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ABSTRACT. The effectiveness of spinosad for larval mosquito control is summarized based on available published literature and some heretofore unpublished studies. Spinosad is highly active against larvae of all mosquito species tested thus far. It is effective at similar dosages for all larval mosquito instars, with peak cumulative mortality occurring at 72 h posttreatment. More studies are needed to fully define spinosad's ovicidal properties and its impact on the pupal stage. High levels of organic matter and full sunlight are both factors that can negatively impact spinosad efficacy and longevity and should be considered when making use rate and retreatment decisions. Studies clearly show that spinosad technical active ingredient and current crop formulations are suboptimal for larval mosquito control and underrepresent spinosad's true activity. A series of spinosad formulations specialized for larval mosquito control will be sold commercially. Prior to its launch and widespread use, there is a need for additional baseline studies to clarify the natural geographic variation in susceptibility of field mosquito populations. Spinosad represents a new and effective natural product for the integrated management of larval mosquitoes. It possesses a unique mode of action not shared by any other insecticide and is shown to be minimally disruptive to most nontarget species tested thus far at its proposed field use rates.

KEY WORDS Spinosad, mosquito control, larvicide, insecticide

INTRODUCTION

Mosquitoes transmit many important human diseases and serve as a source of serious nuisance and irritation (Brogdon and McAllister 1998). Control is targeted at both adult and larval stages using numerous techniques, including the prudent use of insecticides (US EPA 2007a). There are, however, few insecticide products that can fulfill the stringent public and governmental expectations for a public health insecticide. A successful product must demonstrate sustained efficacy against the target pest under a broad range of environmental and use conditions, display low environmental impact, and exhibit wide margins of safety for humans and nontarget organisms. In the United States only a limited number of pesticide products are currently registered as mosquito larvicides. These include the organophosphate temephos, the insect growth regulator S-methoprene, the microbial larvicides containing *Bacillus thuringiensis israelensis* de Barjac or *Bacillus sphaericus* Neide, and certain oils and molecular films (US EPA 2007b). Emerging insecticide resistance issues, label use restrictions stemming from nontarget toxicity or human health risks, or a lack of residual control pose further limitations on the widespread use of even these registered larvicides (Rose 2001, Zaim and Guillet 2002, Duchet et al. 2008). The paucity of mosquito larvicide treatment options is even more pronounced globally where there is a continued dependence on older organophosphate and pyrethroid products. A new mosquito larvicide meeting the expectations outlined above would represent a much-needed addition to the limited arsenal of mosquito larvicide products (Zaim and Guillet 2002).

Spinosad is an insecticide product from Dow AgroSciences (Indianapolis, IN) derived via fermentation from a naturally occurring soil actinomycete, *Saccharopolyspora spinosa* Mertz and Yao. Spinosad contains 2 insecticidal factors, A and D, present in a ~85:15% ratio within the final product (Mertz and Yao 1990, Kirst et al. 1992, Sparks et al. 1999). Spinosad is highly active by both contact and ingestion to numerous pests in the orders Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, Hymenoptera, and others (Sparks et al. 1995, Bret et al. 1997). In addition, spinosad exhibits highly favorable mammalian toxicology and environmental profiles (Cleveland et al. 2001). Spinosad was registered in 1997 under the US Environmental Protection Agency (EPA) Reduced Risk Pesticide initiative and received the US EPA Presidential Green Chemistry Challenge Award in 1999. Spinosad is considered a naturally derived product by the National Organic Standards Board of the US Department of Agriculture and certain formulations have been approved for

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use in organic agriculture by the Organic Materials Review Institute and numerous other national and international certification bodies (Cleveland 2007, Racke 2007). Spinosad affects the insect nervous system at unique sites on the nicotinic acetylcholine and gamma-aminobutyric acid (GABA) receptors and is non–cross-resistant to any other known insecticide (Salgado 1998, Watson 2001, Salgado and Sparks 2005, Magaña et al. 2007). This combination of efficacy and favorable toxicity and environmental attributes has resulted in rapid growth, with spinosad now registered in 73 countries and on >250 crops.

Although spinosad was initially discovered during the early 1980s in an early-stage insecticide screen that included *Aedes aegypti* (L.) larvae (Thompson et al. 1997), development activities during the first 20 years following its discovery were heavily focused on agricultural uses. In recent years, however, a number of nonagricultural applications, including its use as a mosquito larvicide, have also been developed for spinosad. The first spinosad-containing mosquito larvicide products were recognized by the World Health Organization's Pesticide Evaluation Scheme (WHOPES) during 2007, and first registrations were approved in Morocco and the USA.

Spinosad's suitability for larval mosquito control has been progressively highlighted in a series of scientific publications dating from 2003. This paper draws on both published literature and previously unpublished research results to examine the potential of spinosad as a mosquito larvicide. Information is presented on spinosad's pest spectrum, overall efficacy, mosquito life stage susceptibility, speed of lethal action, nontarget effects, formulations, and on the impact on spinosad of sunlight and levels of organic matter in habitat water.

MATERIALS AND METHODS

Standard laboratory cup/beaker tests against mosquito larvae

The laboratory cup/beaker studies reported in these trials were designed to evaluate the inherent biopotency of various insecticides and insecticide formulations to mosquito larvae, and all were structured according to WHO/WHOPES guidelines for laboratory testing of mosquito larvicides (WHO 2005). Generally, a 1% stock solution was prepared using technical active ingredient (tech AI) dissolved in acetone or ethanol. In some studies stock solutions were prepared using formulated products diluted in water. Stock solutions were then serially diluted and a series of final doses prepared by adding aliquots of the serially diluted stock solution to distilled, osmosed, or tap water held in cups or beakers. Final test solution volumes ranged from 100 ml to 200 ml and 20–25 larvae of a standard age/instar were added to each cup. Larvae were laboratory-reared or the F1 progeny of field-collected mosquitoes. At least 4 replicates were run at each dose. Test cups/beakers were held at 25–28°C under a recommended 12:12 h light:dark photoperiod. Mortality was assessed at various time points postexposure: 24 h, 48 h, 72 h, or up to adult emergence when evaluating delayed effects. For longer exposures larval food was added to the test cups. At evaluation, moribund larvae were counted as dead. Regression analyses were used to calculate 50% and 90% lethal concentrations (LC_{50} and LC_{90}) or 50% and 90% inhibition of adult emergence (IE_{50} and IE_{90}), along with confidence intervals. This set of standard laboratory bioassay conditions will be referred to as "standard WHOPES laboratory test conditions" throughout the remainder of the text. Spinosad LC values generated using non-WHOPES test methods (e.g., Pridgeon et al. 2009) were excluded from these comparisons.

Variations to the standard WHOPES laboratory test conditions occurred in some reported studies. Examples include the use of deionized tap water (DI) or osmosed water instead of distilled water, the use of a more limited exposure period than 24 h, field-collected larvae versus laboratory-reared larvae, or field-collected habitat water high in organic material versus distilled water. Some of these variations were deemed nonsubstantive and the data were combined (e.g., DI versus osmosed versus distilled water); others provided interesting direct comparisons (e.g., limited exposures, field versus laboratory colonies, habitat water versus distilled water).

Comparisons of LC values

The LC_{50} and LC_{90} values were tabulated for 24 studies evaluating spinosad's impact on mosquito larvae. Reported here (see Appendix 1) are a total of 189 LC values derived from evaluations involving 10 mosquito species, 4 spinosad formulation types (tech AI, 120 g AI per liter suspension concentrate (SC) (120 SC), 480 SC, and 0.125% dust), as well as different mosquito instars (L1–L4), bioassay exposure periods (1, 24, 48, or 72 h or at emergence), test insect sources (laboratory colony versus field-collected), and water types (distilled water or DI water versus habitat water). Comparisons of interest are presented as tables or figures and are based on average LC_{50} and LC_{90} values.

Statistics

Because the raw data from which the original LC values were calculated were not reported in most studies it was deemed inadvisable to attempt analysis of variance and mean separation on the

resulting averaged LC values. Regression analyses were performed on individual LC values but did not include the underlying raw data from which the LC values were originally calculated. Minitab (Minitab 12.2.1, Minitab, State College, PA) was used to generate box plots and regression analyses. The standard box plot format uses a horizontal line to designate the median and a dot for the mean; the box captures 50% of the data with the bottom of the box positioned at the first quartile and the top at the third quartile values. The whiskers are the lines that extend from the top and bottom of the box to the adjacent values. The adjacent values are defined as the lowest and highest observations that are still inside the region delineated by 1.5 times the range of values represented by the length of the box.

Sublethal dosage effects on adult emergence

This study was conducted by Lyagoubi and Faraj (2004 unpublished). Methodology followed standard WHOPES laboratory test conditions. A spinosad 120 SC stock formulation was serially diluted in distilled water and then aliquots added to field-collected water to form test solutions with the following concentrations: 0.00375, 0.0075, 0.015, 0.03, 0.045, 0.06, and 0.09 ppm AI. The control consisted of field water only. A total of 20 field-collected L3–L4 *Culex pipiens* L. were added to a beaker containing 1.5 liters of test solution. Four replicates were used at each rate. Test units were held at 25°C and larval mortality assessed after 24, 48, and 72 h of continuous exposure. After the final larval mortality grading at 72 h, surviving larvae were held for an additional 4 days in the treated test solutions, after which numbers of dead pupae or emerged adults were recorded and percentage of adult emergence was calculated.

Mosquito fish testing

This series of studies was conducted by Ben Salah and Alimi (2005 unpublished). A total of 5 tests were conducted during this period, each with a slightly different rate structure. Spinosad 120 SC formulation was serially diluted using field-collected rainwater to form test solutions of concentrations ranging from 0.0075 ppm AI to 491 ppm AI. For comparison, a fenitrothion formulation (Larvos® 50 EC) was diluted in rainwater to form test solutions ranging from 1 ppm AI to 8 ppm AI. The control consisted of rainwater. A single nursery-reared *Gambusia affinis* Baird and Girard female of 5 cm length was added to a 1-liter glass jar containing 500 ml of the treatment test solution. Within each test, 4 replicates were used at each treatment/rate combination. *Gambusia* were kept continuously supplied with mosquito larvae as food. Test units were held at 25°C and fish mortality assessed daily for 10 days. Cumulative mortality is reported at the final 10-day rating. Where dose rates matched, data were combined over different test dates.

RESULTS

Mosquito species susceptibility rankings

Relative susceptibility of larvae of various mosquito species to spinosad is presented in Table 1. Rankings are based on larval LC_{50} values and tabulated separately for different spinosad formulation types. All bioassays were conducted using standard WHOPES laboratory test conditions unless otherwise noted. Larval stage and exposure period were kept constant as much as possible in order to allow for direct comparisons among species. Based on these results, and taking into account expected differences in LC values when using different spinosad formulations, the relative species susceptibility ranking (most sensitive to least) would suggest: (*Anopheles gambiae* Giles = *Anopheles pseudopunctipennis* Theobald) > (*Culex pipiens* L. = *Aedes albopictus* (Skuse)) > *Aedes vigilax* (Skuse) = *Anopheles sinensis* Wied. > *Culex quinquefasciatus* Say > *Aedes aegypti* (L.) > *Anopheles albimanus* Wied. *Anopheles stephensi* Liston > *Ae. albopictus*. *Aedes albopictus* appears twice in this list because of 2 conflicting sets of data (Liu et al. 2004a, Lagneau et al. 2008 unpublished).

Spinosad formulation types

The impact of spinosad formulations on LC_{50} and LC_{90} values for the cosmopolitan pest species *Ae. aegypti* and *Cx. quinquefasciatus* are summarized in Figs. 1 and 2. In the case of *Ae. aegypti*, 3 spinosad formulations were compared: tech AI, 120SC, and 480SC. With *Cx. quinquefasciatus*, only the spinosad tech AI and 120 SC formulations could be directly compared. All studies and their associated LC values in these comparisons were appropriately matched for standard WHOPES laboratory test conditions, including use of L3–L4 instars originating from lab colonies, distilled or DI water test solutions, indoor holding conditions, and a 48-h exposure period. For *Ae. aegypti* (Fig. 1), if the activity of the spinosad tech AI formulation at LC_{50} is normalized to 1×, then the potency of the 480 SC and 120 SC formulations are 1.5-fold and 2.7-fold higher than the tech AI formulation, respectively. At LC_{90}, the 480 SC is 1.1-fold more active than spinosad tech AI, and the 120 SC is 2.5-fold more active. For *Cx. quinquefasciatus* (Fig. 2), the 120 SC formulation is 2.8-fold more potent than

Table 1. Relative susceptibility of larvae of various mosquito species to different spinosad formulations. Rankings are based on larval 50% lethal concentration values (most sensitive to least sensitive).

Species	Form *	Source	Stage	Exposure period	LC-50 ppm AI	LC-90 ppm AI	n ***	LC values from Appendix 1 lines:
Anopheles gambiae Giles	Tech AI	Lab	** L3	24 hr	0.010	-	1	49
Anopheles sinensis Wied.	Tech AI	Field	L3-4	24 hr	0.030	0.074	1	51
Culex quinquefasciatus Say	Tech AI	Lab	L4	24 hr	0.065	0.176	4	94, 96, 97, 98
Aedes aegypti (L.)	Tech AI	Lab	L4	24 hr	0.065	0.123	3	33, 34, 35
Aedes albopictus (Skuse)	Tech AI	Lab	L4	24 hr	0.300	0.900	1	40

Species	Form	Source	Stage	Exposure period	LC-50	LC-90	n	LC values from Appendix 1 lines:
Culex pipiens L.	120 SC	Field	L3	24 hr	0.002	0.006	1	57
Aedes albopictus	120 SC	Lab	L3	48 hr	0.002	0.016	1	42
Aedes vigilax (Skuse)	120 SC	Lab	L4	48 hr	0.004	0.007	1	47
Culex quinquefasciatus	120 SC	Lab	L4	48 hr	0.012	0.028	2	77, 81
Aedes aegypti	120 SC	Lab	L4	48 hr	0.028	0.048	2	17, 18

Species	Form	Source	Stage	Exposure period	LC-50	LC-90	n	LC values from Appendix 1 lines:
Culex pipiens	480 SC	Lab	L3	48 hr	0.003	0.013	1	66
Aedes aegypti	480 SC	Lab	L3	48 hr	0.007	0.010	1	23
Anopheles stephensi Liston	480 SC	Lab	L3	48 hr	0.024	0.042	1	53

Species	Form	Source	Stage	Exposure period	LC-50	LC-90	n	LC values from Appendix 1 lines:
Anopheles pseudopunctipennis Theobald	480 SC	Lab	L3-4	1 hr exposure, 24 hr grade	0.010	-	1	50
Anopheles albimanus Wied.	480 SC	Lab	L3-4	1 hr exposure, 24 hr grade	0.024	-	1	48

* AI = active ingredient; SC = suspension concentrate.
** unless otherwise noted, LC values in these comparisons were appropriately matched for standard WHOPES laboratory test conditions. Shaded cells denote unmatched data.
*** n = denotes numbers of LC values comprising the mean.

spinosad tech AI at LC_{50} and 3.4-fold more active at LC_{90}.

Acute and cumulative mortality relative to exposure period

The relationship between the duration of the bioassay exposure period and LC_{50} and LC_{90} values for *Ae. aegypti* and *Cx. pipiens* are summarized in Figs. 3 and 4 and detailed for *Cx. quinquefasciatus* in Table 2. Average LC_{50} values for *Ae. aegypti* L3 larvae treated with spinosad 120 SC show a steady decline from 0.024 ppm AI at the 24-h exposure period to 0.007 ppm AI at 72 h—an apparent 3.4-fold increase in perceived potency between bioassays graded at 24 h versus 72 h. Little difference was observed between the LC_{50} measured at 72 h and that measured later at adult emergence (Fig. 3). Mean LC_{90} values for L3 *Ae. aegypti* exhibited even larger differences as exposure periods lengthened, decreasing from 0.165 ppm AI at a 24-h exposure period to 0.013 ppm AI at 72 h—an apparent 12.7-fold increase in potency. A similar but less pronounced trend is seen with L3–L4 *Cx. pipiens* larvae treated with spinosad 120 SC, but here utilizing field-collected larvae and field-collected habitat water (Fig. 4). The LC_{50} for *Cx. pipiens* declines from 0.031 ppm AI at a 24-h exposure period to 0.020 ppm AI at 72 h—a 1.6-fold decrease, whereas the LC_{90} declines from 0.094 ppm AI at a 24-h exposure period to 0.040 ppm AI at 72 h—a 2.4-fold shift. Examination of these same types of data for *Cx. quinquefasciatus* L4 larvae treated with a spinosad tech AI formulation (Table 2) reveals a 1.5-fold drop in mean LC_{50} between the 24-h and 48-h exposure periods and a similar 1.5-fold drop in mean LC_{90} between these 2 exposure time points.

Instar susceptibility

The relationship between mosquito larval stage and LC_{50} and LC_{90} values was examined for *Ae. aegypti* and *Cx. quinquefasciatus* in 3 separate formulation comparisons. Comparisons included *Ae. aegypti* and *Cx. quinquefasciatus* larvae treated with spinosad tech AI formulations and *Ae. aegypti* larvae treated with spinosad 120 SC. All studies included in these comparisons utilized standard WHOPES laboratory test conditions. *Ae. aegypti* L2 and L4 larvae treated with spinosad tech AI formulations showed exactly the same LC_{50} values—0.052 ppm AI (Fig. 5). Regression analysis detected no relationship between l instar and LC_{50} value (LC_{50} = 0.0520

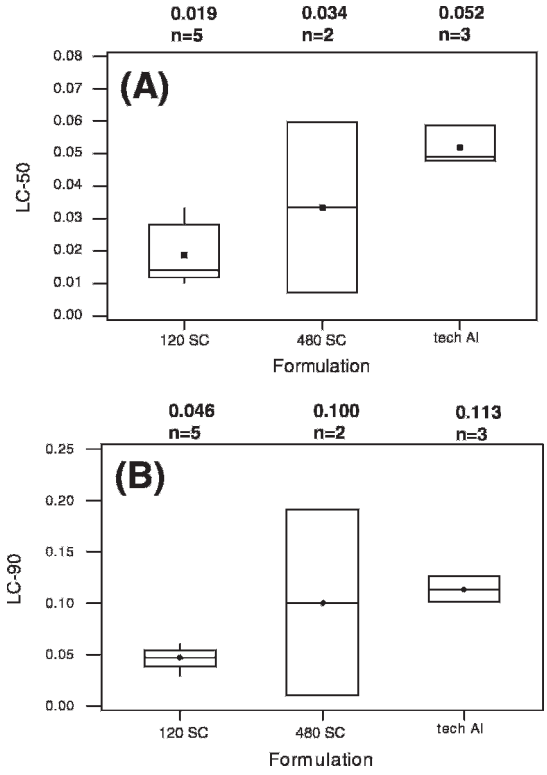

Fig. 1. Impact of spinosad formulation on mean (A) 50% lethal concentration (LC_{50}) and (B) 90% lethal concentration (LC_{90}) values for lab-reared *Aedes aegypti* L3–L4 instars when graded for mortality after a 48-h exposure period. The LC values used in these analyses correspond to the following Table 6 lines: 8–10, 17–18, 23, 28, 36–38.

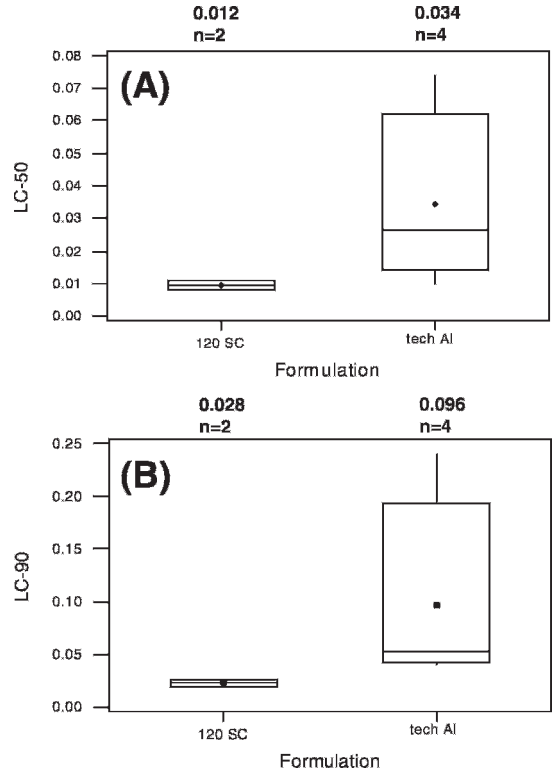

Fig. 2. Impact of spinosad formulation on mean (A) 50% lethal concentration (LC_{50}) and (B) 90% lethal concentration (LC_{90}) values for lab-reared *Culex quinquefasciatus* L3–L4 instars when graded for mortality after a 48-h exposure period. The LC values used in these analyses correspond to the following Table 6 lines: 77, 81, 92, 99–101.

+ 0.00 (instar); r^2 (adjusted) = 0.0%; df 1, 2; P = 1.00) for the tech AI formulation. At the LC_{90} level, *Ae. aegypti* L2 larvae actually had a slightly higher LC_{90} (0.145 ppm AI) than did L4 larvae (0.113 ppm AI) (Fig. 5). Again, there was no strong relationship between instar and LC_{90} value (LC_{90} = 0.177 − 0.0158 (instar); r^2 (adjusted) = 56.0%; df 1, 2; P = 0.160). Similar analyses of *Cx. quinquefasciatus* larvae treated with spinosad tech AI formulations, this time including L2, L3, and L4 instars, suggest no relationship between instar and LC_{50} (LC_{50} = 0.0075 + 0.00783 (instar); r^2 (adjusted) = 0.0%; df 1, 5; P = 0.419) or LC_{90} (LC_{90} = 0.0181 + 0.0223 (instar); r^2 (adjusted) = 0.0%; df 1, 5; P = 0.498) (Fig. 6). A final comparison was made of *Ae. aegypti* L2, L3, and L4 instars treated with spinosad 120 SC formulations. Although LC_{50} and LC_{90} values were generally 2- to 3-fold lower for *Ae. aegypti* larvae treated with spinosad 120 SC formulations versus spinosad tech AI formulations, the regression analyses again failed to detect any evidence of a relationship between instar and either LC_{50} (LC_{50} = 0.0141 + 0.002 (instar); r^2 (adjusted) = 0.0%; df 1, 5; P = 0.665) or LC_{90} (LC_{90} = 0.0467 + 0.00 (instar); r^2 (adjusted) = 0.0%; df 1, 5; P = 1.00) (Table 3).

Sublethal dosage effects on adult emergence

The percentage of *Cx. pipiens* adults that emerged from pupae after previously being exposed to sublethal doses of spinosad 120 SC test solutions as L3–L4 larvae are summarized in Fig. 7. The 0.06 and 0.09 ppm AI rates are omitted because few or no larvae survived these treatments. A clear dose response is apparent, with percentage of adult emergence declining steadily from 79% at a 0.0037 ppm AI rate to 22% at 0.045 ppm AI.

Impact of levels of organic matter in habitat water

A single study in the database directly addressed the impact of water purity on larval LC values (Bahgat et al. 2007). The LC_{50} and LC_{90} values were measured for field-collected *Cx.*

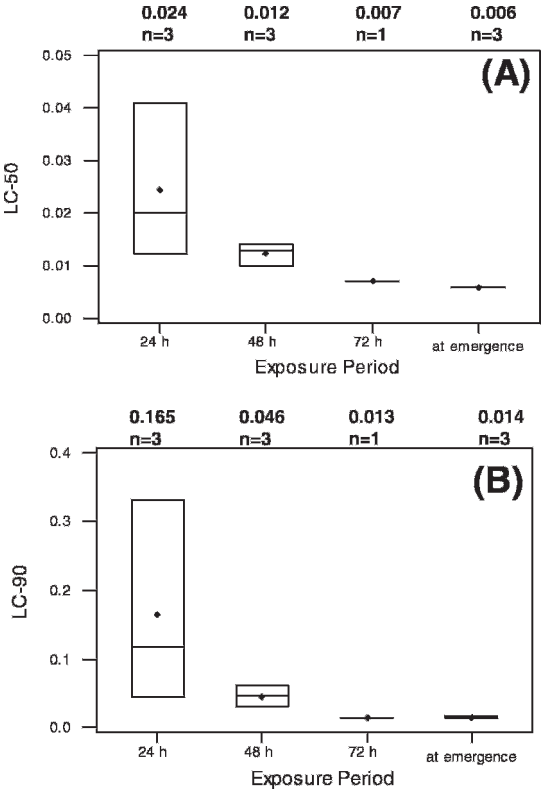

Fig. 3. Impact of exposure period on mean (A) 50% lethal concentration (LC_{50}) and (B) 90% lethal concentration (LC_{90}) values for *Aedes aegypti* L3 instars treated with a spinosad 120 suspension concentrate (SC) formulation. All other bioassay variables conform to standard World Health Organization Pesticide Evaluation Scheme laboratory test conditions. The LC values used in these analyses correspond to the following Appendix 1 lines: 5–14.

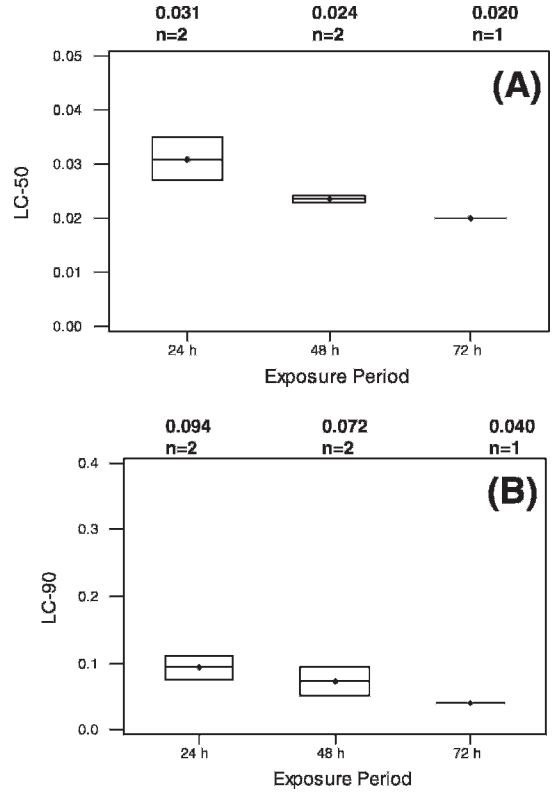

Fig. 4. Impact of exposure period on mean (A) 50% lethal concentration (LC_{50}) and (B) 90% lethal concentration (LC_{90}) values for field-collected *Culex pipiens* L3–L4 instars in a bioassay using field-collected stagnant water treated with a spinosad 120 suspension concentrate (SC) formulation. All other bioassay variables conform to standard World Health Organization Pesticide Evaluation Scheme laboratory test conditions. The LC values used in these analyses correspond to the following Appendix 1 lines: 55, 58–61.

pipiens L3 larvae treated with a spinosad 0.125% dust formulation and with test conditions differing only in the composition of the water test solution—one treatment using field-collected sewage water and the other using DI tap water (Fig. 8). The LC_{50} and LC_{90} values in DI tap water were 0.007 ppm AI and 0.040 ppm AI, respectively. With field-collected sewage water these same values rose to 0.022 ppm AI and 0.175 ppm AI. This represents a 3.1-fold increase in the LC_{50} and a 4.4-fold increase in LC_{90} when bioassays utilized habitat water high in organic matter (Fig. 8).

Field-collected larvae versus laboratory reference colonies

The overall database yielded direct comparisons of *Cx. quinquefasciatus* L2 and L4 larvae treated with spinosad 120 SC formulations, with

Table 2. Impact of exposure period on mean 50% lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) values for lab-reared *Culex quinquefasciatus* L4 instars treated with a spinosad technical active ingredient formulation. All other bioassay variables conform to standard World Health Organization Pesticide Evaluation Scheme laboratory test conditions.

Species	LC_{50} (ppm)	LC_{90} (ppm)	Exposure period	LC values and citations from Appendix 1 (lines)
Culex quinquefasciatus	0.065	0.176	24 h	94, 96, 97, 98
Culex quinquefasciatus	0.042	0.115	48 h	99, 100, 101

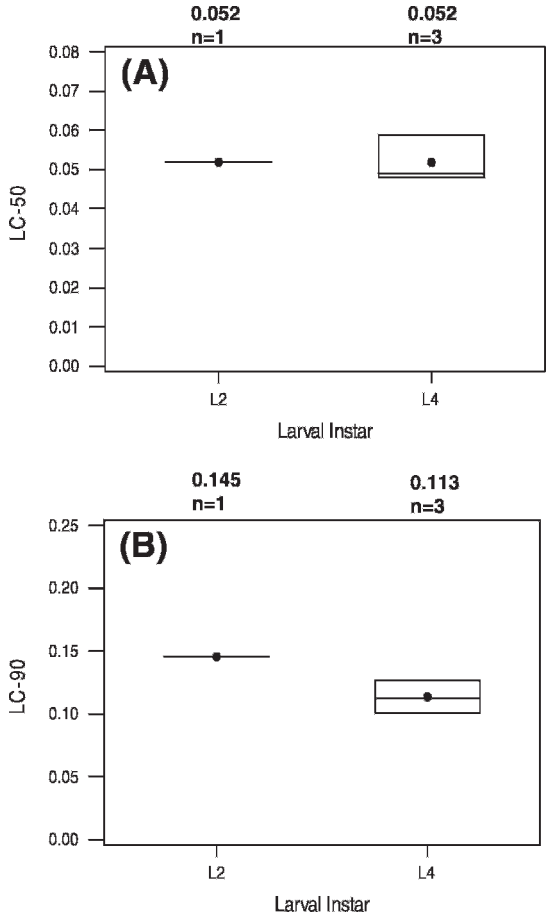

Fig. 5. Impact of larval instar on mean (A) 50% lethal concentration (LC_{50}) and (B) 90% lethal concentration (LC_{90}) values for lab-reared *Aedes aegypti* larvae treated with a spinosad technical active ingredient (AI) formulation and graded for mortality after a 48-h exposure period. The LC values are expressed in ppm AI. LC values used in these analyses correspond to the following Appendix 1 lines: 30, 36–38.

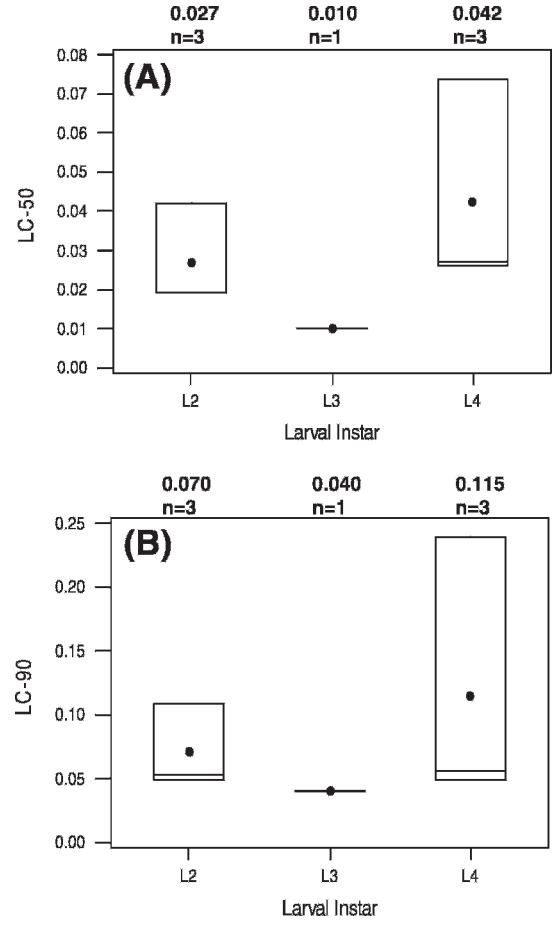

Fig. 6. Impact of larval instar on (A) 50% lethal concentration (LC_{50}) and (B) 90% lethal concentration (LC_{90}) values for *Culex quinquefasciatus* lab-reared larvae treated with a spinosad technical active ingredient (AI) formulation and graded for morality after a 48-h exposure period. The LC values are expressed in ppm AI. LC values used in these analyses correspond to the following Appendix 1 lines: 89–92, 99–101.

all other test conditions equivalent except for the origin of the larvae used—laboratory reference strains versus field-collected larvae. Results reveal a 2.0-fold increase in LC_{50} for field-collected L2 larvae versus laboratory reference strains and a 1.4-fold increase in LC_{50} for L4 larvae (Fig. 9).

Standard WHOPES LC_{50} and LC_{90} values for *Ae. aegypti* and *Cx. quinquefasciatus* L4 larvae

Table 4 summarizes LC_{50} and LC_{90} values for *Ae. aegypti* and *Cx. quinquefasciatus* L4 larvae treated with spinosad tech AI and a specialized

Table 3. Impact of larval instar on mean 50% lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) values for *Aedes aegypti* lab-reared larvae treated with a spinosad 120 suspension concentrate (SC) formulation and graded for mortality after a 48-h exposure period. All other bioassay variables conform to standard World Health Organization Pesticide Evaluation Scheme laboratory test conditions.

Species	Instar	LC_{50} (ppm)	LC_{90} (ppm)	LC values and citations from Table 6 (lines)
Aedes aegypti	L2	0.024	0.048	3,4
Aedes aegypti	L3	0.012	0.046	8, 9, 10
Aedes aegypti	L4	0.028	0.048	17, 18

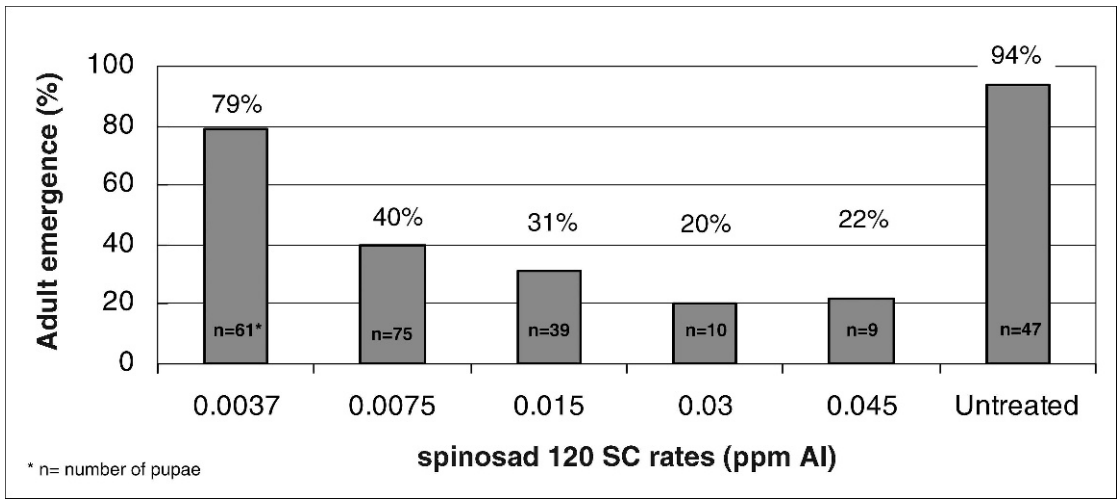

Fig. 7. Percent of emergence of *Culex pipiens* adults from pupae previously exposed to sublethal test solutions of spinosad 120 suspension concentrate (SC) as L3–L4 larvae.

120 SC formulation designed for larval mosquito control. With *Ae. aegypti*, the specialized 120 SC formulation was ~2.0-fold more active than the tech AI formulation (as judged by relative LC_{50} or LC_{90}) at both the 24-h and 48-h exposure periods. Results for *Cx. quinqefasciatus* demonstrated that the specialized 120 SC formulation was ~4-fold more active than the tech AI formulation. These data provide useful benchmarks for future resistance or cross-resistance studies.

Mosquito fish testing

Toxicity of various rates of spinosad 120 SC to the mosquito fish, *G. affinis*, are presented in Table 5 (data for some intermediate tested rates have been omitted). Spinosad caused no mortality at rates up to 50 ppm AI. A fenitrothion (Larvos® 50 EC) standard included for comparison caused 100% morality at rates of 5 ppm AI and above.

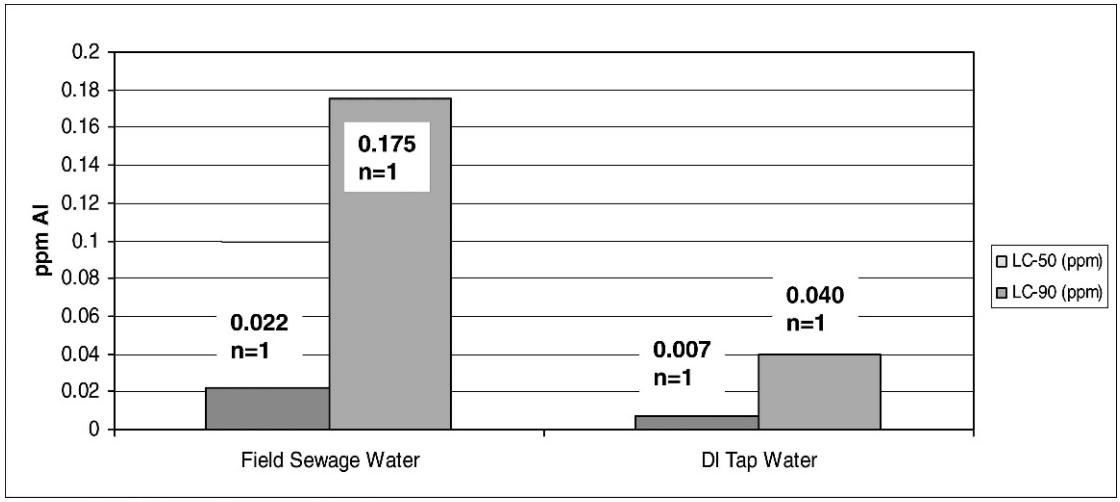

Fig. 8. Impact of field-collected, stagnant water versus deionized tap water on the 50% lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) values of *Culex pipiens* L3 larvae treated with a spinosad 0.125% dust formulation and graded for mortality after a 24-h exposure period. The LC values are expressed in ppm AI. LC values used in these analyses correspond to the following Appendix 1 lines: 69, 70.

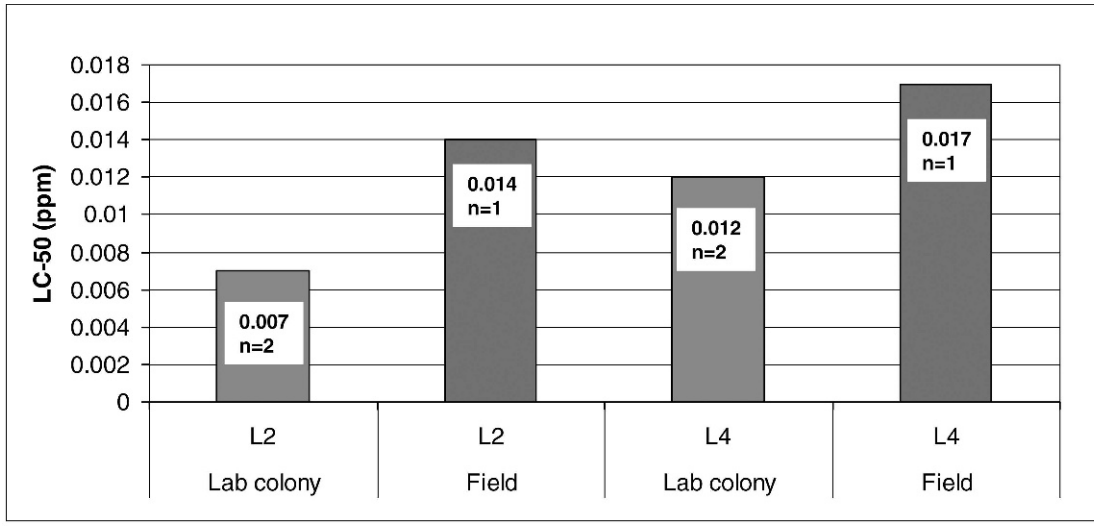

Fig. 9. Impact of field-collected larvae versus lab colony larvae on mean 50% lethal concentration (LC_{50}) values of *Culex quinquefasciatus* L2 and L4 larvae treated with a spinosad 120 suspension concentrate (SC) formulation and graded for mortality after a 48-h exposure period. The LC values are expressed in ppm AI. LC values used in these analyses correspond to the following Appendix 1 lines: 75, 77, 79, 81, 83, 85.

Full database

The full database of spinosad LC values is presented in Appendix 1. This database includes all published literature on spinosad as a mosquito larvicide current to May 2009 and some previously unpublished Dow AgroSciences and cooperator data as well. Studies included in Appendix 1 but not referenced specifically in the text of this paper include Morgan 1997 unpublished, Mavrotas 1997 unpublished, Ritchie and Zborowski 2003 unpublished, Shin et al. 2003, Jiang and Mulla 2005 unpublished, and Sadanandane et al. 2006 unpublished. When test conditions are matched appropriately and comparisons made among LC values, seeming outliers in the database (Appendix 1) appear to be very few. The vast majority of LC values are remarkably consistent, owing no doubt to the standardized WHOPES laboratory guidelines under which these studies were conducted. Data from published (and thus peer reviewed) and unpublished sources agree well. This suggests that LC-values from these different studies can be confidently combined or compared.

DISCUSSION

Mosquito species susceptibility rankings

All mosquito species tested thus far appear to be highly sensitive to spinosad. The LC_{50} values for spinosad SC formulations generally range from 0.002 ppm AI to 0.028 ppm AI and LC_{90}

Table 4. Comparison of mean 50% lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) values of lab-reared *Aedes aegypti* and *Culex quinquefasciatus* L4 larvae treated with either the spinosad technical active ingredient (tech AI) formulation or a 120 suspension concentrate (SC) formulation specially developed for larval mosquito control and graded for mortality after 24- or 48-h exposure periods.

Species	Formulation	Instar	Exposure period	LC_{50} (ppm)	LC_{90} (ppm)	*n*	LC values and citations from Appendix 1 (lines)
Aedes aegypti	Tech AI	L4	24 h	0.065	0.123	3	33, 34, 35
Aedes aegypti	120 SC (specialized)	L4	24 h	0.030	0.061	1	15
Aedes aegypti	Tech AI	L4	48 h	0.052	0.113	3	36, 37, 38
Aedes aegypti	120 SC (specialized)	L4	48 h	0.023	0.049	1	18
Culex quinquefasciatus	Tech AI	L4	24 h	0.065	0.178	4	94, 96, 97, 98
Culex quinquefasciatus	120 SC (specialized)	L4	24 h	0.014	0.035	2	76, 80
Culex quinquefasciatus	Tech AI	L4	48 h	0.042	0.115	3	99, 100, 101
Culex quinquefasciatus	120 SC (specialized)	L4	48 h	0.012	0.028	2	77, 81

Table 5. Percentage of mortality of mosquito fish (*Gambusia affinis*) at various rates of spinosad 120 SC and fenitrothion (Larvos® 50 EC) in laboratory bioassays.

Compound	Rate (ppm active ingredient)	% mortality	*n*
Spinosad 120 SC	200	100	4
Spinosad 120 SC	150	100	4
Spinosad 120 SC	123	100	4
Spinosad 120 SC	60	37.5	8
Spinosad 120 SC	50	0	4
Spinosad 120 SC	40	0	4
Spinosad 120 SC	30	0	4
Spinosad 120 SC	10	0	4
Fenitrothion	6	100	4
Fenitrothion	5	100	4
Fenitrothion	4	50	8
Fenitrothion	3	0	8
Fenitrothion	2	0	8
Untreated		0	16

values from 0.006 ppm AI to 0.048 ppm AI for L3–L4 larvae over all species. There does appear to be some variation in species susceptibility, with LC_{50} values for L3–L4 larvae of different species spanning a range of 6.5- to 14-fold depending on formulation type (tech AI, SC). The composite rank order of species susceptibility to spinosad (based on larval LC_{50}s) presented here is in agreement with those noted by other authors within individual trials (Bond et al. 2004, Darriet et al. 2005, Mulla 2006 unpublished, Romi et al. 2006). Two problematic placements are *Ae. albopictus* and *An. stephensi*. Romi et al. (2006) place the susceptibility of *An. stephensi* below that of *Ae. aegypti* whereas Laddoni (2006 unpublished) places it above that of *Cx. quinquefasciatus*. Results from Liu et al. (2004a) show *Ae. albopictus* to be one of the least susceptible species to spinosad, whereas Lagneau et al. (2008 unpublished) indicates its susceptibility to be on a par with *Cx. pipiens*, one of the most sensitive species. Further research will serve to clarify these questions of relative species susceptibility. Overall difference in susceptibility to spinosad among mosquito species is relatively slight and suggests that spinosad should prove highly effective for the control of most mosquitoes despite wide variations in the habitats, feeding modes, and food resources of these different species. Spinosad has also been documented to be effective against chironomid larvae (Bond et al. 2004, Perez et al. 2007).

Formulation type

Formulation exerts a large influence on spinosad's activity as a mosquito larvicide. Technical spinosad is the least active, the 480 SC is intermediate in activity, and spinosad 120 SC is clearly the most effective formulation. Spinosad 120 SC formulation is approximately 2-fold more active than spinosad 480 SC. Spinosad 480 SC is a crop formulation and particle size was optimized to create a balance between initial knockdown and residual efficacy. The spinosad 120 SC formulation used in most (but not all) of these mosquito larvicide studies was designed to maximize the number of particles falling within a defined particle range based on reported food size preferences in mosquito larvae (Dadd 1971, Merritt et al. 1978, Wallace and Merritt 1980, Merritt et al. 1992, Dahl et al. 1993). Spinosad tech AI and current crop formulations thus appear to be suboptimal for larval mosquito control. The spinosad 120 SC formulation commercialized for larval mosquito control will be a specialized formulation with a particle size average and distribution optimized for mosquito larvae control.

Speed of lethal action

Spinosad is a relatively slow-acting toxicant compared to some other chemical classes used for larval mosquito control such as pyrethroids or organophosphates. Although characteristic symptoms of intoxication occur rapidly and a lethal dose can be acquired within minutes to hours, maximum cumulative mortality can take up to 72 h or more to manifest itself (Viñuela et al. 2001, Cisneros et al. 2002). This gradual onset of mortality is due to a combination of factors including slow cuticular penetration, the preponderance of ingestion versus contact mode of uptake, and spinosad's unique mode of action at nicotinic acetylcholine and GABA receptors (Salgado and Sparks 2005). Composite results reported here clearly show that mortality of larval mosquitoes treated with spinosad accumulates steadily over a period of 72 h. This is confirmed by the decrease in LC values between 24 h and 48 h of exposure and again between 48 h and 72 h. Beyond 72 h the LC values remain relatively constant. Ayesa et al. (2006) also reported no change in LC values of *Ae. aegypti* treated with spinosad after 72 h. Studies by Bond et al. (2004) and Perez et al. (2007) with *Ae. aegypti* demonstrated that larval exposure to spinosad for as little as 1 h still led to substantial and irreversible mortality although the LC_{50}s were appreciably higher (~6-fold) compared to a 72-h exposure (Gaven and Lagneau 2004 unpublished). Laddoni (2006 unpublished) noted that mortality of *Cx. quinquefasciatus* and *An. stephensi* L3 larvae nearly doubled after they were initially exposed to spinosad-treated solutions for 24 h, but then removed and transferred to clean water and graded again at 48 h. These results confirm that even limited exposures to spinosad can result in substantial and irreversible larval mortality.

These results also suggest that bioassays with spinosad should include a 72-h exposure in order to fully reflect the toxicity of spinosad to mosquito larvae.

Toxicity of spinosad to mosquito life stages

Larval mortality: The LC_{50} and LC_{90} values of spinosad are shown to be relatively insensitive to increasing larval size between instars L2 through L4 for *Ae. aegypti* and *Cx. quinquefasciatus*. This same observation was made by Mulla (2006 unpublished) in a study of *Ae. aegypti*. It is possible that this flat dose response versus larval stage is a result of higher levels of ingestion of spinosad by older larvae. Practically, these results suggest that timing of spinosad applications for control of single-brood populations can be flexible and that no major increase in rate will be required in multibrood situations where mixed larval instars or mosquito stages are present at the time of treatment.

Pupal mortality: Mosquito pupae do not feed, so an insecticide needs to have substantial contact activity in order to specifically control pupae. Spinosad is a contact-active insecticide, although its ingestion activity is clearly superior. Whether or not spinosad can cause direct mortality to the mosquito pupal stage is unclear at present. Results presented here (from Lyagoubi and Faraj 2004 unpublished) show that surviving *Cx. pipiens* pupae exposed earlier as L3–L4 larvae to sublethal spinosad test solutions suffer dose-dependent mortality that ultimately results in a 20–79% inhibition of adult emergence. In a field study on a mixed population of *Culex*, *Aedes*, and *Anopheles* in Egypt, Bahgat et al. (2007) found that pupal mortality ranged from 67% to 71% in the first 2 days following treatment with spinosad liquid and dust formulations at rates as low as 0.027 ppm AI; however, it was unclear whether this was direct or indirect (via previous larval exposure) pupal mortality. Other laboratory studies on *Ae. aegypti* reported little or no direct mortality of pupae surviving spinosad exposures as larvae (Darriet and Corbel 2006, Lagneau et al. 2006 unpublished). Other studies have observed the complete elimination of adult emergence from field populations treated with spinosad (Bond et al. 2004, Cetin et al. 2005, Bahgat et al. 2007), but did not differentiate larval from pupal mortality. No study directly measured spinosad's impact on the isolated pupal stage. Future research on this topic seems warranted.

Egg mortality: Spinosad does possess ovicidal properties, although this effect is species-specific (Bret et al. 1997) and spinosad's level of ovicidal activity is oftentimes significantly lower than its larvicidal activity (Charmillot et al. 2007). In susceptible species, the activity of spinosad on newly hatched larvae (neonates) is usually quite high (Pineda et al. 2004) and this can sometimes be confused with direct ovicidal activity. The case for spinosad as a direct ovicide to mosquito eggs is mixed. Perez et al. (2007) reported a lack of ovicidal activity on *Ae. aegypti* eggs at spinosad rates as high as 20 ppm AI. Romi et al. (2006) observed only 18.6% mortality to *Ae. aegypti* eggs after exposure to 1 ppm AI of spinosad, but 100% control of eggs of *An. stephensi* at this same rate. Further studies are needed to clarify the potential for ovicidal activity of spinosad on key mosquito species at realistic field use rates.

Repellency to adults: Based on laboratory cage tests that provided an oviposition site choice between spinosad-treated and untreated water solutions, Romi et al. (2006) suggested that spinosad was a partial repellent to *Ae. aegypti* gravid females at rates above 1 ppm AI based on significant differences in the total numbers of eggs laid over a 72-h period. No repellency was detected for *An. stephensi* gravid females at spinosad rates up to 500 ppm AI in this same series of tests. In a similar cage choice test, Perez et al. (2007) found no evidence of spinosad repellency to *Ae. aegypti* gravid females at rates of 5 ppm AI and 20 ppm AI based either on differences in the numbers of females visiting the oviposition cups during a 60-min exposure period or the total numbers of eggs laid over 24 h versus control water treatments. In fact, significantly more adult females visited spinosad-treated cups versus untreated cups at the higher spinosad rate, and this was conjectured to be due to attraction of adult females to the earthy odor of spinosad evident at this 20 ppm AI rate. Bond et al. (2004) noticed no obvious repellency of spinosad to *Ae. aegypti* adult females at rates up to 10 ppm AI. Müller et al. (2008) detected no repellency of spinosad to adult female *Aedes caspius* Pallas or *Anopheles sergentii* Theobald when spinosad was presented within sugar baits at a concentration of 400 ppm AI (0.04%). Based on these studies, spinosad at its projected field use rates is not expected to be repellent to adult female mosquitoes.

Impact of levels of organic matter in habitat water

Larvicidal efficacy of spinosad can be negatively impacted by adsorption onto particulate matter within habitat waters high in clay or organic matter. This is a function of spinosad's physical properties and rather high adsorption coefficients (Saunders and Bret 1997). Data from Bahgat et al. (2007) show a 3- to 4-fold decrease in efficacy of a spinosad dust formulation versus *Cx. pipiens* larvae in test solutions composed of field-collected sewage water compared to DI tap water. However, Bahgat et al. (2007) found the reverse trend with the 120SC formulation in this same study. A benign impact of high organic matter on spinosad performance is not borne out

by early field studies, which suggest that rates of spinosad will need to be increased in response to habitats with very high levels of liquid or solid sewage such as cisterns (Cetin et al. 2005, Sadanandane et al. 2009) or street drains (Sadanandane et al. 2009). This observed reduction in spinosad's larvicidal efficacy could be due to adsorption, soil microbial degradation, decreased ingestion by larvae, or all—although adsorption is the likelier explanation given the much longer half-lives involved in microbial degradation (Saunders and Bret 1997). Mosquito control abatement professionals should consider the levels of pollution and organic matter content in target habitats when determining recommended label use rates and retreatment intervals for spinosad.

Impact of sunlight

The main degradative pathway for spinosad is photolysis. Laboratory and field studies demonstrate rapid degradation of spinosad in water when exposed to summer sunlight, with a half-life on the order of 1–2 days (Saunders and Bret 1997, Cleveland et al. 2002, Duchet et al. 2008). However, spinosad undergoes very little hydrolysis even over a broad range of pH values (5–9) and thus is very stable in water not exposed to sunlight, with half-lives on the order of months (Saunders and Bret 1997). Perez et al. (2007) used an *Ae. aegypti* larval bioassay system to measure the half-life of a 10-ppm AI solution of spinosad exposed to various degrees of shading and/or protection from ultraviolet (UV) light. Their results demonstrated a half-life of 2.1 days for spinosad solutions exposed outdoors to full sunlight, 24.5 days when exposed outdoors but kept constantly shaded, and 90 days when maintained in a darkened section of a laboratory. In a similar study, Thavara et al. (2009) utilized a larval bioassay system to measure the residual efficacy of spinosad within covered 200-liter earthen water jars held outdoors in Thailand. They showed that spinosad applied at a rate of 0.5 ppm provided 90–100% control of *Ae. aegypti* larvae for 20–27 days (depending on water regimen) and 34–64 days of control at 1.0 ppm. In outdoor pond microcosm studies run in full sunlight, Jiang and Mulla (2009) showed that spinosad 120SC at rates ranging from 0.05 ppm AI to 0.10 ppm AI provided >95% control of *Culex* spp. larvae for 7–14 days. Mosquito control abatement professionals should factor in the impact of sunlight when selecting spinosad formulation types or determining retreatment intervals for spinosad. A number of specialized controlled-release formulations will be available for multibrood situations where extended residual is required. These controlled-release formulations serve to mitigate the impact of UV degradation and are designed to provide control lasting from 30 days to 180 days, depending on formulation and use pattern.

Unique mode of action and lack of cross-resistance

Spinosad acts on the postsynaptic nicotinic acetylcholine and GABA receptors of insects and has been demonstrated to possess a unique mode of action not shared by any other known insecticidal class of chemistry (Salgado and Sparks 2005). Laboratory studies using larval bioassays to compare LC values of susceptible and resistant reference strains of different mosquito species have shown no cross-resistance to spinosad in pyrethroid, organophosphate, or carbamate insecticide–resistant *Cx. quinquefasciatus* (Liu et al. 2004b, Darriet et al. 2005), *Ae. aegypti* (Darriet et al. 2005), *Ae. albopictus* (Liu et al. 2004a), or *An. gambiae* (Darriet et al. 2005). Ayesa et al. (2006) showed that addition of piperonyl butoxide (PBO) to spinosad test solutions did not significantly alter the LC_{50} of spinosad to *Ae. aegypti* larvae, thus suggesting that spinosad is not subject to oxidative metabolism. This finding is consistent with other PBO studies on Diptera (Shono and Scott 2003) and the larger observation that all instances of resistance to spinosad thus far have been target-site mediated (Zhao et al. 2002, Salgado and Sparks 2005, Sarfraz et al. 2005, Perry et al. 2007).

Susceptibility of field versus laboratory populations

The WHOPES guidelines for laboratory LC_{50} and LC_{90} studies recommend the use of larvae from well-characterized laboratory reference strains or, if practicable, the F1 generation of field-collected mosquitoes (WHO 2005). The LC values generated from laboratory reference strains versus field-collected strains can often differ due to the loss of genetic variability over time or the adaptation of laboratory colonies to less-stressful laboratory rearing conditions. Results presented here show that LC_{50} values for field-collected *Cx. quinquefasciatus* L2 and L4 larvae are 2.0-fold and 1.4-fold, respectively, higher than for laboratory reference strains even though spinosad has never been used in the field to control mosquitoes and thus these higher LC_{50} values simply represent variation in LC_{50} values among susceptible field populations. Liu et al. (2004b) found a similar 1- to 3-fold difference in spinosad LC_{50} values between field-collected and lab colony strains of *Cx. quinquefasciatus* larvae, but no field versus lab colony differential in LC_{50} values for *Ae. albopictus* (Liu et al. 2004a). A comparison of the LC_{50} values of laboratory-reared L3–L4 *Ae. aegypti* larvae reported by Bond et al. (2004) and Perez et al. (2007) versus those for field-collected larvae (Antonio et al.

2009) reveal a 2.3-fold increase in LC_{50} of field-collected larvae despite nearly identical test methodology. Kristensen and Jespersen (2004) reported that spinosad LC_{50} values for field populations of the common housefly, *Musca domestica* L., collected from dairy farms in Denmark were 2.2- to 7.5-fold higher than for laboratory reference strains even though these field-collected strains were judged fully susceptible. They caution that these differences, although representing natural variation in susceptible field populations, could be mistaken for incipient resistance or tolerance and recommend that the term resistance is best defined as a reduction in susceptibility beyond natural variation (Schaub et al. 2002). Additional baseline studies that serve to clarify the natural geographic variation in susceptibility of field mosquito populations to spinosad prior to widespread product launch would prove both timely and useful. Baseline susceptibility data catalogued here represent a valuable resource to future studies on spinosad resistance or cross-resistance in mosquito larvae.

Impact on nontarget species

Laboratory results reported here (Ben Salah and Alimi 2005 unpublished) demonstrate that spinosad had no negative impact on the mosquito fish, *G. affinis*, at rates up to and including 50 ppm AI. This would imply a high margin of safety for *Gambusia* given that field use rates of spinosad will be in the range of 0.02–0.11 ppm AI. Similarly, Laddoni (2006 unpublished) observed only slight adverse effects on nontarget species of Dytiscidae, Histeridae, Libellulidae, and Notonectidae in an outdoor artificial pond study when spinosad 120SC was applied at field use rates up to 50 g AI/ha (=0.050 ppm AI at a 10-cm water depth). Duchet et al. (2008) reported an adverse impact of spinosad on *Daphnia pulex* Leydig (Crustacea, Cladocera) in field microcosm studies when spinosad was applied at nominal concentrations of 0.008, 0.017, and 0.033 ppm AI. *Daphnia* populations recovered to densities similar to those measured in the untreated control by 7 days after treatment at the 0.008-ppm AI rate, but did not recover when treated with the 0.017-ppm AI or 0.033-ppm AI nominal concentrations. More studies are desirable to understand the impact of spinosad on nontarget organisms sharing mosquito larval habitats. However, spinosad appears minimally disruptive to most of the nontarget species tested thus far when applied at or near its proposed field use rates.

WHO/WHOPES listings and specifications

Spinosad 120 SC and 0.5% granule formulations have been fully evaluated and accepted for listing by the WHOPES working group, the official WHO body charged with assessment of pesticides for their effectiveness and safety (WHO 2007). Additionally, an extended-release dispersible tablet formulation of spinosad (7.48% DT) was recently approved by WHO/WHOPES for the residual control of *Ae. aegypti*, the main vector of dengue, in natural and artificial containers (WHO 2008). Associated specifications have also been approved and published for these formulations. WHOPES recommended field use rates are similar, although not identical, to those specified in the US label. In 2007 spinosad was registered for mosquito larvicide uses in Morocco under the tradename Mozkill 120 SC® and subsequently has been registered for use in Turkey, Tunisia, and Spain. Additional spinosad registrations are currently being pursued in Algeria, Greece, Saudi Arabia, and United Arab Emirates, with additional countries to follow.

US spinosad registrations and label rates

Spinosad was officially approved for use as a mosquito larvicide by the US EPA in October of 2007 as a reduced-risk pesticide. Initially approved formulations included a 0.5% granule, 120 g AI/liter EC (1 lb AI/gallon), and 240 g AI/liter EC (2 lb AI/gal), all of which are single-brood (quick-release) formulations. Additionally, 3 multibrood (extended release) formulations were recently approved by the US EPA during June of 2008 including a 2.5% granule and an 8.33% tablet designed to provide ~30–40 days of extended residual control, and a 6.25% tablet with a planned residual life of ~170–180 days. A 7.48% extended-release tablet designed for 60-day residual control of mosquito larvae in natural and artificial container breeding sites (exclusive of potable water sites) was also recently approved by the US EPA.

The US label rates will range from 20 g AI/ha to 50 g AI/ha (=0.018–0.045 lbs AI/acre; =0.020–0.050 ppm AI at a 10-cm water depth), with a provision for higher use rates of 50–112 g AI/ha (=0.045–0.1 lbs AI/acre; =0.050–0.112 ppm AI at a 10-cm water depth) in waters high in organic content or mosquito habitats having deep water or dense surface cover.

Spinosad represents a valuable new natural product for the integrated management of larval mosquitoes. Upon product launch, a number of specialized formulations will be available to control mosquito larvae in a broad array of habitats.

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REFERENCES CITED

Antonio GE, Sanchez D, Williams T, Marina C. 2009. Paradoxical effects of sublethal exposure to the naturally derived insecticide spinosad in the dengue vector mosquito, *Aedes aegypti*. *Pest Manag Sci* 65:323–326.

Ayesa P, Harrington LC, Scott JG. 2006. Evaluation of novel insecticides for control of the dengue vector, *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 43:55–60.

Bahgat IM, El Kady GA, Temerak SA, Lysandrou M. 2007. The natural bio-insecticide spinosad and its toxicity to combat some mosquito species in Ismailia Governorate, Egypt. *World J Agric Sci* 3:396–400.

Bond JG, Marina CF, Williams T. 2004. The naturally derived insecticide spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. *Med Vet Entomol* 18:50–56.

Bret BL, Larson LL, Schoonover JR, Sparks TC, Thompson GD. 1997. Biological properties of spinosad. *Down Earth* 52:6–13.

Brogdon WG, McAllister JC. 1998. Insecticide resistance and vector control. *Emerg Infect Dis* 4:605–613.

Cetin HA, Yanikoglu A, J.E. Cilek JE. 2005. Evaluation of the naturally-derived insecticide spinosad against *Culex pipiens* L. (Diptera: Culicidae) larvae in septic tank water in Antalya, Turkey. *J Vector Ecol* 30:151–154.

Charmillot P-J, Pasquier D, Salamin C, Ter-Hovannesyan A. 2007. Ovicidal and larvicidal effectiveness of insecticides applied by dipping apples on the small fruit tortrix *Grapholita lobarzewskii*. *Pest Manag Sci* 63:677–681.

Cisneros J, Goulson D, Derwent LC, Penagos DI, Hernández O, Williams T. 2002. Toxic effects of spinosad on predatory insects. *Biol Control* 23: 156–163.

Cleveland CB. 2007. Environmental and health assessments for spinosad against the backdrop of organic certification. In: Felsot AJ, Racke KD, eds. *Certified organic and biologically-derived pesticides: environmental, health, and efficacy assessment*. Washington DC: American Chemical Society Symposium Series. p 109–130.

Cleveland CB, Bormett GA, Saunders DG, Powers FL, McGibbon AS, Reeves GL, Rutherford L, Balcer JL. 2002. Environmental fate of spinosad. 1. Dissipation and degradation in aqueous systems. *J Agric Food Chem* 50:3244–3256.

Cleveland CB, Mayes MA, Cryer SA. 2001. An ecological risk assessment for spinosad use on cotton. *Pest Manag Sci* 58:70–84.

Dadd RH. 1971. Effects of size and concentration of particles on rate of ingestion of latex particulates by mosquito larvae. *Ann Entomol Soc Am* 64:687–692.

Dahl C, Sahlen G, Grawe J, Johannisson A, Amneus H. 1993. Differential particle uptake by larvae of three mosquito species (Diptera: Culicidae). *J Med Entomol* 30:537–543.

Darriet F, Corbel V. 2006. Laboratory evaluation of pyriproxyfen and spinosad, alone and in combination, against *Aedes aegypti* larvae. *J Med Entomol* 43:1190–1194.

Darriet F, Duchon S, Hougard JM. 2005. Spinosad: a new larvicide against insecticide-resistant mosquito larvae. *J Am Mosq Control Assoc* 21:495–496.

Duchet C, Larroque M, Caquet Th, Franquet E, Lagneau C, Lagadic L. 2008. Effects of spinosad and *Bacillus thuringiensis israelensis* on a natural population of *Daphnia pulex* in field microcosms. *Chemosphere* 74:70–77.

Jiang Y, Mulla MS. 2009. Laboratory and field evaluations of spinosad, a biorational natural product, against larvae of *Culex* mosquitoes. *J Am Mosq Control Assoc* 25: 456–466.

Kirst HA, Michel KH, Mynderse JS, Chio EH, Yao RC, Nakatsukasa WM, Boeck LD, Occlowitz J, Paschel JW, Deeter JB, Thompson GD. 1992. Discovery, isolation and structure elucidation of a family of structurally unique fermentation-derived tetracyclic macrolides. In: Baker DR, Fenyes JG, Steffens JJ, eds. *Synthesis and chemistry of agrochemicals*. Volume 3. Washington, DC: American Chemical Society. p 214–225.

Kristensen M, Jespersen JB. 2004. Susceptibility of spinosad in *Musca domestica* (Diptera: Muscidae) field populations. *J Econ Entomol* 97:1042–1048.

Liu H, Cupp EW, Guo A, Liu N. 2004a. Insecticide resistance in Alabama and Florida mosquito strains of *Aedes albopictus*. *J Med Entomol* 41:946–952.

Liu H, Cupp EW, Micher KM, Guo A, Liu N. 2004b. Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefaciatus*. *J Med Entomol* 41:408–413.

Magaña C, Hernández-Crespo P, Ortego F, Castañera P. 2007. Resistance to malathion in field populations of *Ceratitis capitata*. *J Econ Entomol* 100:1836–1843.

Merritt RW, Dadd RH, Walker ED. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu Rev Entomol* 37:349–376.

Merritt RW, Mortland MM, Gersabeck ER, Ross DH. 1978. X-ray diffraction analysis of particles ingested by filter-feeding animals. *Entomol Exp Appl* 24:27–34.

Mertz FP, Yao RC. 1990. *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar rum still. *Int J Syst Bacteriol* 40:34–39.

Müller GC, Kravchenko VD, Schlein Y. 2008. Decline of *Anopheles sergentii* and *Aedes caspius* populations following presentation of attractive toxic (spinosad) sugar bait stations in an oasis. *J Am Mosq Control Assoc* 24:147–149.

Perez CM, Marina CF, Bond JG, Rojas JC, Valle J, Williams T. 2007. Spinosad, a naturally-derived insecticide, for control of *Aedes aegypti* (Diptera: Culicidae): efficacy, persistence, and elicited ovipositional response. *J Med Entomol* 44:631–638.

Perry T, McKenzie JA, Batterham P. 2007. A D-α-6 knockout strain of *Drosophila melanogaster* confers a high level of resistance to spinosad. *Insect Biochem Mol Biol* 37:184–188.

Pineda S, Budia F, Schneider MI, Gobbi A, Viñuela E, Valle J, Del Estal P. 2004. Effects of two biorational insecticides, spinosad and methoxyfenozide, on *Spodoptera littoralis* (Lepidoptera: Noctuidae) under laboratory conditions. *J Econ Entomol* 97:1906–1911.

Pridgeon JW, Becnel JJ, Clark GG, Linthicum KJ. 2009. A high-throughput screening method to identify potential pesticides for mosquito control. *J Med Entomol* 46:335–341.

Racke KD. 2007. A reduced risk insecticide for organic agriculture. In: Felsot AJ, Racke KD, eds. *Certified organic and biologically-derived pesticides: environmental, health, and efficacy assessment*. Washington DC: American Chemical Society Symposium Series. p 92–108.

Romi RS, Proietti S, Di Luca M, Cristofaro M. 2006. Laboratory evaluation of the bioinsecticide spinosad for mosquito control. *J Am Mosq Control Assoc* 22:93–96.

Rose RI. 2001. Pesticides and public health: integrated methods of mosquito management. *Emerg Infect Dis* 7:17–23.

Sadanandane C, Boopathi Doss PS, Jambulingam P, Zaim M. 2009. Efficacy of two formulations of the bioinsecticide spinosad against *Culex quinquefasciatus* in India. *J Am Mosq Control Assoc* 25:66–73.

Salgado VL. 1998. Studies on the mode of action of spinosad: insect symptoms and physiological correlates. *Pestic Biochem Physiol* 60:91–102.

Salgado VL, Sparks TC. 2005. The spinosyns: chemistry, biochemistry, mode of action and resistance. In: Gilbert LI, Iatrou K, Gill S, eds. *Comprehensive insect molecular science*. Volume 6 Control. New York: Elsevier B.V. p 137–173.

Sarfraz M, Dosdall LM, Keddie BA. 2005. Spinosad: a promising tool for integrated pest management. *Outlook Pest Manag* April:78–84.

Saunders DG, Bret BL. 1997. Fate of spinosad in the environment. *Down Earth* 52:14–20.

Schaub L, Hardy S, Capkun G. 2002. Natural variation in baseline data: when do we call a new sample resistant? *Pest Manag Sci* 58:959–963.

Shin EH, Park YI, Lee HI, Lee WJ, Shin YH, Shim JC. 2003. Insecticide susceptibilities of *Anopheles sinensis* (Diptera: Culicidae) larvae from Paju-shi, Korea. *Korean J Entomol* 33:33–37.

Shono TS, Scott JG. 2003. Spinosad resistance in the housefly, *Musca domestica*, is due to a recessive factor on autosome 1. *Pestic Biochem Physiol* 77:1–7.

Sparks TC, Thompson GD, Kirst HA, Hertlein MB, Mynderse JS, Turner JR, Worden TV. 1999. Fermentation-derived insect control agents. In: Hall FR, Menn JJ, eds. *Biopesticides: use and delivery*. Totowa, NJ: Humana Press. p 171–188.

Sparks TC, Thompson GD, Larson LL, Kirst HA, Jantz OK, Worden TV, Hertlein MB, Busacca JD. 1995. Biological characteristics of the spinosyns: new naturally derived insect control agents. In: Proceedings of the Beltwide Cotton Conference. 1995 January 4–7; San Antonio, TX: Memphis, TN: National Cotton Council of America. p 903–907.

Thavara U, Tawatsin A, Asavadachanukorn P, Mulla M. 2009. Field evaluation in Thailand of spinosad, a larvicide derived from *Saccharopolyspora spinosa* (Actinomycetales) against *Aedes aegypti* (L.) larvae. *Southeast Asian J Trop Med Public Health* 40:235–242.

Thompson GD, Michel KH, Yao RC, Mynderse JJ, Mosburg CT, Worden TV, Chio EH, Sparks TC, Hutchins SH. 1997. The discovery of *Saccharopolyspora spinosa* and a new class of insect control products. *Down Earth* 52:1–5.

US EPA [Environmental Protection Agency]. 2007a. *Joint statement on mosquito control in the United States from the U.S. Environmental Protection Agency (EPA) and the U.S. Centers for Disease Control and Prevention (CDC)* [Internet]. Available from: http://www.epa.gov/pesticides/health/mosquitoes/mosquitojoint.htm#ipm (Accessed January 20, 2010).

US EPA [Environmental Protection Agency]. 2007b. *Larvicides for mosquito control* [Internet]. Available from: http://www.epa.gov/opp00001/health/mosquitoes/larvicides4mosquitoes.htm (Accessed January 20, 2010).

Viñuela E, Medina MP, Schneider M, Gonzalez M, Budia F, Adan A, Del Estal P. 2001. Comparison of side-effects of spinosad, tebufenozide and azadirachtin on the predators *Chrysoperla carnea* and *Podisus maculiventris* and the parasitoids *Opius concolor* and *Hyposoter didymator* under laboratory conditions. *Bull IOBC/WPRS* 24:25–34.

Wallace JB, Merritt RW. 1980. Filter-feeding ecology of aquatic insects. *Annu Rev Entomol* 25:103–132.

Watson GB. 2001. Actions of insecticidal spinosyns on γ-aminobutyric acid responses from small-diameter cockroach neurons. *Pestic Biochem Physiol* 71:20–28.

WHO [World Health Organization]. 2005. *Guidelines for laboratory and field testing of mosquito larvicides* World Health Organization (WHO) communicable disease control, prevention and eradication WHO Pesticide Evaluation Scheme (WHOPES). Geneva, Switzerland. p 1–41.

WHO [World Health Organization]. 2007. *Review of: spinosad 0.5% GR and 12% SC*. Report of the Tenth WHOPES Working Group meeting. Document WHO/CDS/INTD/WHOPES/2007. Geneva, Switzerland: WHO.

WHO [World Health Organization]. 2008. *Review of: spinosad 7.48% DT*. Report of the Eleventh WHOPES Working Group meeting. Document WHO/HTM/NTD/WHOPES/2008.1. Geneva, Switzerland: WHO.

Zaim M, Guillet P. 2002. Alternative insecticides: an urgent need. *Trends Parasitol* 18:161–163.

Zhao JZ, Li YX, Collins HL, Gusukuma-Minuto L, Mau RFL, Thompson GD, Shelton AM. 2002. Monitoring and characterization of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad. *J Econ Entomol* 95:430–436.

Appendix 1. Database of larval lethal concentration (LC) values (in ppm) for various mosquito species treated with spinosad formulations. Data included here were sourced from published literature or from previously unpublished studies. LC values were generated using standard WHOPES[1] laboratory guidelines unless otherwise noted.

Line	Species	Investigator	Year	Citation	Formulation	Instar	Larvae source	Water type[1]	LC_{50}[1] (ppm)	LC_{90}[1] (ppm)	Exposure period
1	*Aedes aegypti*	Mulla	2006	WHOPES unpublished report	120 SC	L2	Lab colony	Distilled	0.031 (0.02–0.04)	0.084 (0.06–0.20)	24 h
2	*Aedes aegypti*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L2	Lab colony	Distilled	0.039 (0.034–0.045)	0.060 (0.053–0.070)	24 h
3	*Aedes aegypti*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L2	Lab colony	Distilled	0.024 (0.021–0.029)	0.035 (0.031–0.042)	48 h
4	*Aedes aegypti*	Mulla	2006	WHOPES unpublished report	120 SC	L2	Lab colony	Distilled	0.024 (0.02–0.03)	0.06 (0.04–0.12)	48 h
5	*Aedes aegypti*	Lagneau et al.	2006	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.020 (0.017–0.024)	0.117 (0.082–0.191)	24 h
6	*Aedes aegypti*	Lagneau et al.	2006	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.012 (0.011–0.014)	0.045 (0.037–0.057)	24 h
7	*Aedes aegypti*	Lagneau et al.	2006	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.041 (0.023–0.076)	0.332 (0.087–1.38)	24 h
8	*Aedes aegypti*	Lagneau et al.	2006	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.013 (0.011–0.016)	0.060 (0.039–0.094)	48 h
9	*Aedes aegypti*	Lagneau et al.	2006	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.014 (0.012–0.015)	0.047 (0.039–0.060)	48 h
10	*Aedes aegypti*	Lagneau et al.	2006	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.010 (0.009–0.011)	0.030 (0.026–0.035)	48 h
11	*Aedes aegypti*	Gaven and Lagneau	2004	Unpublished[2]	120 SC	L3	Lab colony	DI Tap water	0.007 (0.0062–0.0070)	0.013 (0.0116–0.0142)	72 h
12	*Aedes aegypti*	Lagneau et al.	2006	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.006 (0.0056–0.0066)	0.014 (0.0129–0.0161)	at emergence
13	*Aedes aegypti*	Lagneau et al.	2006	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.006 (0.0051–0.0060)	0.015 (0.0135–0.0172)	at emergence
14	*Aedes aegypti*	Lagneau et al.	2006	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.006 (0.0052–0.0061)	0.014 (0.0125–0.0157)	at emergence
15	*Aedes aegypti*	Mulla	2006	WHOPES unpublished report	120 SC	L4	Lab colony	Distilled	0.030 (0.027–0.033)	0.061 (0.05–0.07)	24 h
16	*Aedes aegypti*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L4	Lab colony	Distilled	0.048 (0.043–0.055)	0.069 (0.061–0.81)	24 h
17	*Aedes aegypti*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L4	Lab colony	Distilled	0.033 (0.029–0.040)	0.046 (0.040–0.058)	48 h
18	*Aedes aegypti*	Mulla	2006	WHOPES unpublished report	120 SC	L4	Lab colony	Distilled	0.023 (0.02–0.03)	0.049 (0.04–0.08)	48 h

Appendix 1. Continued.

Line	Species	Investigator	Year	Citation	Formulation	Instar	Larvae source	Water type[1]	LC_{50}[1] (ppm)	LC_{90}[1] (ppm)	Exposure period
19	*Aedes aegypti*	Bond et al.	2004	*Med Vet Entomol* 18	480 SC	L3–L4	Lab colony	DI tap water	0.025 (0.023–0.027)	–	24 h (with 1 h exposure only)
20	*Aedes aegypti*	Perez et al.	2007	*J Med Entomol* 44(4)	480 SC	L3–L4	Lab colony	DI tap water	0.026 (NR[1])	–	24 h (with 1 h exposure only)
21	*Aedes aegypti*	Antonio et al.	2009	*Pest Manag Sci* 65	480 SC	L3	Field	Dechlorinated tap water	0.060 (0.045–0.079)	–	24 h (with 1 h exposure only)
22	*Aedes aegypti*	Romi et al.	2006	*J Am Mosq Control Assoc* 22(1)	480 SC	L3	Lab colony	DI tap water	0.010 (NR)	0.015 (NR)	24 h
23	*Aedes aegypti*	Romi et al.	2006	*J Am Mosq Control Assoc* 22(1)	480 SC	L3	Lab colony	DI tap water	0.007 (NR)	0.010 (NR)	48 h
24	*Aedes aegypti*	Gaven and Lagneau	2004	Unpublished[2]	480 SC	L3	Lab colony	DI tap water	0.004 (0.0033–0.0046)	0.008 (0.0061–0.0106)	72 h
25	*Aedes aegypti*	Morgan	1997	Unpublished[2]	480 SC	L3–L4	Lab colony	DI tap water	0.029 (0.026–0.032)	0.096 (0.087–0.106)	24 h
26	*Aedes aegypti*	Morgan	1997	Unpublished[2]	480 SC	L3–L4	Lab colony	DI tap water	0.149 (0.062–0.298)	0.493 NA	24 h
27	*Aedes aegypti*	Morgan	1997	Unpublished[2]	480 SC	L3–L4	Lab colony	DI tap water	0.084 (0.036–0.170)	0.260 NA	24 h
28	*Aedes aegypti*	Morgan	1997	Unpublished[2]	480 SC	L3–L4	Lab colony	DI tap water	0.060 (0.032–0.105)	0.191 NA	48 h
29	*Aedes aegypti*	Mulla	2006	WHOPES unpublished report	Tech AI[1]	L2	Lab colony	Distilled	0.088 (0.074–0.11)	0.208 (0.16–0.38)	24 h
30	*Aedes aegypti*	Mulla	2006	WHOPES unpublished report	Tech AI	L2	Lab colony	Distilled	0.052 (0.02–0.08)	0.145 (0.09–1.02)	48 h
31	*Aedes aegypti*	Darriet and Corbel	2006	*J Med Entomol* 43(6)	Tech AI	L3	Lab colony	Distilled	0.055 (0.047–0.064)	–	at emergence
32	*Aedes aegypti*	Darriet et al.	2005	*J Am Mosq Control Assoc* 21(4)	Tech AI	L3	Lab colony	DI tap water	0.350 (0.31–0.40)	–	24 h
33	*Aedes aegypti*	Jiang and Mulla	2005	Unpublished[2]	Tech AI	L4	Lab colony	DI tap water	0.055 (0.044–0.070)	0.098 (0.075–0.193)	24 h
34	*Aedes aegypti*	Jiang and Mulla	2005	Unpublished[2]	Tech AI	L4	Lab colony	DI tap water	0.055 (0.005–0.059)	0.116 (0.102–0.138)	24 h
35	*Aedes aegypti*	Mulla	2006	WHOPES unpublished report	Tech AI	L4	Lab colony	Distilled	0.084 (0.08–0.09)	0.155 (0.14–0.18)	24 h

Appendix 1. Continued.

Line	Species	Investigator	Year	Citation	Formulation	Instar	Larvae source	Water type[1]	LC_{50}[1] (ppm)	LC_{90}[1] (ppm)	Exposure period
36	*Aedes aegypti*	Jiang and Mulla	2005	Unpublished[2]	Tech AI	L4	Lab colony	DI tap water	0.049 (0.038–0.067)	0.101 (0.072–0.239)	48 h
37	*Aedes aegypti*	Jiang and Mulla	2005	Unpublished[2]	Tech AI	L4	Lab colony	DI tap water	0.048 (0.043–0.052)	0.113 (0.098–0.138)	48 h
38	*Aedes aegypti*	Mulla	2006	WHOPES unpublished report	Tech AI	L4	Lab colony	Distilled	0.059 (0.05–0.07)	0.126 (0.10–0.18)	48 h
39	*Aedes aegypti*	Ayesa et al.	2006	*J Med Entomol* 43(1)	Tech AI	L4	Lab colony	Distilled	0.160 (0.12–0.20)	–	72 h
40	*Aedes albopictus*	Liu et al.	2004a	*J Med Entomol* 41(5)	Tech AI	L4	Lab colony	DI tap water	0.300 (0.2–0.4)	0.900 (0.6–1.5)	24 h
41	*Aedes albopictus*	Lagneau et al.	2008	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.005 (0.004–0.006)	0.068 (0.040–0.096)	24 h
42	*Aedes albopictus*	Lagneau et al.	2008	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.002 (0.0015–0.0025)	0.016 (0.012–0.020)	48 h
43	*Aedes albopictus*	Lagneau et al.	2008	Unpublished[2]	120 SC	L3	Lab colony	Osmosed	0.002 (0.0014–0.0023)	0.010 (0.007–0.013)	72 h
44	*Aedes vigilax*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L2	Lab colony	Distilled	0.014 (0.012–0.017)	0.027 (0.023–0.033)	24 h
45	*Aedes vigilax*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L2	Lab colony	Distilled	0.003 (0.002–0.003)	0.005 (0.004–0.006)	48 h
46	*Aedes vigilax*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L4	Lab colony	Distilled	0.024 (0.018–0.033)	0.045 (0.036–0.063)	24 h
47	*Aedes vigilax*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L4	Lab colony	Distilled	0.004 (0.004–0.005)	0.007 (0.006–0.008)	48 h
48	*Anopheles albimanus*	Bond et al.	2004	*Med Vet Entomol* 18	480 SC	L3–L4	Lab colony	DI tap water	0.024 (NR)	–	24 h (with 1 h exposure only)
49	*Anopheles gambiae*	Darriet et al.	2005	*J Am Mosq Control Assoc* 21(4)	Tech AI	L3	Lab colony	DI tap water	0.010 (0.009–0.012)	–	24 h
50	*Anopheles psuedo-punctipennis*	Bond et al.	2004	*Med Vet Entomol* 18	480 SC	L3–L4	Lab colony	DI tap water	0.010 (NR)	–	24 h (with 1 h exposure only)
51	*Anopheles sinensis*	Shin et al.	2003	*Korean J Entomol* 33(1)	Tech AI	L3–L4	Field	DI tap water	0.030 (0.024–0.037)	0.074 (NR)	24 h
52	*Anopheles stephensi*	Romi et al.	2006	*J Am Mosq Control Assoc* 22(1)	480 SC	L3	Lab colony	DI tap water	0.039 (NR)	0.101 (NR)	24 h
53	*Anopheles stephensi*	Romi et al.	2006	*J Am Mosq Control Assoc* 22(1)	480 SC	L3	Lab colony	DI tap water	0.024 (NR)	0.042 (NR)	48 h
54	*Anopheles stephensi*	Laddoni et al.	2006	WHOPES unpublished report	Tech AI	L3	Lab colony	DI tap water	0.004 (SE ± 0.002)	0.020 (SE ± 0.004)	48 h
55	*Culex pipiens*	Cetin et al.	2005	*J Vector Ecol* 30(1)	120 SC	L3–L4	Field	Septic tank water	0.027 (0.002–0.057)	0.111 (0.054–5.38)	24 h

Appendix 1. Continued.

Line	Species	Investigator	Year	Citation	Formulation	Instar	Larvae source	Water type[1]	LC_{50}[1] (ppm)	LC_{90}[1] (ppm)	Exposure period
56	*Culex pipiens*	Bahgat et al.	2007	*World J Agric Sci* 3(4)	120 SC	L3	Field	Field sewage water	< 0.00098 (NA[1])	<0.00098 (NA)	24 h
57	*Culex pipiens*	Bahgat et al.	2007	*World J Agric Sci* 3(4)	120 SC	L3	Field	DI tap water	0.002 (0.002–0.003)	0.006 (0.004–0.010)	24 h
58	*Culex pipiens*	Lyagoubi and Faraj	2004	Unpublished[2]	120 SC	L3–L4	Field	Sewage water	0.035 (NR)	0.076 (NR)	24 h
59	*Culex pipiens*	Lyagoubi and Faraj	2004	Unpublished[2]	120 SC	L3–L4	Field	Sewage water	0.024 (NR)	0.051 (NR)	48 h
60	*Culex pipiens*	Lyagoubi and Faraj	2004	Unpublished[2]	120 SC	L3–L4	Field	Sewage water	0.020 (NR)	0.040 (NR)	72 h
61	*Culex pipiens*	Ben Salah and Alimi	2005	Unpublished[2]	120 SC	L3–L4	Field	Stagnant groundwater	0.023 (NR)	0.093 (NR)	48 h
62	*Culex pipiens*	Mavrotas	1997	Unpublished[2]	480 SC	L3–L4	Lab colony	DI tap water	–	0.056 (0.044–0.074)	24 h
63	*Culex pipiens*	Mavrotas	1997	Unpublished[2]	480 SC	L3–L4	Lab colony	DI tap water	–	0.044 (0.032–0.060)	24 h
64	*Culex pipiens*	Mavrotas	1997	Unpublished[1]	480 SC	L3–L4	Lab colony	DI tap water	–	0.046 (0.030–0.066)	24 h
65	*Culex pipiens*	Romi et al.	2006	*J Am Mosq Control Assoc* 22(1)	480 SC	L3	Lab colony	DI tap water	0.006 (NR)	0.018 (NR)	24 h
66	*Culex pipiens*	Romi et al.	2006	*J Am Mosq Control Assoc* 22(1)	480 SC	L3	Lab colony	DI tap water	0.003 (NR)	0.013 (NR)	48 h
67	*Culex pipiens*	Ben Salah and Alimi	2005	Unpublished[2]	480 SC	L3–L4	Field	Stagnant groundwater	0.013 (NR)	0.041 (NR)	48 h
68	*Culex pipiens*	Ben Salah and Alimi	2005	Unpublished[2]	480 SC	L3	Field	Stagnant groundwater	0.0005 (NR)	0.066 (NR)	48 h
69	*Culex pipiens*	Bahgat et al.	2007	*World J Agric Sci* 3(4)	Dust 0.125%	L3	Field	Field sewage water	0.022 (0.013–0.037)	0.175 (0.044–0.691)	24 h
70	*Culex pipiens*	Bahgat et al.	2007	*World J Agric Sci* 3(4)	Dust 0.125%	L3	Field	DI tap water	0.007 (0.005–0.011)	0.040 (0.020–0.083)	24 h
71	*Culex pipiens*	Ben Salah and Alimi	2005	Unpublished[2]	Tech AI	L1	Field	Stagnant groundwater	0.002 (NR)	0.010 (NR)	48 h
72	*Culex pipiens*	Ben Salah and Alimi	2005	Unpublished[2]	Tech AI	L3	Field	Stagnant groundwater	0.009 (NR)	0.046 (NR)	48 h
73	*Culex pipiens*	Ben Salah and Alimi	2005	Unpublished[2]	Tech AI	L1	Field	Stagnant groundwater	0.009 (NR)	0.024 (NR)	24 h
74	*Culex quinquefasciatus*	Mulla	2006	WHOPES unpublished report	120 SC	L2	Lab colony	Distilled	0.004 (0.003–0.005)	0.015 (0.012–0.019)	24 h

Appendix 1. Continued.

Line	Species	Investigator	Year	Citation	Formulation	Instar	Larvae source	Water type[1]	LC_{50}[1] (ppm)	LC_{90}[1] (ppm)	Exposure period
75	*Culex quinquefasciatus*	Mulla	2006	WHOPES unpublished report	120 SC	L2	Lab colony	Distilled	0.003 (0.001–0.005)	0.012 (0.009–0.02)	48 h
76	*Culex quinquefasciatus*	Mulla	2006	WHOPES unpublished report	120 SC	L4	Lab colony	Distilled	0.013 (0.009–0.019)	0.038 (0.03–0.09)	24 h
77	*Culex quinquefasciatus*	Mulla	2006	WHOPES unpublished report	120 SC	L4	Lab colony	Distilled	0.011 (0.008–0.014)	0.026 (0.02–0.04)	48 h
78	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	120 SC	L2	Lab colony	DI tap water	0.012 (0.011–0.013)	0.026 (0.022–0.032)	24 h
79	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	120 SC	L2	Lab colony	DI tap water	0.010 (0.009–0.012)	0.023 (0.020–0.029)	48 h
80	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	120 SC	L4	Lab colony	DI tap water	0.014 (0.013–0.016)	0.032 (0.029–0.037)	24 h
81	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	120 SC	L4	Lab colony	DI tap water	0.013 (0.010–0.016)	0.030 (0.023–0.045)	48 h
82	*Culex quinquefasciatus*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L2	Field	Distilled	0.022 (0.009–0.337)	0.036 (0.022–0.929)	24 h
83	*Culex quinquefasciatus*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L2	Field	Distilled	0.014 (0.012–0.017)	0.022 (0.019–0.027)	48 h
84	*Culex quinquefasciatus*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L4	Field	Distilled	0.023 (0.016–0.040)	0.038 (0.027–0.075)	24 h
85	*Culex quinquefasciatus*	Ritchie and Zborowski	2003	Unpublished[2]	120 SC	L4	Field	Distilled	0.017 (0.015–0.020)	0.027 (0.023–0.032)	48 h
86	*Culex quinquefasciatus*	Mulla	2006	WHOPES unpublished report	Tech AI	L2	Lab colony	Distilled	0.065 (0.04–0.09)	0.174 (0.12–0.40)	24 h
87	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	Tech AI	L2	Lab colony	DI tap water	0.024 (0.016–0.037)	0.052 (0.035–0.163)	24 h
88	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	Tech AI	L2	Lab colony	DI tap water	0.021 (0.013–0.037)	0.051 (0.032–0.158)	24 h
89	*Culex quinquefasciatus*	Mulla	2006	WHOPES unpublished report	Tech AI	L2	Lab colony	Distilled	0.042 (0.04–0.05)	0.109 (0.10–0.13)	48 h

Appendix 1. Continued.

Line	Species	Investigator	Year	Citation	Formulation	Instar	Larvae source	Water type[1]	LC_{50}[1] (ppm)	LC_{90}[1] (ppm)	Exposure period
90	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	Tech AI	L2	Lab colony	DI tap water	0.019 (0.017–0.021)	0.053 (0.044–0.068)	48 h
91	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	Tech AI	L2	Lab colony	DI tap water	0.019 (0.012–0.032)	0.049 (0.030–0.057)	48 h
92	*Culex quinquefasciatus*	Laddoni et al.	2006	WHOPES unpublished report	Tech AI	L3	Lab colony	DI tap water	0.010 (SE ± 0.002)	0.040 (SE ± 0.007)	48 h
93	*Culex quinquefasciatus*	Darriet et al.	2005	*J Am Mosq Control Assoc* 21(4)	Tech AI	L3	Lab colony	DI tap water	0.093 (0.065–0.13)	–	24 h
94	*Culex quinquefasciatus*	Liu et al.	2004b	*J Med Entomol* 41(3)	Tech AI	L4	Lab colony	DI tap water	0.100 (0.08–0.1)	0.300 (0.2–0.4)	24 h
95	*Culex quinquefasciatus*	Sadanandane et al.	2006	WHOPES unpublished report	Tech AI	L4	Field	Habitat water	0.085 (0.082–0.088)	0.189 (0.179–0.198)	24 h
96	*Culex quinquefasciatus*	Mulla	2006	WHOPES unpublished report	Tech AI	L4	Lab colony	Distilled	0.094 (0.06–0.15)	0.281 (0.17–1.07)	24 h
97	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	Tech AI	L4	Lab colony	DI tap water	0.031 (0.024–0.041)	0.062 (0.046–0.211)	24 h
98	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	Tech AI	L4	Lab colony	DI tap water	0.033 (0.025–0.042)	0.060 (0.046–0.105)	24 h
99	*Culex quinquefasciatus*	Mulla	2006	WHOPES unpublished report	Tech AI	L4	Lab colony	Distilled	0.074 (0.07–0.08)	0.240 (0.19–0.27)	48 h
100	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	Tech AI	L4	Lab colony	DI tap water	0.027 (0.018–0.037)	0.056 (0.040–0.127)	48 h
101	*Culex quinquefasciatus*	Jiang and Mulla	2009	*J Am Mosq Control Assoc* 25(4)	Tech AI	L4	Lab colony	DI tap water	0.026 (0.024–0.029)	0.049 (0.044–0.056)	48 h

[1] WHOPES, World Health Organization Pesticide Evaluation Scheme (WHOPES) Phase II–III evaluation; tech AI, technical active ingredient; DI, deionized; LC_{50}, 50% lethal concentration; LC_{90}, 90% lethal concentration; NR, not reported; NA, noncalculable.

[2] Available upon request to Dow AgroSciences