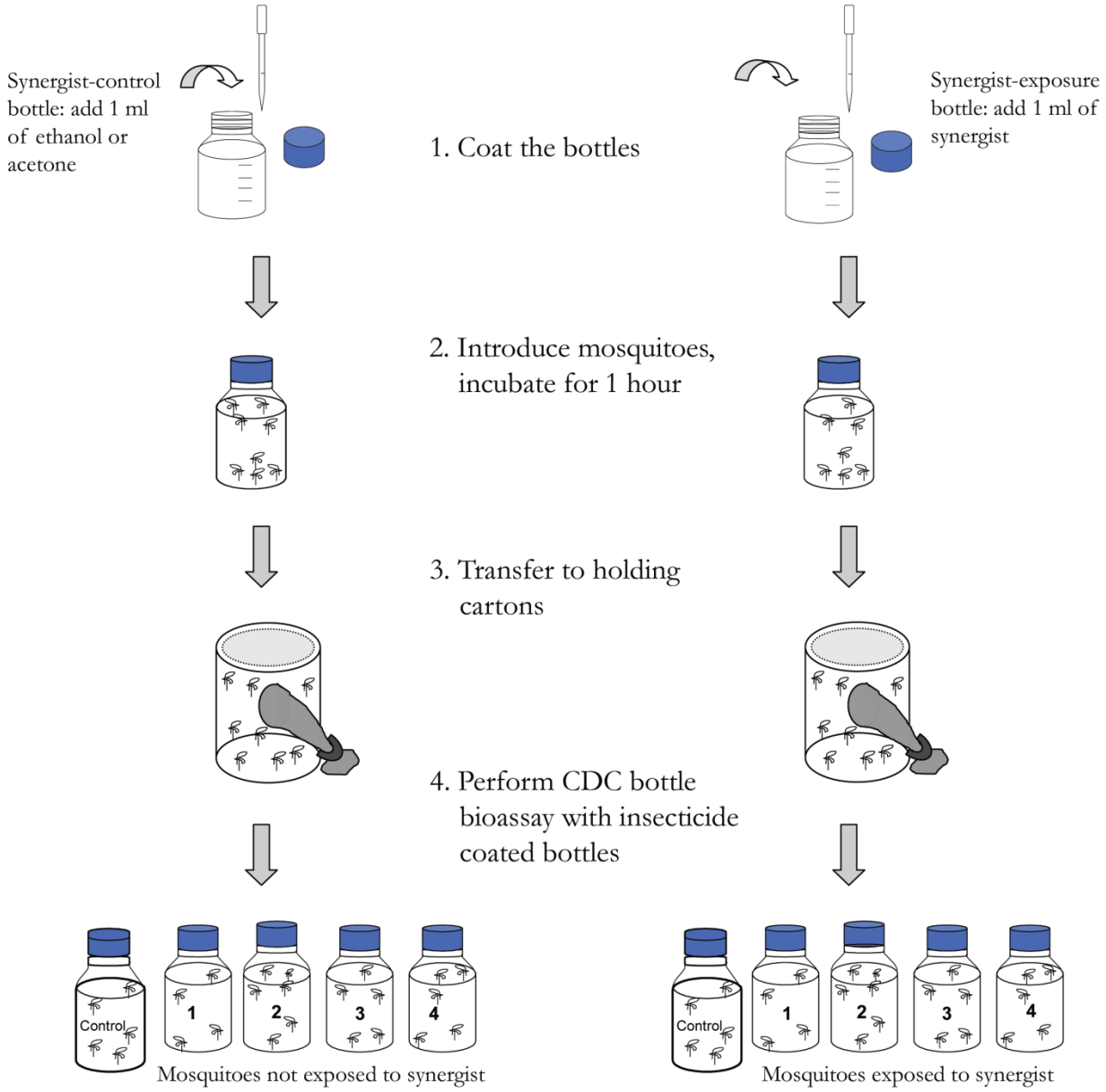

Synergist-control bottle: add 1 ml of ethanol or acetone
1. Coat the bottles
Synergist-exposure bottle: add 1 ml of synergist
2. Introduce mosquitoes, incubate for 1 hour
3. Transfer to holding cartons
4. Perform CDC bottle bioassay with insecticide coated bottles
Control
1
2
3
4
Mosquitoes not exposed to synergist
Control
1
2
3
4
Mosquitoes exposed to synergist

Fig. 10a.

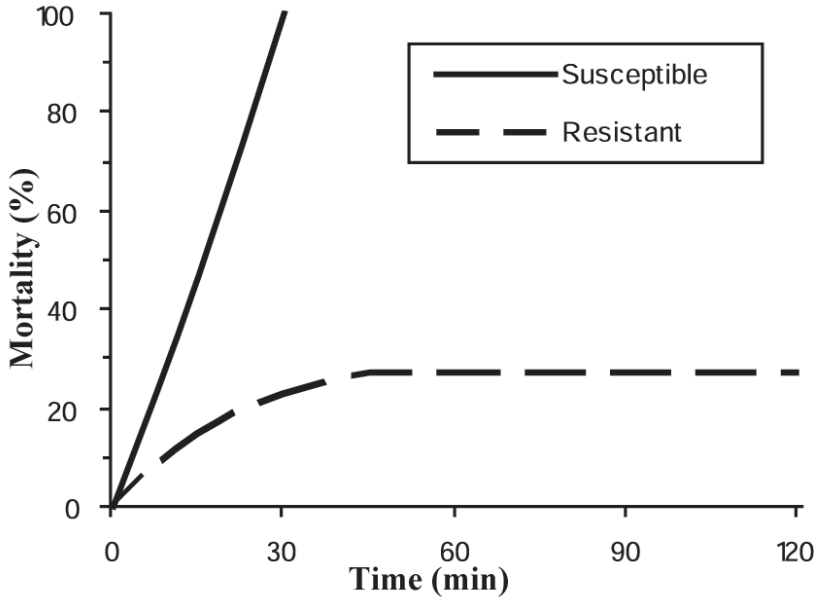
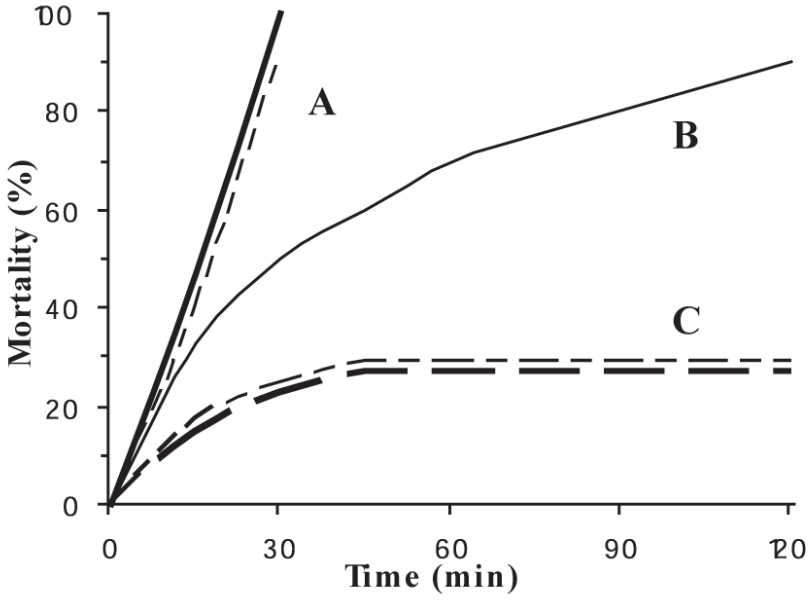


Fig. 10b.



Figures 10a and 10b. Effects of synergists on resistant vector populations.

Figure 10a shows data for a population of resistant vectors compared to a susceptible population.

Figure 10b shows the three possible outcomes of synergist exposure:

Line A: Resistance to the insecticide is abolished

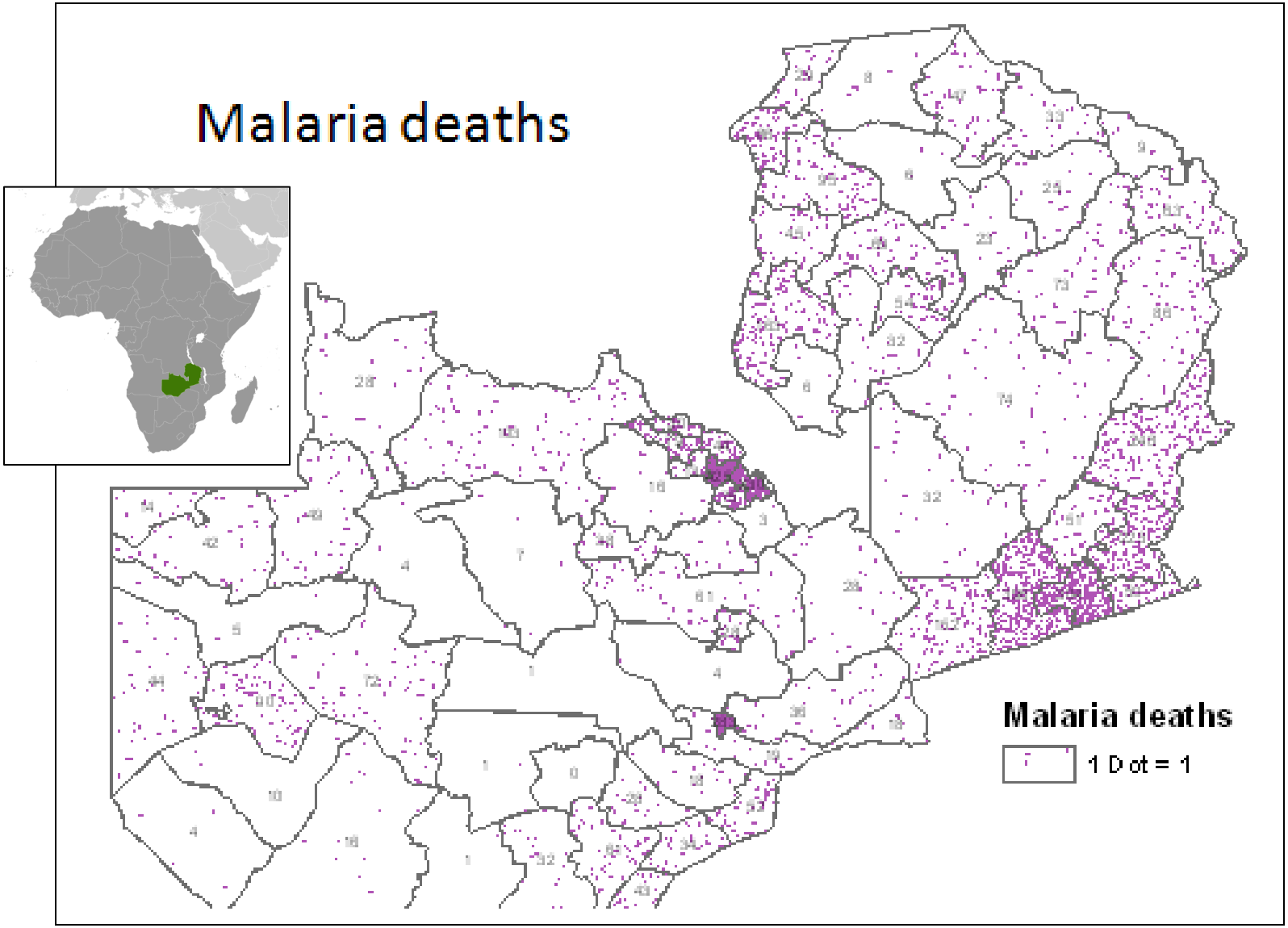
Line B: Resistance to the insecticide is partially abolished

Line C: Resistance to the insecticide is unaffected

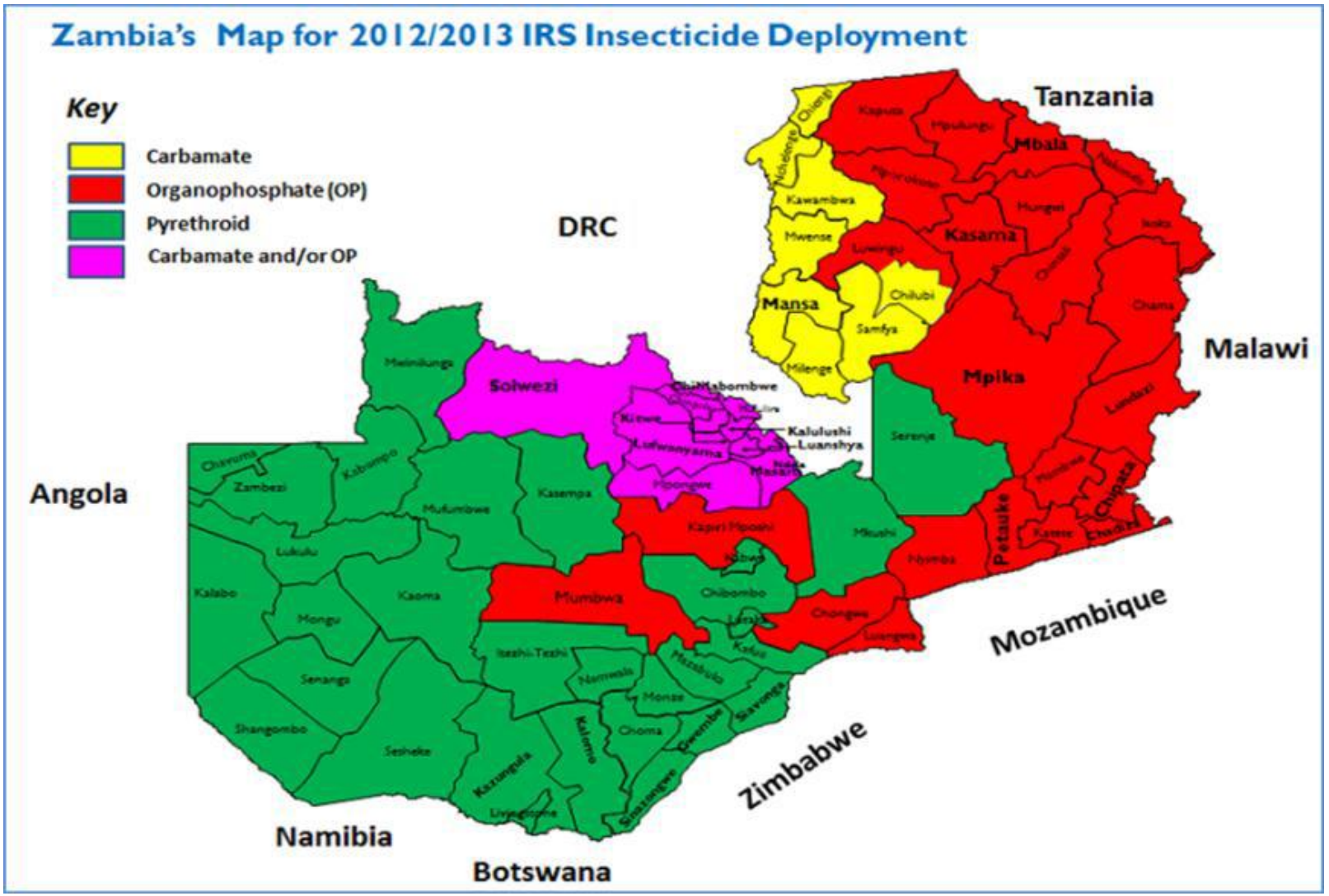
CDC Bottle Bioassay

- Resistance **INTENSITY**
- Resistance intensity bioassay
- Standard bioassay protocol
- Bottles treated with:
  - Dosages in diagnostic dosage multiples
    - e.g., 1X, 2X, 5X, 10X
- Can also perform after synergist exposure
- Protocol to be included in future editions of manual

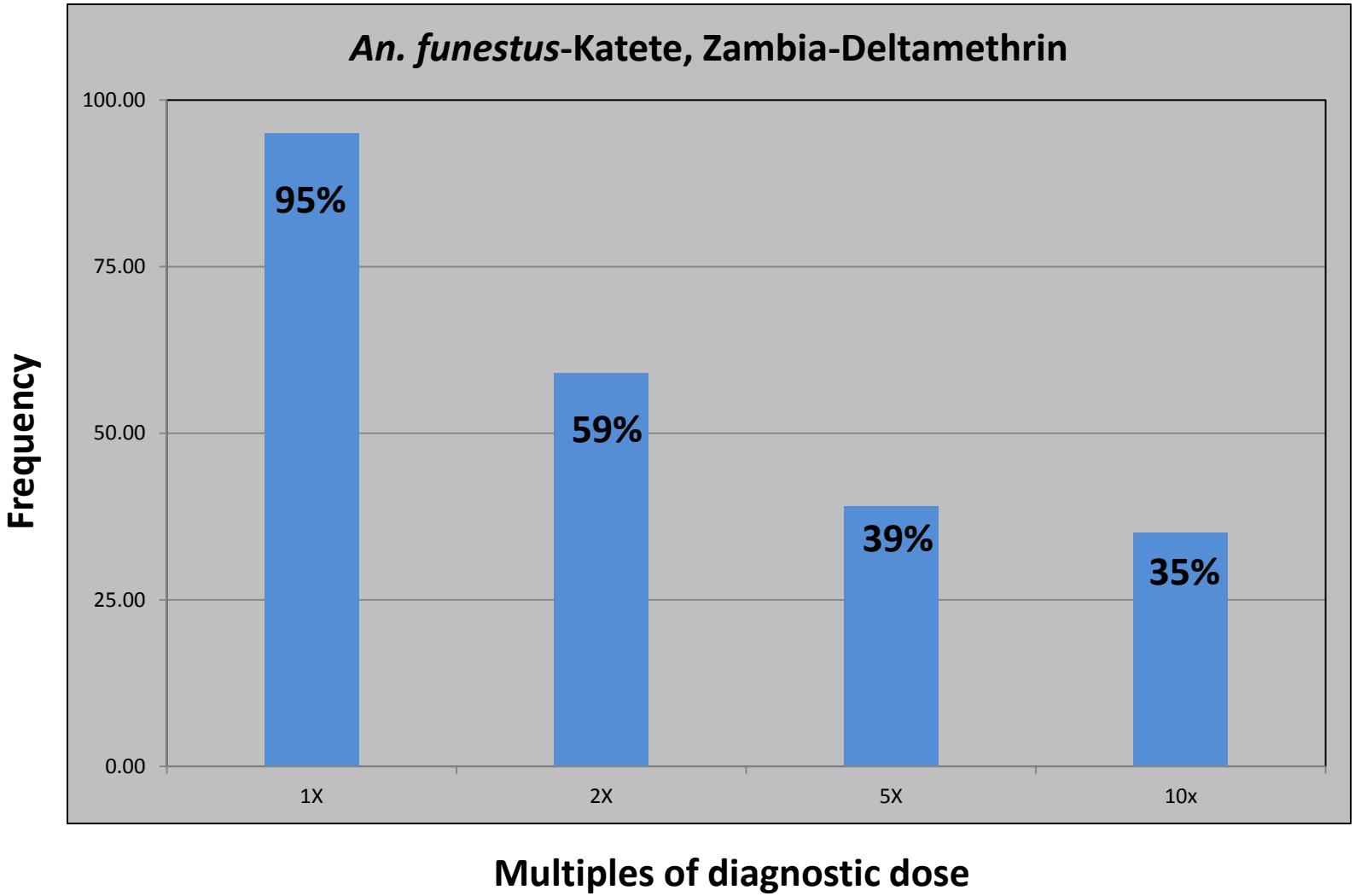
Case Studies



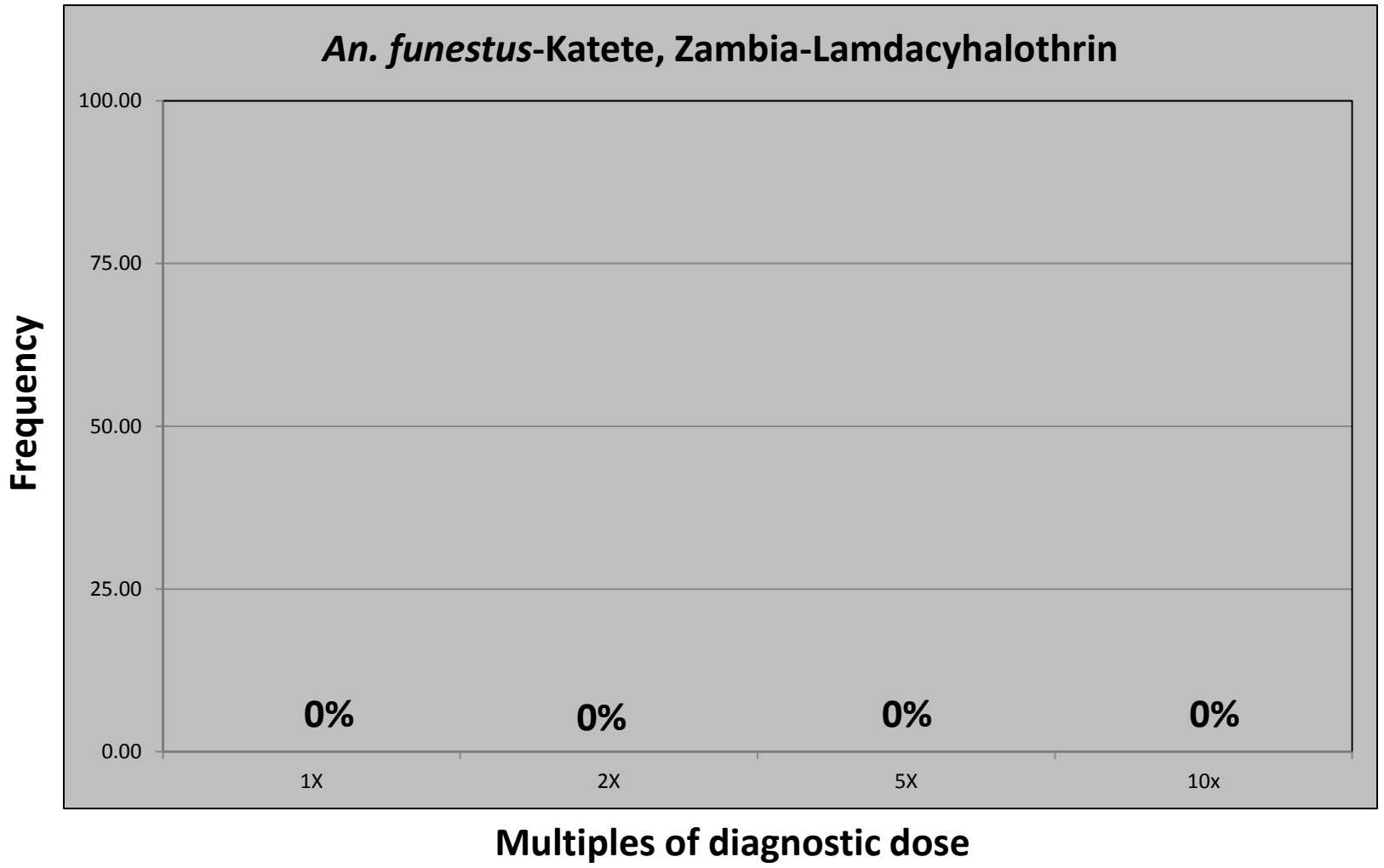
Case Studies



Case Studies



Case Studies



Case Studies

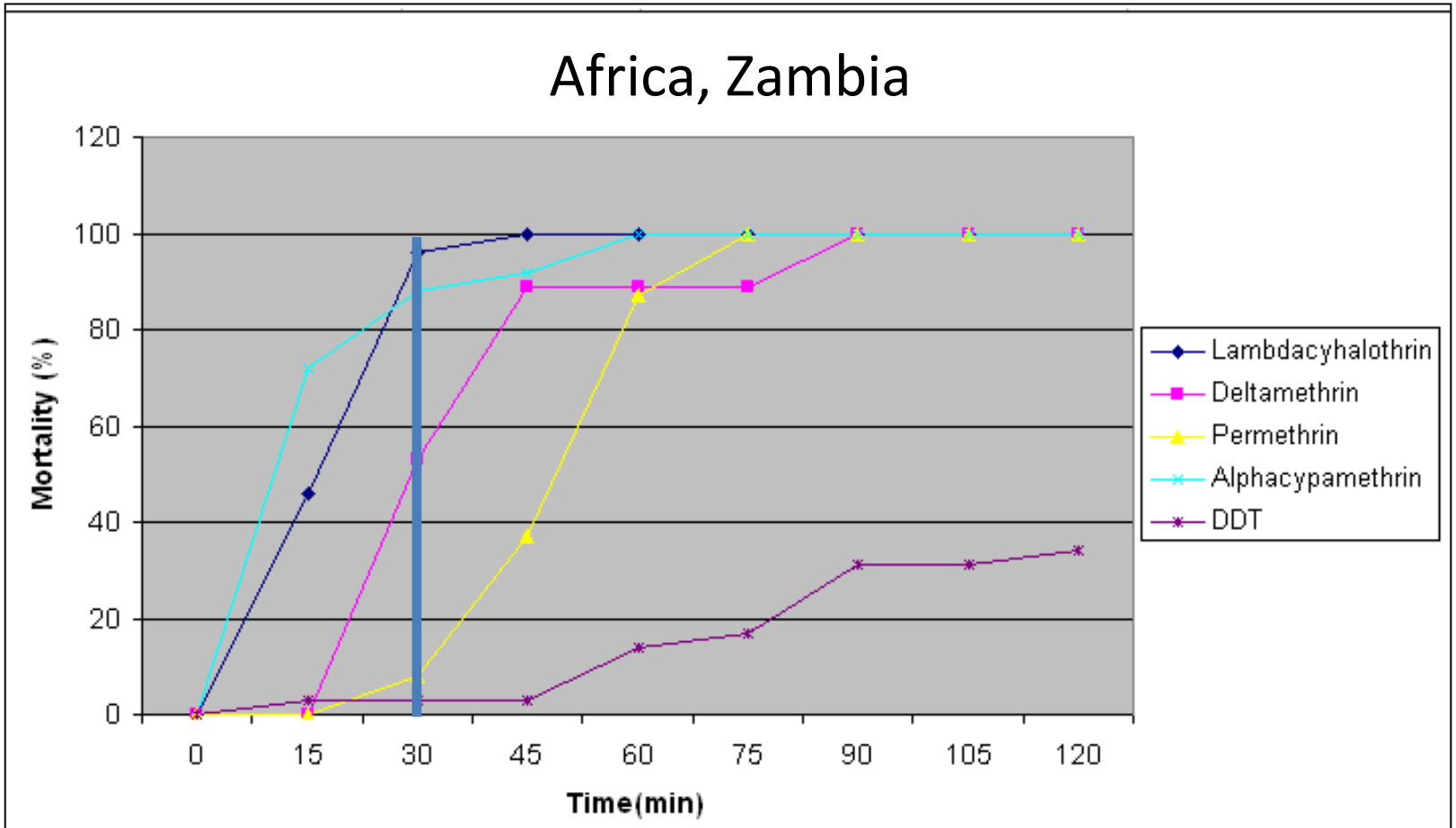
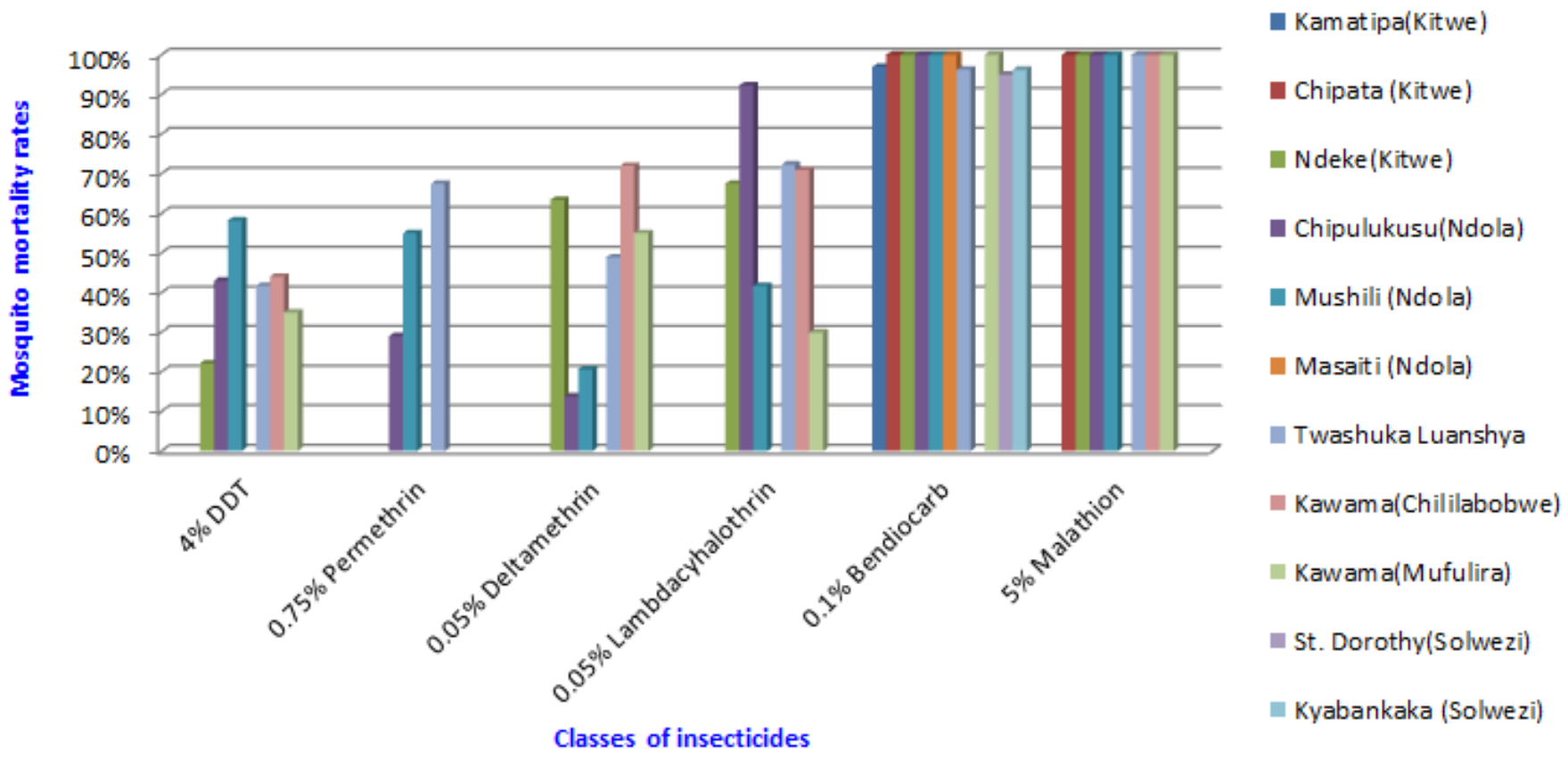


Fig 1. Exposure time vs Mortality(%) for *An gambiae* s.s from Mushili-commando

Case Studies

Africa, Zambia

Fig 1: Percentage mortality in field sample of *An. gambiae* s.l 24 hours after a 1-hour exposure to inscetcide impregnated papers in WHO test tubes.



Discussion

- Managing resistance:

  IPM Approach

  - Rotation of chemical classes
  - Time application of chemicals
  - Mixtures or combinations
  - Mosaic application of insecticides to provide refugia
  - Periodically monitor susceptibility/resistance using bioassays***

Discussion

- Resistance issues in malarious countries/Africa well documented
- How is resistance management being addressed in the U.S.?
  - Need for U.S. species diagnostic times/doses; e.g., *Culex* etc.
- Politics and economics of vector control here and abroad may differ
- Budgets and authority to implement IPM strategies/resistance monitoring

Resistance Training

- CDC PMI entomologists conduct resistance training overseas (Ivory Coast, Kenya, Zambia, Nigeria, Senegal, Tanzania)
- Currently 8 day resistance monitoring course in Ethiopia
- Offer training at CDC on bottle bioassay (contact me (VIT1@CDC.GOV) or Bill Brogdon (WGB1@CDC.GOV)

On-line Information

- Bioassay Feature: http://www.cdc.gov/malaria/
- Education and Training (including the permanent home of the bioassay): http://www.cdc.gov/parasites/education_training/
- If your institution or program would like to order a bottle bioassay kit, which contains bottles, insecticide formulations, manual, and instructional video, please contact CDC to discuss the collaboration at bottleassay@cdc.gov. To order only the bottle bioassay insecticide resistance formulation, please send your request to bottleassay@cdc.gov. The formulations are free.

QUESTIONS?