VectoBac (Bti) WDG Biological Mosquito Larvicide Applications for Control of Zika Vectors

Frequently Asked Questions

1) **What is Bti?**
    a. Bti (*Bacillus thuringiensis* sub-species *israelensis*) is a naturally occurring bacteria found in the soils worldwide. It is a bacterial insecticide, not a chemical. In addition, Bti is different than any other type of bacteria in the world. It produces crystal shaped proteins that only kills the larval stage of mosquitoes, black flies (this specific biting fly is sometimes called a "buffalo gnat"), and other closely related flies.
        i. Latin Meaning: "*Bacillus*" means that this is a bacteria and "*thuringiensis*" relates to the province of Thuringia where a German scientist actually named this type/family of bacteria. "*israelensis*" is the name of the actual species of Bt, and there are dozens of different Bt types. This specific organism, *Bt israelensis* was actually found infecting mosquitoes in a puddle after a rainstorm in an Israeli desert.
    b. Bti is produced through fermentation, similar to making spirits or yogurt. The bacteria are fed nutrients and grow in large stainless steel tanks. After several days, nutrients are cut off. The bacteria die, leaving dead cells, crystal proteins, spores in the broth. The broth from these tanks is collected and then processed to make the various formulations of Bti that we produce.

2) **How does Bti work?**
    a. Bti produces a protein crystal during fermentation. These microscopic crystals are ingested by mosquito larvae as they feed. In the mosquito's alkaline digestive system, the crystals are dissolved and converted by specific enzymes into protein molecules that bind with unique receptor sites on the mosquito gut wall. This binding causes changes which destroy the walls of the mosquito larvae's gut. The larvae quit feeding within hours and quickly die.
    b. Bti affects only a select insect type (mosquitoes and other closely related flies in the sub-order of Diptera known as Nematocera); it must be ingested to work (hence it only works against larvae that are actively feeding), and it only sets up a reaction in their alkaline gut. It doesn't impact beneficial insect populations, as Bti works a bit like a 'lock and key' in the gut of susceptible insects. Other insects don't have the same structure and physiology for Bti to work on them.

3) **Is Bti safe?**

    a. Bti has undergone nearly 40 years of lab/field research and over 30 years of large-scale operational use in a variety of public health programs around the globe. Bti is very target-specific with activity largely restricted to mosquitoes and related flies (in the sub-order Nematocera of the order Diptera).

    b. In addition to Bti's non toxicity to beneficial insects, numerous toxicology studies have shown Bti to be non-pathogenic and non-toxic to other forms of wildlife (birds, fish, mammals, etc.) and humans. The World Health Organization has approved the use of Bti for drinking water. This is particularly important in many regions around the globe that depend on potable water or rain water stored in vessels in and around their home.

Unfortunately, these vessels are often prime locations for mosquito vectors of Zika virus to lay their eggs. The same species of mosquito also transmits the diseases dengue, chikungunya and yellow fever.

4) What is VectoBac?

a. VectoBac is the brand name for a specific strain of Bti (strain AM65-52). All Bti strains can be 'lumped' into a category known as H14, but there are several strains within this category, and while all Bti products share some common characteristics, there are significant differences amongst the strains of Bti that are used to produce the final products.
b. Strain is important to assure the bacteria's capacity to produce only the right proteins and express those proteins properly during fermentation, in fact VBC's strain AM65-52 is only 1 of 2 strains evaluated and recommended by the World Health Organization as a mosquito larvicide in its public health pesticide evaluation program
c. While genetics plays a crucial role, the environment in which a bacteria grows can also have a significant impact on performance. While many traits may be similar, different environments can result in distinctly different individuals. The same holds true for the same species of bacteria being produced by two different manufacturers/environments. VectoBac's unique strain number for this bacterial mosquito larvicide is a critical link to product performance and quality expectations. The published literature shows that identical strains produced under different conditions can affect performance similar to different yoghurt cultures tasting different.
d. In addition, quality control (QC) requirements applied for fermentation of Bti strain AM65-52 are critical to ensure consistency and safety of VectoBac branded larvicides by ensuring that other potentially harmful pathogens are not produced during fermentation. The same QC measures are not utilized by all manufacturers of bacterial-based larvicides. As such, it is critical to differentiate these products based on the manufacturer's unique strain number. The use of generic strain identifers does not guarantee identical product performance or environmental safety.

5) What is VectoBac WDG?

a. VectoBac WDG (also known as WG) Bacterial Larvicide is a Water Dispersible Granule formulation of Bti, strain AM65-52 for control of mosquito larvae. It is designed for use in wide area larvicide spraying and direct larvicide application to water containers that produce Zika virus vectors (the same mosquito species that transmit dengue, chikungunya and yellow fever.)
b. VectoBac WDG was the first bacterial larvicide to complete the World Health Organization (WHO) pesticide evaluation scheme.
c. VectoBac WDG consists only of Bti strain AM65-52 and food-grade ingredients; it is gluten-free, egg-free and nut-free. It is approved by the Organic Materials Review Institute [OMRI] and the US Department of Agriculture for acceptance in the USDA National Organic Program [NOP].

6) What are container mosquitoes?

a. Container mosquito species prefer to lay eggs in artificial containers (e.g., waste tires, flowerpots, gutters, trash cans, etc.) or natural containers (bromelaid leaf axils, tree holes, fallen leaves etc.) that can hold water. Egg laying takes place just above water level. The species *Aedes aegypti* and *Aedes albopictus* are the primary disease vectors of concern worldwide, as they can transmit Zika, dengue, chikungunya and yellow fever. *Aedes aegypti* is primarily an urban mosquito which prefers artificial container types. *Aedes albopictus* is a more opportunistic species and inhabits both artificial and natural containers.

7) Why are container mosquitoes so difficult to control?

a. Programs that target *Aedes aegypti* and *Aedes albopictus* are not considered by mosquito control professionals as "general mosquito control". In Florida, the bulk of programs focus intensely on saltmarsh mosquitoes (*Aedes taeniorhynchus*) which, if left untreated, would result in severe mosquito annoyance and large tracts of uninhabitable land.
b. *Ae. aegypti/albopictus* have a distinctly different biology. They are referred to often as "container mosquitoes" because of their affinity for laying eggs in clean or fresh water that is protected/containerized, and their development in urban and suburban areas; these mosquitoes will not be found laying eggs in the salt marshes of Florida. In addition, they will lay their eggs in very small containers; in fact as little as a teaspoonful is needed. Aedes aegypti and albopictus are not strong fliers and typically they remain in the neighborhoods in which they develop. Of course, imagine all the "containers", both artificial and natural, that abound in and around human dwellings. Waste tires, bromeliads, flower pots, plastic trash, buckets, etc. all collect rain water and are ideal locations for *Aedes aegypti/albopictus*.
c. As such, it would be impractical to physically locate and treat each and every water container used by these mosquito species, so wide-area spray application, is needed for effectively reach the many small and hard-to-find habitats of these vectors.
d. Aerial application procedures that are used for VectoBac WDG wide area larvicide spray to control container mosquitoes are significantly different than the techniques used to treat open water bodies and wetlands for general mosquito control.
e. Valent BioSciences (VBC) has over 30 years' experience in aerial application of larvicides to control public health and forest health insect pests and has adapted the techniques developed for forest insect control for wide area larvicide spray to control container mosquitoes. Specific aircraft parameters, including nozzle type and output, flight patterns and flight altitude allows the WDG spray mixture to penetrate through vegetation, and into the targeted containers.
f. In addition, VBC has over 10 years' experience with consistent success of VectoBac® WDG ground spray programs for control of container mosquitoes. Several publications have been made confirming ground based larval efficacy and adult suppression. These types of container mosquito control programs have been fully operational in Singapore and Malaysia for many years.

8) Why are there so many issues regarding the control of container mosquitoes?

a. The control of any disease vector can be a challenge; the starting point for container mosquito control should be an integrated approach that utilizes source reduction, as well as larviciding and adulticiding. Habitat and species determine the application methods to be used, and this in turn drives the selection of a product solution that addresses those specific needs.
b. **Mosquito Species Challenges**
    i. Daytime flying and biting behavior limits opportunity for ULV spraying.
    ii. Anthropophilic and anthropophagic behavior (they like to live around and bite humans)
    iii. Extremely adaptable to urban envrironments.
    iv. Success at colonizing both artificial man-made and natural containers

v. It takes very little water for container mosquito larvae to survive to become blood seeking adults

c. **Mosquito Habitat Challenges**
   i. Container mosquitoes prefer man-made habitats such as roof gutters, water-holding refuse, plastic toys, and flower pots, as well as natural, plant-based 'containers' such as leaf axils, tree holes, and fallen leaves
   ii. These habitats are in abundance in urban/semi-urban areas
   iii. In many situations, people need to store water on their property to survive
   iv. While many of these sites are obvious and water can either be removed or treated with larvicides, other sites can be small, hard-to-find, and hard-to-treat.

d. **Insecticide Application Challenges**
   i. **Source Reduction Intervention**
   Source Reduction is considered the most permanent form of mosquito control because it eliminates or reduces habitats where mosquitoes develop. Since many mosquito species lay their eggs in standing water, source reduction often includes the removal of debris or other vessels in which water collects — items such as buckets or discarded tires.
      1. ***The Challenge:*** For this strategy, “reduction” is the operative word since it is often impossible to eliminate all sources of standing water in an affected area. And since container mosquitoes often lay their eggs in sites that are extremely small and difficult to find, complete source reduction is impossible on a large-scale. The labor cost of source reduction is substantial in most urban areas, and many properties cannot be accessed for source reduction.

   ii. **Larviciding Intervention**
   Larviciding is widely considered the most effective form of mosquito control. Applied using ground or aerial treatments, larvicides kill mosquito larvae before they become biting adults. Larviciding as a base-line intervention is gaining widespread acceptance because it efficiently suppresses mosquito populations in their most vulnerable stages of development, and improves the efficiency of adulticides when needed. Reducing the number of mosquito larvae ultimately reduces the number of adult mosquitoes thereby reducing the number of adults and also helping improve the efficacy of the adult sprays.
      1. ***The Challenge:*** Similar to the challenges highlighted for source reduction, traditional larviciding application methods require full knowledge of the locations of all water sources and breeding sites. Since container mosquito larval habitats are often temporary and cryptic, historically it has been difficult to find and address all habitats within a given area.

   iii. **Adulticiding Intervention**
   Ultimately, it is adult mosquitoes that are responsible for disease transmission, so it follows that any program actively combating vector-borne disease over wide areas will include an important ULV adulticiding component. While daytime activity and night time resting of container mosquitoes can render ULV adulticiding less efficient than other forms of control, adulticides serve as a necessary last line of defense – often the 'go-to' approach when disease outbreaks occur.

1. ***The Challenge:*** While many common mosquitoes are active at dusk, aggressive, container breeding mosquito species such as *Aedes aegypti* and *Aedes albopictus* look

for blood sources during the day. Limiting container breeding interventions only to adulticides becomes complicated, since the atmospheric conditions for ULV spraying of adulticides are not optimal during daylight hours when these species are most active. In addition, beneficial insects such as bees are also active during daylight hours, so ULV adulticide labels often limit applications during the day.

9) Is there any evidence that VectoBac WDG wide area sprays can control container mosquitoes and help stop Zika transmission?

a. **Yes, case studies below:**
In Malaysia, where the primary habitat is urban, natural/man-made containers, larvae were treated with ground and backpack spray applications of VectoBac WDG. The result: Dengue cases reduced 93%

In Cambodia, where the primary habitat is man-made water jars in villages, larvae were treated with ground spray and direct treatment applications of VectoBac WDG. The result: Human Dengue cases reduced 43%

In the Florida Keys, where the primary habitat is natural/man-made containers, helicopters routinely spray applications of VectoBac WDG. The result: No local transmission of Dengue since 2010

b. First US cases of locally acquired Zika virus
    i. Florida Governor Rick Scott first declared on July 29, 2016 that the Zika cases identified from residents of the Wynwood district in Miami Florida were a result of being bitten locally with mosquitoes that had the Zika virus. Starting August 6, 2016, a large scale, integrated vector management program was launched headed by the aerial wide area larvicide spraying of VectoBac WDG to treat Zika virus vector larval habitats. After an intensive spray program, Governor Scott declared on September 19, 2016 (in combination with the Center for Disease Control) that the Wynwood district was "Zika transmission" free.

c. VectoBac WDG aerial spray has been used in urban aerial operations with demonstrated success for >5 years at the Florida Keys Mosquito Control District (FKMCD)
    i. (>50) applications of VectoBac WDG over urban areas.
    ii. Efficacy of VectoBac WDG has been repeatedly demonstrated using bioassay of containers within the treatment zones showing very high levels of *Aedes aegypti* larval mortality following individual sprays.
    iii. The impact of repeated VectoBac WDG sprays on *Aedes aegypti* adults in treatment zones has been demonstrated with BG trapping and ovitraps in a multi-year study.
    iv. VectoBac WDG sprays have consistently driven *Aedes aegypti* populations down over time.
    v. FKMCD continues to expand their use of this technology.

d. VectoBac WDG aerial spray was evaluated with clear efficacy results in a multi-year study in Grand Cayman
    i. Individual sprays showed very high mortality (>95%) of *Aedes aegypti* in container bioassays after spraying.
    ii. Adult *Aedes aegypti* populations were suppressed by >70% following repeated sprays.

e. Due to the successes in Miami-Dade County and the Florida Keys, many other jurisdictions have made preparations for VectoBac WDG wide area spray programs to control container mosquitoes that can vector the Zika virus.

f. VectoBac WDG ground and aerial sprays have been used globally for more than 10 years with consistent success against container mosquitoes (e.g. *Aedes aegypti* and *Aedes albopictus*) that can transmit Zika virus
    i. Several publications have been made confirming larval efficacy and adult suppression
    ii. Fully operational programs in Singapore and Malaysia(?) for many years

10) Should I be concerned and what should I do if a VectoBac WDG wide area spray program is conducted in or over my neighborhood?

a. There is nothing harmful or toxic in VectoBac WDG; it is gluten-free, egg-free, and nut-free. It won't affect you, your family or your pets. The VectoBac spray deposit may show as very small droplets on outside surfaces of your home and your vehicle. The spray deposit will not affect your home and garden, outdoor furniture or your vehicles. If you have any medical issues, we would suggest that you consult your medical doctor and if you are still concerned or feel nervous for any reason, stay inside during the short time that your property/neighborhood is being treated. Spraying is conducted early in the day, before most residents have started their day, and it happens quickly; many residents aren't even aware of it.

11) Where can I get more information/studies regarding Bti and VectoBac WDG wide area spray programs?

a. World Health Organization – Environmental Health Criteria 217 for *Bacillus thuringiensis* (http://www.who.int/ipcs/publications/ehc/en/EHC217.PDF?ua=1)
b. What is Bti? Q&A list on Bti from the Environmental Protection Agency (EPA) (https://www.epa.gov/mosquitocontrol/bti-mosquito-control).
c. World Health Organization specifications for Bti strain AM65-52 (http://www.who.int/whopes/quality/Bti_eval_spec_Jun_07.pdf)
d. World Health Organization mosquito larvicide recommendations (http://www.who.int/whopes/Mosquito_larvicides_March_2016.pdf?ua=1)
e. VectoBac WDG product brochure (http://publichealth.valentbiosciences.com/docs/public-health-resources/vectobac-wg-technical-use-sheet)
f. World Health Organization's (WHO) Approved Mosquito Larvicides Products Table. The WHO has approved the use of Bti in drinking water (http://www.who.int/water_sanitation_health/gdwqrevision/Fourth_Edition_Bacillus_thuringiensis_Apr2010.doc)
g. VectoBac WDG US Environmental Protection Agency Label (recently updated label [Aug 10, 2016] available upon request).
h. Organic Material Review Institute (OMRI) Certificate for VectoBac WDG (available upon request).