**From:** Karen Larson [mailto:klarson@clarke.com]   
**Sent:** Wednesday, April 30, 2014 1:16 PM  
**To:** Graham, Alan  
**Cc:** Giguere, Cary; Wally Terrill; Jeff Hottenstein; Josh Matta; Jonathan Ostrowski  
**Subject:** RE: Natular G30

Alan,

I apologize I have been unable to find species-specific LC50 and LC90 data for you.  I had provided published work which summarizes the available data (referenced below).  It is discussed in that report the importance of formulation in LC50/90 work.  You’ll note the difference in mosquito susceptibility to technical spinosad versus a formulated suspension concentrate.  Since spinosad itself is not particularly soluble nor stable in open natural bodies of water, formulation enhancements that improve delivery to and solubility within the water column will enhance bioactivity.  Although I don’t presently have formula-specific LC50 work, it is evident from our field work that the G30 formulation is effective for the full 30-day control period against initially exposed populations of mixed L1 – L4 and against subsequent early instars.

For slow-release/sustained-release products like Natular G30 it is the young neonates which are targeted.   Published data largely references LC50 work on 3rd and early 4th instar mosquito larvae, as is the WHO protocol.  There is substantial need to quantify susceptibility to earliest instars, as well as to elucidate the importance of formulation type.  We are preparing methods to identify product-specific LC50 and LC90 data against Ae. aegypti (the most robust colony available to us presently), initially following the WHO protocols with late instars and following with early instar challenges.  I won’t have this data complete before your meeting next week, but we hope it will be the beginning of a robust body of work to steward this product.

Good luck next week. I will be attending the AMCA Washington Conference, but will be available by cellphone and by email to provide any support I can.

Best Regards,

Karen

Mobile (630) 675-0936

**From:** Graham, Alan [<mailto:Alan.Graham@state.vt.us>]   
**Sent:** Wednesday, April 30, 2014 10:51 AM  
**To:** Karen Larson  
**Cc:** Giguere, Cary  
**Subject:** RE: Natular G30

Hi Karen,

I just spoke with Wally Terrill yesterday at a meeting here in Vermont.  He suggested that you may have already sent us all the available information that you have for Naturlar G30.  If that is the case, please don’t go to any extra efforts.  I will simply use the information that you have provided us and see if we can get it approved by the Vermont Pesticides Advisory Council.  I appreciate your help and hope that we will be able to trial the product this year.  Naturlar G was approved and that will be welcome addition to our list of approved mosquito larvicides.

Best Regards,

Alan

**From:** Graham, Alan   
**Sent:** Monday, April 21, 2014 2:03 PM  
**To:** 'Karen Larson'  
**Cc:** Cary Giguere ([Cary.Giguere@state.vt.us](mailto:Cary.Giguere@state.vt.us)); Schmalz, Tim  
**Subject:** RE: Natular G30

Hi Karen,

On May 6 we have another Vermont Pesticides Advisory Council meeting to review the pesticide Natular G30 for use here in Vermont.  I would appreciate any additional information you might be able to provide.   Your data on larval instar susceptibility was helpful.

Thanks for your help,

Alan

**From:** Karen Larson [<mailto:klarson@clarke.com>]   
**Sent:** Tuesday, March 25, 2014 5:31 PM  
**To:** Graham, Alan  
**Cc:** Wally Terrill; Schmalz, Tim; Giguere, Cary; Grifith Lizarraga  
**Subject:** RE: Natular G30

Alan,

I won’t be able to review your attachments with much specificity today; I will try to get you at least a brief reply by the end of the week.  I was able, however, to review your two instar susceptibility questions with our field science team:

Does Natular G30 have different effects on each of the mosquito instars?   Early instars (Neo-nates, First and second instar) consume more % of their body weight than later (third and fourth) instars; and may be presumed to be more affected through ingestion as well as contact exposure.  Although the mechanism of effect may be different, Natular G30 is effective at labeled rates for all larval mosquito instars, with peak cumulative mortality occurring at 72 hours post-exposure.

Do you have LD50 values for Natular G30 for first and second mosquito larvae.  Traditional assessments of dose response are done in third (L3) and fourth (L4) instar larvae.  We’ve not seen prolific LD50 publications for the earliest instars, but some comparative work is presented in the attached.  Hertlein et al (2010, J. Am Mosquito Control Association 26(1):67-87) discusses larval instar susceptibility to spinosad:

**Larval instar susceptibility.** The relationship between mosquito larval instar and LC-50 and LC-90 values were 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 120SC.  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).  Regression analysis detected no relationship between larval instar and LC-50 value (LC-50 = 0.0520 + 0.00 [Larval instar]; r2 (adj) = 0.0%; DF 1, 2; p=1.00) for the tech AI formulation. Similar analyses of *Cx. quinquefasciatus*  larvae treated with spinosad tech AI formulations, this time including L2, L3 and L4 instars, suggest no relationship between larval instar and LC-50 (LC-50 = 0.0075 + 0.00783 [Larval instar]; r2 (adj) = 0.0%; DF 1, 5; p=0.419) or LC-90 (LC-90 = 0.0181 + 0.0223 [Larval instar]; r2 (adj) = 0.0%; DF 1, 5; p=0.498). A final comparison was made of *Ae. aegypti* L2, L3, and L4 larval instars treated with spinosad 120 SC formulations. Regression analyses again failed to detect any evidence of a relationship between larval instar and either LC-50 (LC-50 = 0.0141 + 0.002 [Larval instar]; r2 (adj) = 0.0%; DF 1, 5; p=0.665) or LC-90 (LC-90 = 0.0467 + 0.00[Larval instar]; r2 (adj) = 0.0%; DF 1, 5; p=1.00).

I hope this little bit is helpful.  I will try to review & comment further as soon as I can.

Karen

**From:** Graham, Alan [<mailto:Alan.Graham@state.vt.us>]   
**Sent:** Tuesday, March 25, 2014 2:24 PM  
**To:** Karen Larson  
**Cc:** Wally Terrill; Schmalz, Tim; Giguere, Cary  
**Subject:** Natular G30

Karen,

I would to see Naturlar G30 permitted for use in Vermont.   The Vermont Pesticides Advisory Council (VPAC) reviews such requests and has looked at your Natular G30 Exposure Assessment Summary.   Rick Levey reviewed your summary and made the following comments (see attachment).   I have also looked at your summary and tried to address concerns Rick made (attachment).  At some point, would you be able to quickly answer Rick’s major concerns and correct any mistakes I may have made in my reasoning (attachment)?

Perhaps the most basic two questions to be answered right away are as follows.

Does Natular G30 have different effects on each of the mosquito instars?

Do you have LD50 values for Natular G30 for first and second mosquito larvae.

Any quick answers to my basic two questions above would be helpful for me to have.  I will be presenting a summary of the 2013 Vermont mosquito surveillance and suppression activities at the VPAC tomorrow at 1 pm.  A discussion of Natular G30 is also on the agenda.  Your thoughts about Rick’s comments on Naturlar G30 would be helpful, but not necessary for tomorrow’s meeting.

I will understand if you do not have time to respond on such short notice.  Thanks for your time.

Alan

Alan C. Graham

State Entomologist

Vermont Agency of Agriculture

322 Industrial Lane

Barre, VT  05641

telephone: 802-828-1319

email:  [alan.graham@state.vt.us](mailto:alan.graham@state.vt.us)

website:  <http://agriculture.vermont.gov/plant_pest/mosquitoes_ticks/mosquitoes>