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# Assessing Insecticide Resistance in Adult Mosquitoes: Perspectives on Current Methods

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**ABSTRACT:** Mosquito insecticide resistance (IR) is a growing global issue that must be addressed to protect public health. Vector control programs (VCPs) should regularly monitor local mosquito populations for IR and plan control measures accordingly. In some cases, state/federal resources financially support this testing with expertise and/or training programs. Standardization of methods (eg, Centers for Disease Control and Prevention bottle bioassay, World Health Organization tube testing, dose-mortality bioassay) for monitoring IR must be prioritized. One solution is regional hubs of IR monitoring at the state or other level. Training programs on methodology and interpretation of results should be developed and routinely offered to local VCPs conducting IR testing in mosquitoes. Here, current methods for assessing mosquito IR are discussed and insights into a variety of questions from VCPs are considered. It is critical that methods for IR monitoring and data interpretation are standardized through routine training, with the goal of evidence-driven decision making to improve control of mosquitoes and mosquito-borne disease.

**KEYWORDS:** CDC bottle bioassay, mosquito, insecticide resistance

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## Introduction

Mosquito-borne diseases remain a global public health issue and effective vaccines and treatments are often unavailable. Nevertheless, these diseases are preventable, as avoiding mosquito bites and/or controlling potential vectors using insecticides remain a primary avenue for disease reduction as part of integrated mosquito management (IMM).<sup>1</sup> However, control failures could be due to insecticide resistance (IR), improper insecticide application procedures, and/or other factors and this is underreported, in part, due to limited resources.<sup>2</sup> It is essential that mosquitoes are routinely and effectively monitored for IR to inform control decisions, as the specific reasons for mosquito control failures, or inefficiencies, need to be understood and corrected.<sup>1,3</sup> Despite the knowledge of IR, few vector control programs (VCPs) monitor this as part of their plans to protect the public from mosquito-borne disease.<sup>3</sup> Insecticide resistance emerges from multi-generational selection from exposure to sublethal doses of insecticides.<sup>4</sup> In each population of insects, some individuals have alleles for resistance to insecticides, possibly from interactions with plant allelochemicals.<sup>4,5</sup> Alleles for IR are selected when the population of insects is exposed to insecticides, ultimately fixing the alleles in the insect population, and resulting in the failure of chemical-based control.

Mosquitoes are exposed to insecticides through various sources such as: government (public) VCPs, private pest control (household/urban/commercial), homeowner application, and/or agricultural applications (using multiple active ingredients [AI] and formulated products [FP]).<sup>2,6-9</sup> Adulticide exposures

are likely more prevalent from agricultural and household/urban sources, compared to applications by public VCPs, depending on target species.<sup>10,11</sup> Active ingredients currently registered for use as mosquito adulticides in the United States (US) include organophosphates (eg, malathion, naled, chlorpyrifos) and pyrethroids (eg, permethrin, sumethrin [d-phenothrin], prallethrin, deltamethrin, etofenprox, and pyrethrins). Etofenprox lacks the ester bond found in most pyrethroids (it has an ether bond instead) and is sometimes called a pseudo-pyrethroid. Mechanisms of IR have been identified as primarily target site mutations (knockdown resistance) and increases in insecticide metabolism,<sup>9</sup> although other interacting mechanisms are possible and are not discussed in detail here.

Currently, public health investigators are exploring the option of insecticide co-formulations containing more than 1 AI with different modes of action<sup>9</sup> that could potentially be used in areas where mosquitoes are showing cross resistance to different insecticide classes. Some FPs include synergists that are not, themselves, fatal to mosquitoes, but can effectively increase the potency of an AI. Synergists are designed to improve efficacy of pyrethroid AIs (eg, synergist piperonyl butoxide [PBO] inhibits enzymes [eg, oxidase] that detoxify pyrethroids), organophosphate AIs (eg, synergist S.S.S-tributylphosphorotrithioate inhibits enzymes [eg, esterase] that detoxify organophosphates), or more generally improve the ability of several AIs to cause mortality in mosquitoes (eg, synergist diethyl maleate inhibits enzymes [eg, glutathione transferase] that detoxify several insecticides).<sup>12</sup> Alternative methods for mosquito control that do not use insecticides are also being



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developed (eg, *Wolbachia* infection, sterile insect techniques) but are currently not widely used and will not be covered here.<sup>13</sup>

A 2017 survey by the National Association of County and City Health Officials (NACCHO) was sent to 1,906 VCPs in the US (1048 respondents, 57% response rate) identified by Centers for Disease Control and Prevention [CDC], American Mosquito Control Association (AMCA), and NACCHO in an attempt to identify the capabilities of VCPs.<sup>14</sup> While the specific VCPs surveyed were anonymized in the report, 50 states were represented in the survey results. The same survey identified IR testing as the most significant competency that needed to be addressed in VCPs due to either lack of training and/or capacity to carry out testing.<sup>14</sup> Of 1048 programs with respondents answering the survey question specifically about IR testing, only 14% indicated conducting any type of IR testing, although no details were given about the type and/or frequency of testing carried out.<sup>14</sup>

The Worldwide Insecticide Resistance Network was formed in 2016 and is currently a group of 19 international partners (<https://win-network.ird.fr/>). This network encourages collaboration between countries experiencing IR to improve awareness of this global issue.<sup>2,8</sup> This network suggests 5 foundations needed to develop and implement an IR monitoring program: (1) surveillance (eg, monitoring), (2) research (eg, basic and applied), (3) management (eg, risk assessment, decision making), (4) innovation (eg, partnerships to develop new tools for assessment), and (5) support (eg, communicating results to advocate for funding).<sup>3</sup>

The Innovative Vector Control Consortium (<https://www.ivcc.com/>) began in 2005 to develop new insecticides, new formulations/combinations of existing insecticides, and/or plans for rotating between AI with different modes of action to improve mosquito control for malaria vectors.<sup>15</sup> Among other tasks, this Consortium develops and implements tools to help with decision making and disseminating information to communities. The Consortium works on issues of IR in malaria vectors and neglected tropical diseases associated with mosquitoes and other types of insects.

In some cases, it may be beneficial to assess IR in both FPs and AIs. It is necessary to calibrate bioassay conditions to determine diagnostic doses (DDs) and diagnostic times (DTs) for FPs and AIs using susceptible mosquitoes prior to testing field mosquito populations. The DD is the insecticide dose that causes 100% mortality in a susceptible mosquito population within a certain time period (the DT). The dose applied in the field is listed on the product label (available online for all registered products), usually given in a range from low to high application rates. As most FPs include synergists and other ingredients, it may not be possible to directly compare DD and DT for technical grade AIs and the associated FP that includes the AI. Furthermore, there are some FPs that may contain ingredients impacting mosquito behavior that may not be reflected in a laboratory bioassay. For example, Duet™ contains 2 pyrethroid AIs (prallethrin + sumethrin) and a synergist

(PBO). In this case, prallethrin agitates mosquitoes from resting areas and sumethrin (plus PBO) improves overall population mortality with an increased likelihood of contact with airborne droplets.<sup>16</sup> This behavior response is not directly assessable through a bottle bioassay. Another case, such as Fyfanon™, uses pure malathion (no synergists) in the FP, although other ingredients may be present that would not be found in the technical grade AI. It is also important to note differences between IR monitoring and biological effectiveness testing that may be used in different situations.

It is not fully understood how laboratory assessments of IR correlate to field populations under a variety of biological and environmental conditions, hence caution is advised when interpreting laboratory data for making operational field decisions. This is particularly an issue with a simplified assay such as the CDC bottle bioassay.<sup>17-19</sup> Although this assay is kinetic and multiple timepoints are evaluated, the protocol does not call for evaluation of sublethal and/or delayed effects. This is a significant limitation of the assay. Understanding the lab-to-field connections for IR are critical for operational VCPs and public health officials.<sup>20</sup> Modeling may be a tool used to understand the larger system of IR and this should be evaluated. Initially, implementing IR testing in VCPs may be costly, but long-term benefits in mosquito-borne disease control and preservation of AIs that can be used effectively outweigh the initial costs.<sup>21</sup> This perspective article examines the currently accepted methods for assessment of IR in mosquitoes. We use specific examples to demonstrate some key points; however, the information here may be applied to additional insecticides and mosquito populations/species.

## Comparative analysis of current methods of resistance testing

*Centers for Disease Control and Prevention*



CDC bottle bioassay.  
Image credit: J. Balanay.

Centers for Disease Control and Prevention bottle bioassays were developed to assess IR in any species of insect, including mosquitoes.<sup>1,17,19,22</sup> In this bottle bioassay, adult female mosquito mortality is measured at different time intervals over a 2 hour period. Up to 25 treatment mosquitoes (in each of 4 replicate bottles) are exposed to the interior of each glass bottle that contains residue of a technical grade (pure) AI or FP that may contain additional ingredients to enhance effectiveness (stock usually made in acetone). Control mosquitoes from the same population are exposed to bottles that have previously been coated with acetone (the acetone evaporates, leaving a “clean” surface). In treatment bottles, the longer an insecticide takes to kill a mosquito, the more likely the mosquitoes are to be resistant or developing resistance to the insecticide. Diagnostic doses and DTs for each AI or FP are determined before conducting the CDC bottle bioassay by testing baseline mosquito populations that are susceptible to the insecticide. The DD and/or DT may vary between different mosquito species and populations.<sup>17,18</sup> The CDC lists starting point DDs and DTs for 10 AIs (bendiocarb, cyfluthrin, cypermethrin, DDT, deltamethrin, fenitrothion, lambda-cyhalothrin, malathion, permethrin, pirimiphos-methyl) to test populations of *Anopheles* and *Aedes* mosquitoes (species not given) in bottle bioassays, but stresses that DDs and DTs for mosquitoes from different geographic regions would need to be determined.<sup>17,18</sup> In the CDC bottle bioassay procedure,<sup>17</sup> the (now former) WHO recommendations for assessing IR are used: susceptible is 98% to 100% mortality at DT; possible development of resistance is 80% to 97% mortality at DT; resistant is <80% mortality at DT.

In 2016, additional guidelines for classification of IR using the CDC bottle bioassay were published<sup>21</sup> that focus on assessment of IR in only *Aedes albopictus* Skuse and *Aedes aegypti* L. The CDC now uses the following updated and more stringent guidelines to assess resistance: susceptible is  $\geq 97\%$  mortality at DT; possible development of resistance is 90 to 96% mortality at DT; resistant is < 90% mortality at DT (CDC 2016). Additional CDC guidelines were published in 2019 that clarified this would also apply to all mosquito species in the continental US.<sup>18</sup> This protocol/manual is sent to VCPs that request free IR test kits from the CDC (<https://www.cdc.gov/dengue/mosquito-control/insecticide-resistance.html>). Testing kits provided to VCPs by CDC also include lyophilized insecticides of a predetermined dose (depending on AI) and glass bottles; however, the user must create the insecticide stocks and coat the bottles when ready for use. If the user requires a certain DD that is more dilute than what is provided in the kit, it may be necessary for the user to conduct dilution calculations before preparing insecticide stocks.

#### *World Health Organization pesticide evaluation scheme*

The World Health Organization Pesticide Evaluation Scheme (WHOPES) provides guidelines primarily related to control of malaria (*Anopheles* spp.) vectors, for example, risk assessment for

indoor residual spray and bed nets, as well as guidelines for items such as laboratory/field testing of bed nets, and spatial repellents.<sup>9</sup> The WHO describes a cone bioassay where mosquitoes are exposed to treated fabrics (ie, bed nets) for 3 minutes below a plastic cone.<sup>23</sup> In the same assay, mosquitoes are transferred to clean cages post-exposure and mortality is assessed after 1 hour and 24 hours. The WHO also has a tube test where insecticide treated material (eg, bed net, clothing, or filter paper) is placed around the circumference of the tube before mosquitoes are introduced.<sup>9</sup> In this case, 20 to 25 mosquitoes are introduced into each of 6 replicate tubes. After a 1 hour exposure period, mosquitoes are transferred to clean cages and mortality is assessed after 1 hour and 24 hours (the protocol specifies observing mosquitoes for a longer period for slow-acting compounds). Like the CDC bottle bioassay, mosquitoes that cannot fly, but may still be moving, are considered dead. The WHO has centralized units for production and supply of resistance testing materials (eg, pre-treated filter papers for use in tube tests) by the Vector Control Research Unit, University of Malaysia that helps provide uniformity between tests ([https://www.who.int/malaria/areas/vector\\_control/WHO\\_test\\_kit\\_catalogue\\_and\\_requisition\\_form\\_may2013.pdf?ua=1](https://www.who.int/malaria/areas/vector_control/WHO_test_kit_catalogue_and_requisition_form_may2013.pdf?ua=1)). These test kits are provided at a cost to the user, depending on what supplies are requested. Since the filter papers come pre-treated with insecticides, this method does not require the user to handle liquid insecticide stocks, thus reducing handling and measurement variability and decreasing the likelihood of exposures due to spills. However, if different insecticide concentrations are needed other than what is provided by the WHO, the user may need to create stocks and treat filter papers themselves. Current WHO recommendations to assess resistance are: susceptible is  $\geq 98\%$  mortality at 1 hour and/or 24 hours; possible development of resistance is 90 to 97% mortality; resistant is <90% mortality.<sup>9</sup>

#### *Dose-mortality bioassay*

Another insecticide bioassay includes exposure of mosquitoes to a variety of insecticide doses to determine the dose (lethal concentration [LC]) that kills 50% (LC<sub>50</sub>) or 95% (LC<sub>95</sub>) of mosquitoes.<sup>24,25</sup> In this type of assay, a susceptible (control) population is used to determine the resistance ratio (RR). The RR (LC<sub>50</sub> field population/LC<sub>50</sub> susceptible population) can be used to help monitor changes in resistance over time (ie, RR<5: susceptible or low resistance/tolerance; RR=5-10: moderately resistant; RR>10: highly resistant.<sup>23</sup> These types of bioassays can help determine resistance mechanisms that could be involved and, since multiple doses are used, can increase accuracy when interpreting susceptibility results in field populations.

#### *Other considerations for current methods of resistance testing (advantages, disadvantages)*

In field conditions, the tarsi of resting mosquitoes may contact foliage that has been treated with insecticides via residual

barrier sprays (this type of control approximates CDC bottle bioassay and WHOPEs conditions). Alternatively, some body parts of flying mosquitoes may come into direct contact with drifting droplets applied via ultra-low volume spray machines (most like caged field trial conditions) and this type of topical droplet contact is not assessed via CDC bottle bioassay or WHOPEs. Some VCPs conduct caged field trials to evaluate FPs; however, this is not always the case. Lab and field assessments of insecticide efficacy may have different purposes, and this should be considered on a case-by-case basis when deciding which type of assay to use. In either case, research studies are not an exact approximation of field conditions (eg, differences in weather conditions, mosquito age, resting time on foliage, insecticide application method, and other unknown variables) and this should be considered when interpreting data. This is particularly relevant as older parous mosquitoes found in the field are generally more likely to be infected/infectious and may be more susceptible to insecticides than lab-reared young nulliparous mosquitoes.

Another factor to consider is that the nature of data collection for the CDC bottle bioassay (constant exposure evaluated over multiple time points) and WHOPEs (brief exposure evaluated at 2 time points) is different. The dose-mortality assay ( $LC_{50}$ ,  $LC_{95}$ ) is more complicated than the CDC bottle bioassay and WHOPEs assays since it tests multiple doses, rather than a single DD that had been previously shown to kill 100% of a susceptible population.<sup>24</sup> These differences should be considered when evaluating and comparing results of different assays.<sup>26,27</sup>

Synergists (eg, PBO) can be used in CDC bottle bioassays and WHOPEs (prepared by user—not included in standard kit) to further assess mosquitoes that have been classified as resistant (<90% mortality at DD and DT). For example, if a mosquito population characterized as resistant to an AI is pre-exposed to PBO prior to CDC bottle bioassay is subsequently re-classified as susceptible, enzyme based (ie, monooxygenase) resistance may be involved.<sup>9</sup> If PBO does not “restore” susceptibility, there may be other resistance mechanisms involved.

### Current issues and suggestions

During the last 3 years, we have taught short courses on the CDC bottle bioassay technique for VCPs. These courses are typically 1 day long and involve both didactic and hands-on sessions for participants. During these sessions, we receive questions and comments relating to both thematic and specific limitations of IR monitoring. Below, we have grouped questions we have received into 6 categories to improve the IR evaluation process and help understand limitations of the current methods.

#### *Category #1: Need for a standard susceptible population*

We often get questions about the source of control mosquito populations for determining DD and DT. For instance, to

improve standardization, a susceptible *Ae. albopictus* population from North Carolina or California should not vary in the DD and DT for permethrin. It is appropriate to use baseline mosquitoes (used to establish DD and DT for each AI) that have been phenotypically characterized as susceptible. It is also appropriate to use baseline populations that have been enzymatically characterized prior to use to determine the degree of susceptibility to each class of insecticide. A single baseline susceptible population of each species of interest should be fully characterized using molecular techniques to assess different mechanisms of IR. The same population (for each species) should be used to establish (globally) universal DDs and DTs for all AIs and FPs. Then, these species-specific DDs and DTs should be used to assess field populations. This would take considerable work initially, but the information would be used by all VCPs assessing resistance.

#### *Category #2: Standardized source of AIs*

Questions about where to obtain AIs for bioassays are also common. A standard commercial source (ie, Sigma Aldrich, Chem Service) for technical grade AIs should be used to control quality of the insecticides used in bioassays, hence reducing variability in assay results between different laboratories. There may be variability (eg, quality, purity, age/handling-related degradation) between AIs and/or FPs purchased from different sources and this should be considered when comparing results between laboratories or even between years within the same laboratory. A standard chemical analysis (eg, gas chromatograph/mass spectrometry) should be required of chemicals prior to the bottle bioassay, if VCPs have this capability. Standard protocols for storage conditions (ie, refrigeration in amber colored containers), storage time (ie, 1 season), and handling of insecticide stock mixtures used in bottle bioassays should also be established. Similarly, bottle coating and washing procedures should be standardized to limit human error.

#### *Category #3: Diagnostic dose and diagnostic time*

We also receive questions about the wide variation observed in DD and DT for different AIs and FPs. In a CDC bottle bioassay, if it takes a 15  $\mu\text{g}/\text{mL}$  DD, 30 minutes DT for AI #1 and 400  $\mu\text{g}/\text{mL}$  DD, 30 minutes DT for AI #2, that does not necessarily mean AI #1 is more efficient than AI #2. This point may be well-intended but represents a poor understanding of molecular mass and stoichiometry. However, higher concentrations are required to kill mosquitoes for some AIs and/or FPs and this should be considered when interpreting bioassay results and the environmental footprint of field applications. Similarly, if it takes a 15  $\mu\text{g}/\text{mL}$  DD, 30 minutes DT for AI #1 and 5  $\mu\text{g}/\text{mL}$  DD, 60 minutes DT for the same AI, that does not necessarily impact the assessment of IR. This example shows why baseline assessments of a susceptible population are

important for establishing the DD and DT that will be used for field populations. Both the 15 µg/mL DD, 30 minutes DT and 5 µg/mL DD, 60 minutes DT may be appropriate, depending on results from the baseline susceptible population. Furthermore, if a mosquito population is classified as resistant, the VCP should assess the level of IR intensity, that is, in follow up testing, the VCP can use approximately 5 to 10 times the DD to further assess the level of IR.<sup>9</sup> If possible, additional testing of upregulated resistance genes and/or enzymes should be considered to assess underlying mechanisms of IR, especially in cases of geographically widespread IR.

#### *Category #4: Data analysis and interpretation of results*

The interpretation of bioassay results are also important questions from VCPs. If mosquitoes are classified as resistant to an AI and/or FP at the DD and DT (in the 2 hours CDC bottle bioassay format), but die within 24 to 48 hours post-exposure, that should be considered on a case-by-case basis by VCPs. In a disease outbreak situation, where the potential for pathogen transmission is high, fast-acting insecticides would be most beneficial to reduce human-mosquito contact; however, in other cases, a slower rate of mortality may be acceptable. Information about each insecticide should be investigated prior to use to determine whether it is slow acting. If delayed mortality is not expected with an AI or FP, then this may be an indication of some level of IR development. Furthermore, the semi-quantitative cut-off values used in bioassays (eg, susceptible ≥97% mortality at DT; possible development of resistance 90%-96% mortality at DT; resistant <90% mortality at DT) should be considered when analyzing results. Mortality <90% (wide range 0%-89%) indicates resistance and mosquito populations falling within the low or high end of this range may be exhibiting significantly different levels of resistance. It should be noted that the CDC bottle bioassay and current WHOPES tube test includes counting mosquitoes that are incapacitated (cannot fly or stand but may still be active) or dead during the 2 hour exposure period.<sup>9</sup> Because the CDC bottle bioassay is kinetic (time-mortality curve), the data can be used in a more sophisticated manner instead of endpoint evaluation of the proportion killed (as in WHOPES). A semi-quantitative measurement can be analyzed for the CDC bottle bioassay by comparing time-mortality curves of susceptible or resistant populations. Analyses can be done on time-mortality and dose-mortality data that can be used for RR calculations.<sup>25</sup> Significance in RR calculations can be separated by 95% confidence intervals in bioassay data. The dose-mortality data is more robust and quantitative than both the CDC bottle bioassay DT and WHO assay one-point reading format. The statistical power and uncertainty of the assays, based on sample size, should likewise be included in any operational decision making by VCPs.

#### *Category #5: Field mosquito populations and life stages*

Some VCPs have questions about operational considerations when testing IR. Depending on goals, adult mosquitoes (of different ages) can be collected from the field and introduced into bioassay bottles or eggs/larvae can be collected, reared to adult, and similar-aged mosquitoes be used in bioassays. Differences in IR occur depending on chronological and physiological mosquito age,<sup>28</sup> hence, similar aged mosquitoes should ideally be used in bottle bioassays. If adult mosquitoes are collected from the field and tested, it is likely that IR will be underestimated.<sup>9</sup> However, if a VCP is simply spot checking to see if a field population is susceptible to an AI or FP they plan to use, adult mosquitoes can be collected from the field for bioassay. This type of biological assessment (not necessarily IR monitoring) would be representative of the variation in chronological and physiological ages, physiological conditions, and other variables happening in a VCP's local field populations. Programs should also understand that a population of, for example, *Culex pipiens* collected from 1 neighborhood will show variation in IR (measured via percent resistance observed) compared to a second population of the same species collected from another neighborhood as different populations are exposed to different environmental conditions and insecticide pressures.<sup>2,24</sup> The IR profile of a mosquito population from the same area sampled over successive months may change over time, hence repeated assessments (ie, beginning, middle, and end of season) of IR are recommended.

If a mosquito population is classified as resistant to an AI, the length of time a VCP should wait until they can use that AI effectively again depends on the AI and consistent and long-term testing is essential. Some mosquito populations may revert to susceptibility more quickly when exposed to 1 AI versus another AI. The degree of genetic variation related to IR in natural mosquito populations should be considered, as well as environmental and other unknown factors. A study in Brazil showed continued IR of *Ae. aegypti* to pyrethroids during 10-year period, even though VCPs were no longer using this AI for control.<sup>29</sup>

#### *Category #6: Assessing differences between AIs and FPs*

VCPs often question if an AI-based IR assessment means they can no longer use a FP including that AI. If a mosquito population is categorized as resistant or susceptible to an AI, that does not necessarily translate to field efficacy of a FP.<sup>19</sup> Formulated products often contain synergists and/or other ingredients that increase effectiveness. Hence, a FP used in a CDC bottle bioassay can mask the development of IR to an AI. With this limitation in mind, it is valid to use FPs in CDC bottle bioassays in addition to bioassays with AIs, provided there is consistency in using either AIs or FPs in both reference

and unknown mosquito populations for comparison. If the user is interested in evaluating product performance, the FP should be used.

### Summary and recommendations

Our collective goal is to mitigate the impacts of IR, hence protecting public health from mosquito-borne disease by improving the efficacy of mosquito control. It is important to have standardized susceptible mosquito populations and sources of AIs used in bioassays. Data analysis and interpretation of results are important with consideration to differences in DD and DT for different AIs, FPs, mosquito species, and types of mosquitoes (lab-reared uniform chronological/physiological age versus field-collected varied ages). Results of bioassays for AIs are expected to differ from FPs due to the addition of synergists and other ingredients (eg, agitants) in FPs. Thus, AI bioassays are used as only a starting point for prediction of field-performance of FPs. As IR monitoring should be standard practice in all VCPs, standardization of methodology, interpretation of results, and an understanding of practical applications of different types of susceptibility/resistance testing is needed. While the CDC bottle bioassay has been the gold standard for monitoring IR in mosquito populations and is a suitable assessment platform, clearer protocols (eg, on DDs and DTs for baseline populations, bottle processing, updated protocols easily accessible to all), additional interpretive guidance for making field decisions, and routine hands on training may be needed to guide VCPs. Improving our understanding of these protocols will advance our abilities to assess and mitigate IR.

As IR continues to grow globally, methods for IR monitoring and interpretation of results should be streamlined through routine training that will lead VCPs to informed decision making about control. There are advantages to a user-friendly IR test (eg, CDC bottle bioassay) for VCPs to assess mosquitoes. Laboratory and field studies are needed to assess variation in DDs and DTs between different species and populations for different AIs and/or FPs. It is important to understand that protocols for laboratory and field assessments of insecticide efficacy may have different purposes and this should be considered when interpreting results. Field studies and/or operational trials are advised to validate the results of laboratory experiments.

There may also be non-lethal (biological, behavioral) effects of insecticides on mosquitoes, such as changes in blood feeding habits, fecundity, fertility, and/or other effects and this should be considered as part of the testing model. We recommend that VCPs are informed about the variables influencing IR assessments and, ultimately, mosquito mortality. This should be considered when interpreting results and making decisions on which FPs to use. Large-scale VCPs should consider providing services assessing IR (using CDC bottle bioassay or other method) for smaller VCPs in their area and/or providing

pre-treated bottles to programs wishing to assess IR. Established VCPs with trained personnel are needed to maintain an IR monitoring program and protect public health. More should be done to investigate the occurrence and potential causes of IR to improve policies aimed at mitigating resistance.

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### Author Contributions

Conceived and designed the perspective theme: SR, BB, MH AW. Analyzed the data/reviewed the literature: SR, BB, MH AW. Wrote the first draft of the manuscript: SR. Contributed to the writing of the manuscript: SR, BB, MH AW. Agreed with manuscript results and conclusions: SR, BB, MH AW. Jointly developed the structure and arguments for the paper: SR, BB, MH AW. Made critical revisions and approved the final version: SR, BB, MH AW. All the authors reviewed and approved the final manuscript.

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### Peer Review Statement

The Editor of *Environmental Health Insights* is an author of this paper, therefore, the peer review process was managed by alternative members of the Board and the submitting Editor had no involvement in the decision-making process.

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