Field Application of the Intensity Bottle Bioassay

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Insecticide resistance

“a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species” - Insecticide Resistance Action Committee

- Contributed to failure of disease control programs
  - Global Malaria Eradication Programme (1955-1969)
  - Resistance of headlice to permethrin/organophosphates
Types of resistance

- **Metabolic**
  - Esterases
  - Oxidases
  - Glutathione S-transferases

- **Target-site mutation**
  - Sodium channel (kdr)
  - Acetylcholinesterase (Ace1<sup>R</sup>)
  - GABA

- **Other**
  - Cuticular thickening
  - Behavior change

Wood et al. 2010, Parasites & Vectors
Resistance monitoring

- Detection of resistance mechanisms (genotypic)
  - Genotyping (target site mutations)
  - Biochemical analysis (metabolic resistance)
  - Semi-field/field data (behavior change)

- Bioassays (phenotypic)
  - Larval bioassays
  - Topical application
  - Timed exposure (WHO cone test, Bottle bioassay)
Bottle bioassay
## Diagnostic time

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

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Insecticide concentration per species (µg/bottle)</th>
<th>Diagnostic time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anopheles</td>
<td>Aedes</td>
</tr>
<tr>
<td>Bendiocarb</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>12.5</td>
<td>10</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>12.5</td>
<td>10</td>
</tr>
<tr>
<td>DDT</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>12.5</td>
<td>10</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Lambda cyhalothrin</td>
<td>12.5</td>
<td>10</td>
</tr>
<tr>
<td>Malathion</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Permethrin</td>
<td>21.5</td>
<td>15</td>
</tr>
<tr>
<td>Pirimiphos-methyl</td>
<td>20</td>
<td>—</td>
</tr>
</tbody>
</table>
Synergists

- Piperonyl butoxide
  - Inhibits oxidase activity

- S.S.S-tributylphosphorotrithioate (DEF)
  - Inhibits esterase activity

- Ethnacrynic acid (EA), diethyl maleate (DM/DEM), and chlorfenethol (CF)
  - Inhibit glutathione S-transferase activity
Possible outcomes in bottle bioassays using synergists
Does resistance mean control failure?

• Sometimes

Medical and Veterinary Entomology (2000) 14, 181–189

Impact of DDT re-introduction on malaria transmission in KwaZulu-Natal

R Maharaj, D J Mthembu, B L Sharp

Objectives. To determine whether the re-introduction of DDT in KwaZulu-Natal had any effects on malaria transmission in the province.

Outcome measures. The notified malaria cases and the distribution of A. funestus were measured to determine the effects of DDT re-introduction on malaria transmission.
Does resistance mean control failure?

- Sometimes not

PROTECTIVE EFFICACY OF LAMBDA-CYHALOTHIRIN TREATED NETS IN *ANOPHELES GAMBIAE* PYRETHROID RESISTANCE AREAS OF CÔTE D’IVOIRE

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Abstract. The efficacy of nets treated with lambda-cyhalothrin, a pyrethroid insecticide, on malaria infection and disease was assessed for the first time at the community level in *Anopheles gambiae* pyrethroid resistance areas. The study was carried out in northern Côte d’Ivoire, which is an area of *kdr* resistance. Four pairs of villages were selected and matched according to demographic, sociological, and ecological criteria. Among each pair, a village was randomly allocated to receive mosquito nets. More than 80% of beds were covered with nets treated with lambda-cyhalothrin and retreated after 6 months. In each village, 54 children aged 0–59 months were randomly selected and clinically monitored for 8 periods of 7 days throughout the year. Results showed that the efficacy of treated nets was maintained with a reduction of the prevalence of asymptomatic malaria infection by 12% and an estimated protective efficacy against malaria disease of 56%.
How can we tell when resistance is having an effect?

Intensity assay
Mushili, Ndola – *Anopheles gambiae*

Deltamethrin

<table>
<thead>
<tr>
<th>Multiples of Diagnostic Dose</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1X</td>
<td>50%</td>
</tr>
<tr>
<td>2X</td>
<td>0%</td>
</tr>
<tr>
<td>5X</td>
<td>0%</td>
</tr>
<tr>
<td>10X</td>
<td>0%</td>
</tr>
</tbody>
</table>
Mufulira – *Anopheles gambiae*

**DDT**

- **1X**: 100%
- **2X**: 90%
- **5X**: 80%
- **10X**: 55%

**Frequency**

**Multiples of Diagnostic Dose**
Chipata – *Anopheles funestus*  
Deltamethrin  
February, 2012

- 1X: 36%  
- 2X: 38%  
- 5X: 8%  
- 10X: 6%

Frequency

Multiples of Diagnostic Dose
Chipata – Anopheles funestus
Deltamethrin May, 2012

Multiples of Diagnostic Dose

Frequency

0 25 50 75 100

1X 2X 5X 10X

36% 0% 0% 0%
Conclusions

- The bottle bioassay is a simple, standardized test which can detect resistance
- The use of synergists allows preliminary detection of the resistance mechanisms present in a population
- The intensity bioassay may be an effective way of determining “operationally significant” resistance
- These bioassays provide a sound basis for implementing resistance management strategies