Diagnostic Doses and times for *Phlebotomus papatasi* and *Lutzomyia longipalpis* sandflies using the CDC bottle bioassay to assess insecticide resistance

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### Introduction

Insecticide resistance to synthetic chemical insecticides is becoming a worldwide (Fig. 3) concern in phlebotomine sand flies (Diptera: Psychodidae), the vectors of *Leishmania* parasites (Fig. 1 & 2). The CDC bottle bioassay assesses resistance by testing populations against verified diagnostic doses and diagnostic times for an insecticide, but the assay has been used limitedly with sand flies. The objective of this study was to determine diagnostic doses and diagnostic times for laboratory *Lutzomyia longipalpis* (Lutz and Nieva) and *Phlebotomus papatasi* (Scopoli) to ten insecticides, including pyrethroids, organophosphates, carbamates, and DDT, that are used worldwide to control sandflies (Fig. 1).

### Methodology

Bioassays were conducted in 1000-ml glass bottles each containing 10-25 sand flies from laboratory colonies of *L. longipalpis* or *P. papatasi* (Fig. 4). Four pyrethroids, three organophosphates, two carbamates, and one organochlorine were evaluated. A range of concentrations were tested for each insecticide, and four replicates were conducted for each concentration. Diagnostic doses were determined only during the exposure bioassay for the organophosphates and carbamates. For the pyrethroids and DDT, diagnostic doses were determined for both the exposure bioassay and after a 24-hour recovery period.

### Principal Findings

<table>
<thead>
<tr>
<th>L. longipalpis</th>
<th>P. papatasi</th>
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<tr>
<td><strong>Diagnostic dose (µg/mL)</strong></td>
<td>6</td>
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<tr>
<td><strong>Diagnostic time (min)</strong></td>
<td>40</td>
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*Table 1. Diagnostic doses and times for bendiocarb (carbonate) and fenitrothion (organophosphate) to L. longipalpis and P. papatasi sand flies.*

*Lutzomyia longipalpis* and *Phlebotomus papatasi* sand flies are both highly susceptible to the carbamates, such as bendiocarb, as their diagnostic doses are under 7.0 µg / ml (Table 1, Fig.5). Both species are very susceptible to DDT during the exposure assay as their diagnostic doses are 7.5 µg / ml, yet their diagnostic doses for the 24-h recovery period are 650.0 µg / ml for *L. longipalpis* and 470.0 µg / ml for *P. papatasi*.

### Conclusions/Significance

Diagnostic doses and diagnostic times can now be incorporated into vector management programs that use the CDC bottle bioassay to assess insecticide resistance in wild populations of *L. longipalpis* and *P. papatasi* (Fig.6). These findings provide initial starting points for determining diagnostic doses and diagnostic times for other sand fly vector species and wild populations using the CDC bottle bioassay.

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