Trap-N-Kill® Lethal Ovitrap

The Trap-N-Kill® mosquito control system is a product of the US Military Lethal Ovitrap invention and patents. It is licensed exclusively to SpringStar Inc. The method targets those mosquitoes which may transmit diseases such as Zika, dengue, chikungunya and west Nile virus. The Trap-N-Kill® is US EPA registered and registered in every state.

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Use of Lethal Ovitraps for Areawide Control of *Aedes*

EXECUTIVE SUMMARY

Subject: This paper provides a rationale and background for incorporating lethal ovitraps ("LOTs") into existing municipal mosquito abatement, facilities management or area-wide programs.

Background: In recent decades, mosquito abatement districts worldwide have increasingly needed to relieve the annoyance and degradation of human outdoor activities caused by day time biting mosquitoes, principally *Aedes albopictus*. In more tropical areas, districts must also be on guard against Zika, dengue, chikungunya, and related viruses which are transmitted by *Aedes aegypti, Ae. albopictus* and other *Aedes* species. *Aedes aegypti* is particularly lethal; because it prefers feeding on humans and has adapted to surviving in and around people's homes, this species is a highly efficient vector of human disease. Current mosquito control methods are insufficient for solving these problems. The Trap-N-Kill® ("TNK") is a reusable lethal ovitrap that attracts and kills container-breeding mosquitoes, including *Ae. aegypti* and *Ae. albopictus*.

Discussion: The most common form of mosquito control is aerial spraying or fogging with pesticides. Spraying of pesticides is, by the nature of the process, an area-wide treatment, which results in unnecessary and potentially harmful non-target and environmental exposures. These conventional methods of mosquito control are, for the most part, reactive solutions to preventable problems. Spraying and fogging to kill adult mosquitoes correlates to a failure to interrupt population growth earlier in the life cycles. As demonstrated by the spread of chikungunya through the Caribbean, traditional techniques are unable to mitigate the spread of arboviruses.

Alternatives: The most viable approach to reducing the need for spraying and fogging as the primary form of mosquito control is the integration of lethal ovitrap into the program. Population growths of these *Aedes* mosquitoes could be substantially curtailed if trained pest management personnel deployed these traps in significant numbers beginning early in the breeding season. SpringStar's Trap-N-Kill® is the only EPA registered and commercially available lethal ovitrap.

Recommendation(s): The integration of TNK into mosquito control programs would give technicians an additional tool that is more effective and simultaneously reduces pesticide exposures to applicators, residents, non-target species, and the environment. As a general protocol, abatement districts should acquire a stockpile of ovitraps in advance of the breeding season and begin deployments several weeks before mosquitoes begin to appear in significant numbers.

Use of Trap-N-Kill Lethal Ovitrap by Mosquito Abatement Districts

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INTRODUCTION

SpringStar Inc. wishes to incorporate their lethal ovitrap product, branded "Trap-N-Kill®", into mosquito abatement programs worldwide. Trap-N-Kill® is a reusable lethal ovitrap that attracts and kills day-biting container-breeding mosquitoes such as *Aedes albopictus* and *Aedes aegypti*.

THE NUISANCE OF DAY-BITING MOSQUITOES

Many species of mosquitoes feed after dark or late at night. Avoiding mosquito nuisance by staying indoors can be relatively simple to do without impacting the quality of daily life. *Aedes* species, however, are day biters; in the case of *Ae. albopictus*, the species is highly aggressive and in some areas will feed in the middle of the day. Their presence is an annoyance that reduces morale and quality of life for people in their invasive range. A recent survey of households in two counties in New Jersey quantified the mosquito problem due (primarily) to *Ae. albopictus* as more important than neighborhood safety or cleanliness, and equivalent to living with worrisome health risk factors.[1]

Ae. albopictus is also highly invasive and has recently spread throughout most of the Eastern seaboard and Southeast US. Controlling the spread of these mosquitoes is thus vital for quality of life reasons, not just the associated disease risks[2].

VECTOR-BORNE DISEASES WORLDWIDE

> "Because dengue is more widespread globally than any other vector-borne disease, and the current pandemic of dengue continues unabated with about 100 million human cases annually in ~125 countries, citizens worldwide have increased needs to guard against dengue transmission by *Aedes aegypti, Ae. albopictus* and *Ae. polynesiensis*."[3]

Chikungunya is another emerging infectious disease, carried by the same mosquito species, and has begun to spread rapidly out of its African and Asian origins. Chikungunya, which is traditionally spread by *Ae. aegypti,* jumped to *Ae. albopictus* in 2005, ultimately spreading from Reunion Island (250,000 cases, or 1/3 of the population[4]) to India (1.2 million cases[5], now endemic) and then to

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[1] Halasa, YA, DS Shepard, Dm Fonseca, A Farajollahi, S Healy, R Gaugler, K Bartlett-Healy, DA Strickman and GG Clark. 2014. Quantifying the impact of mosquitoes on quality of life and enjoyment of yard and porch activities in New Jersey. *PLoS ONE* **9**(3):e89221.

[2] Mitchell, CJ. 1995. Geographic spread of *Aedes albopictus* and potential for involvement in arbovirus cycles in the Mediterranean basin. *Journal of Vector Ecology* **20**(1):44-58

[3]Proceedings of the Department of Defense (DoD) Symposium. December 14, 2010 http://www.afpmb.org/sites/default/files/pubs/dwfp/publications/FY11/ESA%20proceedings%202010%20DWFP%20reprint.pdf

[4] Pialoux, G., B. Gaüzère, S. Jauréguiberry and M. Strobel. 2007. Chikungunya, an epidemic arbovirus. *The Lancet Infect Dis* **7(5)**:319-327.

[5] Charrel, R.N., X. de Lamballerie, and D. Raoult. 2007. Chikunguna outbreaks – the globalization of vector-borne diseases. *N Engl J Med* **356**:769-771.

Italy (240 cases in the first outbreak[6], and still present). In December 2013, a different strain of chikungunya was transmitted to the Caribbean, beginning in St. Martin and eventually ending up on the South American mainland[7]. The Caribbean outbreak is ongoing and spreading. There are currently no vaccines to prevent infection with either dengue or chikungunya, and no specific treatment[8]. Control of the mosquito vectors is the only effective way to prevent disease transmission. However, current methods are not effective enough.

There are two primary *Aedes* species of concern in the US: *Aedes aegypti* and *Aedes albopictus*. *Ae. aegypti* mosquitoes are adapted to living in tropical environments and are found throughout the southern states (Figure 1). Because of their strong preference for feeding on human hosts, they are particularly efficient vectors of human arboviruses. *Ae. albopictus* mosquitoes are considered a secondary vector for many of the same viruses spread by *Ae. aegypti*, but they are more aggressive biters, making them a significant nuisance species.[9] In addition, *Ae. albopictus* are more cold-tolerant than *Ae. aegypti*; after being introduced to Texas in the 1980s, they spread throughout the southern US and as far north as Vermont and New Hampshire (Figure 1).

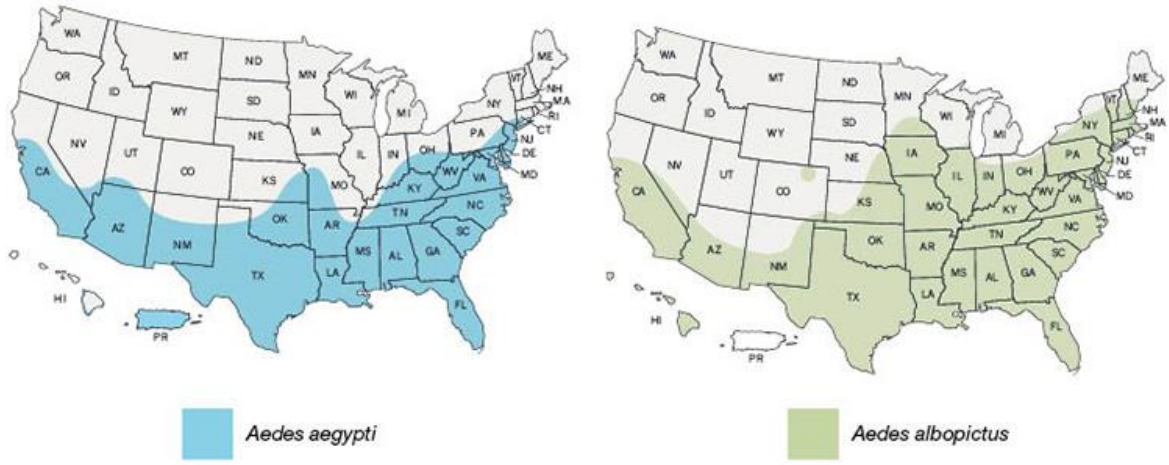

Figure 1. Estimated US distribution of Ae. aegypti *and* Ae. albopictus *mosquitoes. Source: Centers for Disease Control and Prevention (http://www.cdc.gov/zika/vector/range.html, Accessed July 25, 2016)*

[6] Bonalauri, P., R. Bellini, M. Calzolari, R. Angelini, L. Benturi, F. Fallacara, P. Corioli, P. Angelini, C. Venturelli, F. Merialdi and M. Dottori. 2008. Chikunguna virus in *Aedes albopictus*, Italy. *Emerg Infect Dis* **14**:952-954

[7] ProMED-mail Archives. *Chikungunya (21): Caribbean*, published 2014-03-13 23:29:53. Archive No. 20140316.2335886. *International Society for Infectious Diseases*. Available from http://www.promedmail.org

[8] Baragona, Sara. *Army Laboratories Work Together to Defeat Dengue*. U.S. Army Press Release. March 23 2012. p 8-9. http://mrmc.amedd.army.mil/utility/getPDF.cfm?type=point&key=na

[9] Halasa YA, et al. (2014) Quantifying the impact of mosquitoes on quality of life and enjoyment of yard and porch activities in New Jersey. PLoS One 9(3):e89221.

Both *Ae. aegypti* and *Ae. albopictus* populations have been surprisingly robust to traditional mosquito control approaches. While larvicides effectively eliminate immature mosquitoes in treated water sources, these mosquitoes can breed in extremely small volumes of water, making it difficult if not impossible to adequately treat areas large enough to have a significant impact on the population. While aerial adulticides are effective for certain mosquito species, pesticide application often occurs at night to minimize both human and nontarget invertebrate exposure. Since *Aedes* mosquitoes are active during the day, they tend to be missed by nighttime applications. In addition, *Ae. aegypti* are well-adapted to living inside and around human dwellings. Thus even if spraying occurs during the day, the mosquitoes may avoid exposure simply because they are resting indoors or under cover. Finally, because of widespread use of "safer" adulticides like pyrethroids, insecticide resistance is reducing the efficacy of available compounds.

LETHAL OVITRAPS AND MOSQUITO CONTROL

Oviposition traps have been used to monitor mosquito populations since the 1960s.[10] The technology took a step forward in 1999 when army scientists Michael Perich and Brian Zeichner added an insecticide-treated oviposition strip to the trap.[11] The army partnered with SpringStar to commercialize their lethal ovitrap, and the result was the Trap-N-Kill™ Mosquito Trap.

Field trails demonstrated that Perich and Zeichner's lethal ovitrap significantly reduced *Ae. aegypti* populations in Brazil, Peru and Bangladesh.[12] In the Brazilian study, ten lethal ovitraps per house reduced the positive container index by 50-80% and mean pupal counts by more than 90%.[13] The traps also significantly decreased household numbers of adult female *Ae. aegypti* following three months of treatment. A similar study in Thailand found that, once trap maintenance problems were addressed, lethal ovitraps reduced the household pupal index by 30-53% and the mean number of adult *Ae. aegypti* per house by 29-75%.[14] Although significant, the traps' impact on *Ae. aegypti* were less pronounced than in other field trials; the authors suggested that this may have been due to high numbers of alternative water containers and/or mosquito ingress from the surrounding environment.

A number of agencies have integrated lethal ovitraps into their arbovirus control programs. For example, the Queensland Dengue Action Response Team (DART) has used bifenthrin-based lethal ovitraps since 2004 to combat dengue outbreaks and to prevent single cases from spreading. Through the targeted use of traps in a 200 m radius around dengue focal points, DART has been able to virtually eliminate indoor residual spraying, fogging, and all other broadcast chemical

[10] Fay RW, Eliason DA (1966) A preferred oviposition site as a surveillance method for *Aedes aegypti*. *Mosq News*:531–535.
[11] See Perich and Zeichner US Patent Numbers 5,983,557 (1999); 6,185,861 (2001) and 6,389,740 (2002)
[12] Zeichner BC, Debboun M (2011) The lethal ovitrap: a response to the resurgence of dengue and chikungunya. Army Med Dep J Jul-Sep:4–11.
[13] Perich MJ, et al. (2003) Field evaluation of a lethal ovitrap against dengue vectors in Brazil. Med Vet Entomol 17:205–210.
[14] Sithiprasasna R, et al. (2003) Field evaluation of a lethal ovitrap for the control of *Aedes aegypti* (Diptera: Culicidae) in Thailand. J Med Entomol 40(4):455–462.

methods of control for *Aedes aegypti.*[15] The CDC has been evaluating another variant, the autocidal gravid ovtrap (AGO), to control *Ae. aegypti* in Puerto Rico since 2013.[16] Field studies found that use of the AGO reduced *Ae. aegypti* mosquito densities by 85%[17] and chikungunya incidence by 50%.[18] Finally, during the 2016 dengue outbreak in Hawaii, residents of the Big Island deployed the Trap-N-Kill™ Mosquito Trap to help control *Aedes* mosquitoes around their homes.

CURRENT MOSQUITO CONTROL METHODS

Breeding Source Reduction/Prevention

Ae. aegypti and *Ae. albopictus* are container-breeding mosquitoes. This means that they prefer to lay their eggs in small, often man-made receptacles that hold standing water including rain buckets, flower pots, old tires, gutters, and even discarded bottles, cans and other trash. One of the most effective means of mosquito control is source reduction – that is, reducing the number of water sources available for the mosquitoes to breed in. However, with these species, the large number of possible sites makes eliminating or treating all breeding sites time consuming if not impossible.

Insecticide Spraying/fogging

"Aerial adult mosquito control using fixed-wing aircraft or helicopters and/or ground adult mosquito control using truck or boat mounted equipment are often the most visual aspects of an organized mosquito control program. This method of control is called adulticiding. Although it is often expensive in terms of manpower, equipment and inventory, sometimes difficult to accomplish and more likely to affect non-target organisms if mishandled, it is the only method to rapidly reduce infected mosquito numbers or to control pest and nuisance mosquitoes from inaccessible breeding areas that are interfering with normal outdoor activities of a community."[19] Synthetic pyrethroids are commonly used in spraying/fogging programs for targeting adult mosquitoes. However, *Aedes* like to rest under cover and in shaded areas, so are often missed during spraying. Because synthetic pyrethroids leave very little lasting toxic residue on exposed surfaces,[20,21] these females are likely to survive when they do emerge from cover. Trap-N-Kill®

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[15] A full version of the DART Dengue Management Plan is available online at: http://www.health.qld.gov.au/dengue/documents/dengue-mgt-plan.pdf

[16] Barrera R, Amador M, Acevedo V, Hemme RR, Felix G (2014) Sustained, area-wide control of *Aedes aegypti* using CDC Autocidal Gravid Ovitraps. Am J Trop Med Hyg 91(6):1269–1276.

[17] Barrera R, et al. (2014) Use of the CDC Autocidal Gravid Ovitrap to control and prevent outbreaks of *Aedes aegypti* (Diptera: Culicidae). J Med Entomol 51(1):145–154.

[18] Lorenzi OD, et al. (2016) Reduced incidence of chikungunya virus infection in communities with ongoing *Aedes aegypti* mosquito trap intervention studies — Salinas and Guayama, Puerto Rico, November 2015-February 2016. MMWR Morb Mortal Wkly Rep 65.

[19] American Mosquito Control Association. *Adult Control*. http://www.mosquito.org/control Accessed March 17, 2014

[20] Massachusetts Department of Public Health. *Frequently Asked Questions about Spraying and Mosquito Control*. May, 2009 http://www.cmmcp.org/faq_spray_mosquito.pdf

[21] University of Florida. *Mosquito Information Website*. Accessed March 17, 2014. http://mosquito.ifas.ufl.edu/Adulticiding.htm

targets the gravid females which manage to avoid contact with pesticides delivered by aerial dispersal.

Problems associated with spraying/fogging

Spraying of pesticides is, by the nature of the process, an area-wide treatment. The active component must be finely and thoroughly dispersed across the entire area where mosquito control is desired. However, this process means that anything else in the treatment area – human, animal, or other insects - also receives a dose of the pesticide. While the volume and active ingredient used are carefully selected so as to minimize unnecessary exposure, the effects on non-targets cannot be completely eliminated.

Many of the organophosphates and synthetic pyrethroids used for mosquito control are highly toxic to honeybees[22], for example. This precludes their use in times or locations where honeybees may be present. Others, especially the pyrethroids, are also highly toxic to aquatic organisms. The labels for many of these products include language specifically prohibiting applying the products or discharging wastes containing the products into lakes, streams, ponds, estuaries, oceans, or other public waters[23]. That makes them problematic for use in wet areas where mosquitoes breed, especially ecologically sensitive areas like island habitats.

Finally, the constant use of any single pesticide class for treating a pest can lead to pesticide resistance. Synthetic pyrethroids, due to their relative non-toxicity to vertebrates, are commonly used not only by public and professional mosquito control but also in residential and household mosquito products. This can lead to, and has in some areas already led to, strong pyrethroid resistance developing in mosquito populations.

LETHAL OVITRAP

Technical Background

Trade name of the active ingredient(s) with respective concentration levels:

Dichlorvos (DDVP) – 10% w/w; Related compounds – 0.75% w/w

Chemical name of the active ingredient:	Dimethyl 2,2-dichlorovinyl phosphate (CAS #62-73-7)
Type of insecticide:	Impregnated solid resin
Signal Word:	Caution
EPA approved method of application:	For use in insect traps including mosquito traps
EPA registration number:	8730-50-66433, EPA Est. No. 66433-WA-1
EPA-approved pesticide label:	See Appendix A
SDS:	See Appendix B

[22]Johnson, Reed M. et al. *Pesticides and honey bee toxicity – USA* . http://hal.archives-ouvertes.fr/docs/00/89/20/96/PDF/hal-00892096.pdf

[23] E.g. see product label for Duet Dual-action Adulticide (1% Prallethrin, 5% Sumethrin and 5% PBO synergist), used in Norfolk County, MA for adulticiding. http://www.norfolkcountymosquito.org/Documents/Label_Duet.pdf

Brief description of product

The Trap-N-Kill® is a lethal oviposition trap intended for use against gravid female container-breeding mosquitoes. Primary target species are *Aedes aegypti* and *Aedes albopictus*. Secondary target species include other *Aedes* species, *Culex quinquefasciatus* and other *Culex* species. Traps are to be placed outside, preferably under bushes or other sheltered areas where mosquitoes prefer to rest, within three (3) feet of ground level. Recommended placement density is 25 feet apart (for areas with high mosquito populations or numerous alternate breeding sites); in residential areas, traps may be placed at 4-10 per house, depending on lot size. Where possible, alternate breeding sites should be emptied and overturned or treated. Traps should be placed away from places where children or food may be present. Insecticide strips must be replaced every 4-6 weeks. Trap containers may be rinsed and recycled or saved for reuse. Product use may be combined with source reduction, larviciding, and/or adulticiding. Product may not be applied directly to water sources.

Features and Benefits

- There is currently no other EPA registered lethal ovitrap product available
- Trap-N-Kill® provides an alternative and/or complementary active ingredient for areas where pyrethroids are overused and/or not-effective
- Available mosquito adulticides are liquid or intended for liquid formulations of sprays/fogs/ULV applications. This product provides a non-spray adulticide, which substantially reduces exposures to applicators, local personnel, and non-target species.
- Use of this product at label rates can reduce pesticide use in a given area by at least 90% compared to aerial spraying
- The efficacies of current adulticides last only a short time, or require that the adult receive a lethal dose during the initial application. This product lures the adult to the insecticide, and remains efficacious for weeks.
- This product targets day time biting mosquitoes, species which create significant discomfort and aggravation to a urban residents wishing to spend time outdoors
- This product lends itself to both professional and consumer use, which allows for increased coverage with less effort by professional mosquito control personnel

Approach: Prevention vs. Cure

Conventional methods of mosquito control are, for the most part, reactive solutions to preventable problems. Spraying and fogging to kill adult mosquitoes after they become too abundant correlates to a failure to interrupt population growth earlier in the life cycles. Considering the ongoing worldwide incidents of dengue fever outbreaks, as well as the growing nuisance of these day-biters, reactive ground and aerial applications of conventional insecticides are not adequately controlling *Aedes* mosquitoes.

Aside from intensive efforts to eliminate breeding sources, an important component of mosquito population control is interruption of the breeding cycle. Seasonal population growths of these mosquitoes could be substantially curtailed by implementing early-season programs of lethal ovitrap deployments. The Trap-N-Kill® interrupts the breeding cycle at two points by simultaneously targeting gravid females and killing any offspring she may lay in each trap. This process can both prevent population increases and can quickly reduce the size of a population if sufficient traps are used, such as in areas where dengue transmission is a risk.

TNK Development Background

In the 1990s, U.S. military research scientists Michael Perich of the Walter Reed Army Institute of Research (WRAIR) and Brian Zeichner of the U.S. Army Center for Health Promotion and Preventive Medicine (USA-CHPPM), now called the U.S. Army Public Health Command (USAPHC) developed *the Lethal Mosquito Breeding Container*, or Lethal OviTrap (LOT, a black pint-sized cup with water and a strip treated with a small amount of pesticide. These water filled containers mimic the breeding site of container breeding mosquitoes including the Aedes species. These mosquitoes may visit on average 12 or more sites per egg-laying cycle in an act called "skip-oviposition". This ensures that as many eggs as possible will survive if one or more of the breeding sites are damaged or dried up. Because these mosquitoes jump from breeding site to breeding site, the chances that they will find an ovitrap are extremely high if enough ovitraps are used in an area.

The LOT kills gravid female mosquitoes by imparting a lethal dose of insecticide when they enter the trap. The mosquitoes may not perish immediately but they will die within a few hours after exposure. If they survive long enough to lay eggs in the trap, their offspring will also be killed once they develop from eggs to larvae; thus interrupting the life cycle of the local population at two distinct life stages. This process reduces the population of biting mosquitoes, decreasing both the potential for disease transmission and the breeding stock for the next generation.[24] The U.S. Army patented this technology[25] in 1999 and subsequently licensed it to SpringStar in 2008.

The Key West Field Trial: An Illustration of Pyrethroid Resistance

> "Beginning July 2010, in collaboration with the U.S. Army Public Health Command, Navy Entomology Center of Excellence, Armed Forces Pest Management Board, and local Naval Air Station volunteers, approximately 7000 [final count was 5,300] LOTs were placed in Old Town Key West… These LOTs (lethal ovitraps) were checked fortnightly for presence of mosquitoes, while the local dengue vector *Ae. aegypti* population was monitored and subjected to intensive control measures."[26]

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[24] Baragona, Sara. *Army Laboratories Work Together to Defeat Dengue*. U.S. Army Press Release. March 23 2012. p 8-9. http://mrmc.amedd.army.mil/utility/getPDF.cfm?type=point&key=na

[25] *Lethal Mosquito Breeding Container* patent No. US 5983557. http://www.google.com/patents/US5983557

[26] Proceedings of the Department of Defense (DoD) Symposium. *DoD Entomology: Global, Diverse and Improving Public Health. December 14, 2010.*

The first TNK traps deployed in an area-wide program (Fig. 2) used the original open plastic cups of Perich (2003). During the first scheduled trap maintenance, live larvae were found in some of the Trap-N-Kill® (TNK) traps. At the time of the discovery, there was a question as to whether the mosquitoes were resistant to bifenthrin, the pyrethroid used, or whether the ovistrips had been inconsistently treated. Both hypotheses were rapidly and thoroughly investigated.

Figure 2. Key West Treatment (orange) and Untreated Blocks (blue). The 800m flight range of *Aedes* shown in yellow circle.[27]

Each batch of ovistrips had been sampled and confirmed as fully treated via adult bioassays at CHPPM (now USAPHC) before release for field use. The protocol had previously revealed an unexpected adverse reaction between a bittering agent (denatonium benzoate) and bifenthrin which affected efficacy. Ovistrips used in the Key West field trial contained no bittering agent and had passed the bioassay study with efficacy above the acceptable minimum rate.

After the field study, treated oviposition strips (aka ovistrips) were sampled from TNK with and without larvae. Retained ovistrips sampled from the same production batches, as well as new, individually treated ovistrips were compared against the field aged strips in laboratory bioassays with known susceptible mosquitoes. All strips taken from the field (4 weeks old) were found to still

http://www.afpmb.org/sites/default/files/pubs/dwfp/publications/FY11/ESA%20proceedings%202010%20DWFP%20reprint.pdf

[27]Honório, Nildimar Alves, et al. "Dispersal of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) in an urban endemic dengue area in the State of Rio de Janeiro, Brazil." *Memórias do Instituto Oswaldo Cruz* 98.2 (2003): 191-198.

be acceptably lethal to susceptible insects. Retained production strips were also found to be consistently and highly lethal, and equally as lethal as the hand treated strips.

Additionally, eggs were collected from locations around Key West and reared in colony to adulthood. Bioassays with new ovistrips, as well as genetic testing later performed by the CDC, confirmed that the Key West population of *Ae. aegypti* mosquitoes were indeed highly resistant to pyrethroids (and other pesticides). SpringStar had requested a baseline resistance assay prior to initiating the study. Local staff determined that resistance appeared to be less than 0.5% of the wild *Ae. aegypti* tested. At the conclusion of the study, SpringStar staff estimated that 22% of the ovitraps had viable larvae after a six-week deployment. We presume that the pyrethroid used contributed to the resistance observed.

High Efficacy in Key West Deployment:

Despite these resistance issues, the open-cup type devices showed surprisingly good efficacy. In treated areas at day 26, treated areas had 50% fewer homes with pupae present in cups than seen in untreated areas. Pupal counts showed 71% and 84% population reductions at days 12 and 26 respectively (Figure 3). Figure 2 shows the treatment sites where traps were placed. The adult counts during treatment showed only a 27% female count reduction, but surprisingly a 30% reduction for 6 weeks after treatment (Figure 4). There is no doubt that the adult counts were confounded by insects entering the treatment areas from nearby untreated areas.

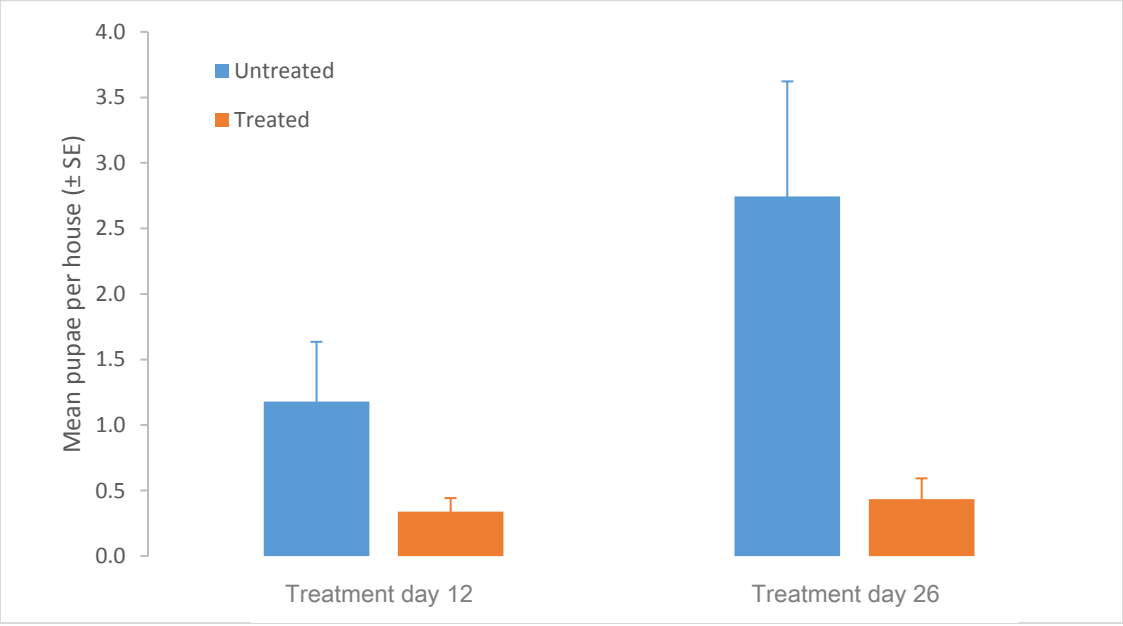

Figure 3. Summary of pupal counts from 1151 homes during the Key West field trial. Bars represent the mean number of pupae per house (± one standard error of the mean) in the treated and untreated (control) areas on two sampling dates. At treatment day 12, there was 71% control observed between the treated and untreated areas (p=0.10). By treatment day 26, the mean pupal count in the untreated areas had increased by 133%, and was significantly higher than the pupal count in the treated areas, demonstrating 84% control (p=0.05).

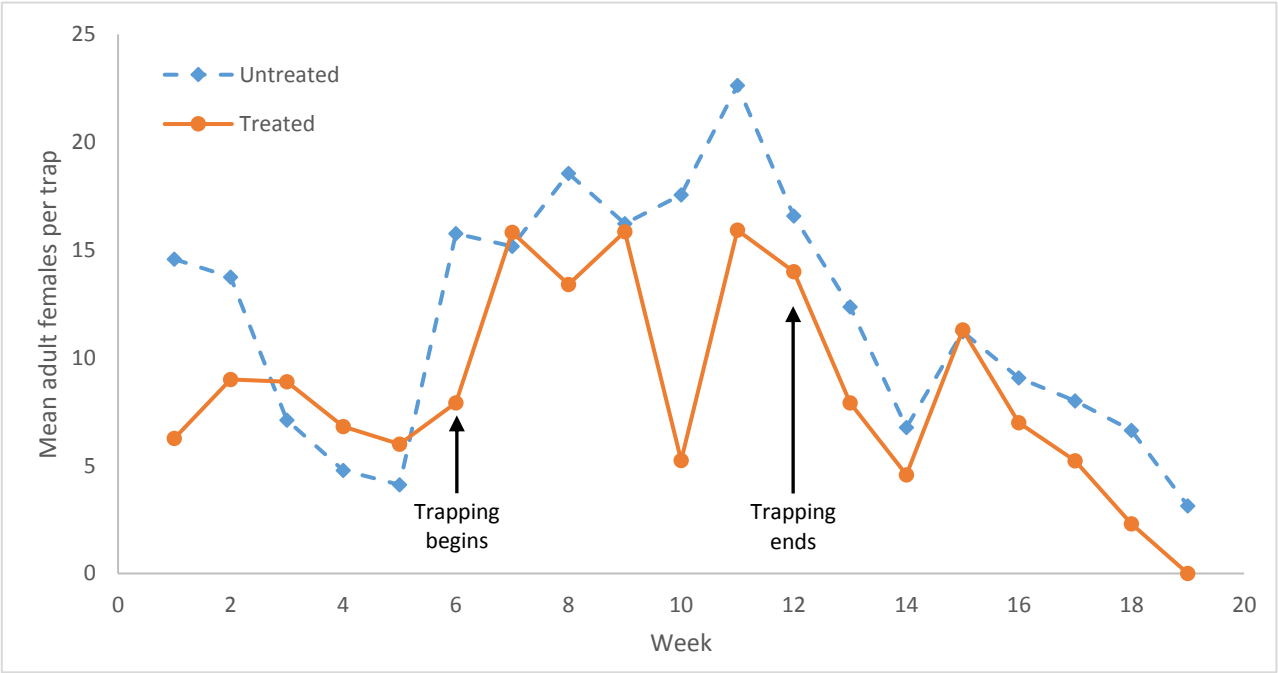

Figure 4. Summary of adult female mosquito counts by week during the Key West field trial. Points represent the mean number of adult female mosquitoes for n=6-19 BG Sentinel Traps in the treated and untreated (control) sites. There was no significant difference between the treated and untreated groups during the pretreatment phase (weeks 1-5, t=0.845, p=0.400). Female mosquito counts were significantly higher in the untreated groups than in the treated groups during both the treatment phase (weeks 6-12, t=2.793, p=0.006) and post-treatment phase (weeks 13-19, t=2.971, p=0.003).

SpringStar and their collaborators at USAPHC have continued to maintain the colony of Key West Resistant mosquitoes. All new versions of the trap and active ingredient formulations have been tested against both susceptible and resistant mosquitoes. Several types of contact pesticides, including other pyrethroids as well as insecticides from different action classes, were assayed to determine which would be most effective. The slow-release formulation of DDVP was found to be the preferred choice in terms of efficacy, formulation safety, duration, and ease of use.

Because of the restrictions on DDVP for all but controlled-release formulations, as well as the continued move away from the organophosphate class of pesticides where possible, DDVP resistance is less likely to be a problem due to active class overuse. It was therefore decided that the next generation of TNK would feature DDVP as the active ingredient.

Product Efficacy: Summary of EPA 810.3400 Product Performance Report

The referenced Product Performance Report was submitted in support of a label amendment to add a new pest, mosquitoes, as well as a distributor sub-label, to the current registration for a DDVP insecticidal strip. Performance data submitted included:

- Laboratory testing conducted on the product for use on mosquitoes; and
- Published, peer-reviewed literature on the efficacy of the active ingredient (dichlorvos/DDVP) against mosquitoes

The dichlorvos-impregnated resin strips were previously registered for use in insect traps. However, the label did not specifically claim use against mosquitoes. The report provided the necessary efficacy data to support adding "mosquitoes" to the label claims. The active ingredient and end product were already known to be highly efficacious against a broad range of insects, including mosquitoes. The efficacy data presented provided additional support specifically for use of the resin strips within a lethal ovitrap device.

Dichlorvos (DDVP) has a long history of use for small-scale control of public health pests, including mosquitoes. The high vapor pressure of the compound allows for efficient treatment of enclosed spaces and complete permeation of difficult-to-treat cracks and crevices, while ensuring a short residual activity for rapid re-entry. Due to the short residual, the material was formulated into resin strips to slow the release of the compound and extend the useful field life. Early studies with 20% DDVP strips maintained killing activity for up to 68 weeks. Current formulations maintain activity for 4-6 weeks (smaller strips); some formulations are active for up to 4 months (large strips).

Test materials were prepared by either SpringStar personnel or USAPHC personnel. All testing was performed in small outdoor cages at USAPCH facilities in Aberdeen Proving Grounds, Maryland. Two strains of Aedes aegypti were used in all tests: a published susceptible strain provided by the USDA, derived from mosquitoes originally collected in Orlando, FL in 1952; and a known pyrethroid-resistant strain, originally collected in Key West, FL in 2010 and reared at USAPHC facilities. Adults were provided blood 3 days before each cage test, to ensure gravid status of the females. Larval bioassays were performed with 3rd and 4th instar larvae.

Laboratory cage tests demonstrated that the 1" x ½" resin strips could provide 100% control of pyrethroid-resistant mosquitoes for at least four weeks, and up to 90% control for at least 6 weeks under expected conditions of use. Larger strips (1" x 1") provided 100% control for at least 10 weeks in outdoor cage assays for susceptible populations. Key West resistant insects showed 100% larval kill (Figures 5 and 6).

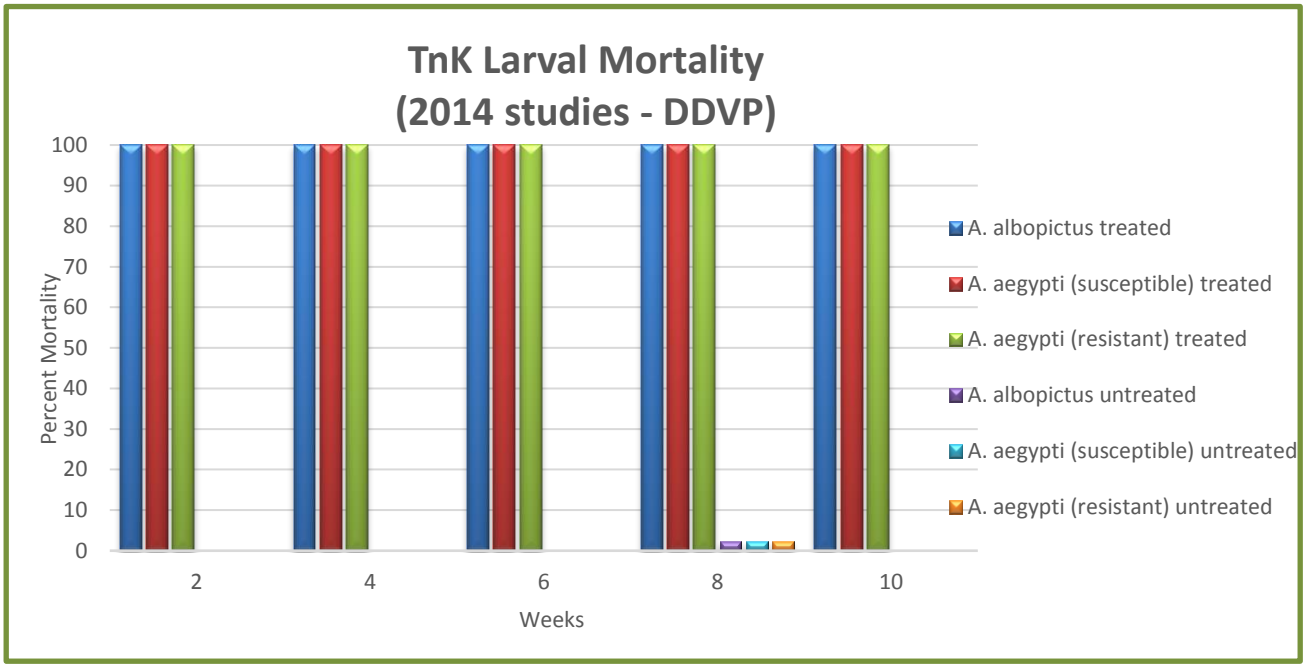

Figure 6. Larval Mortality for *Aedes aegypti* (susceptible and Key West resistant strains) and *Ae. albopictus.* *S*emifield studies (courtesy USAPHC).

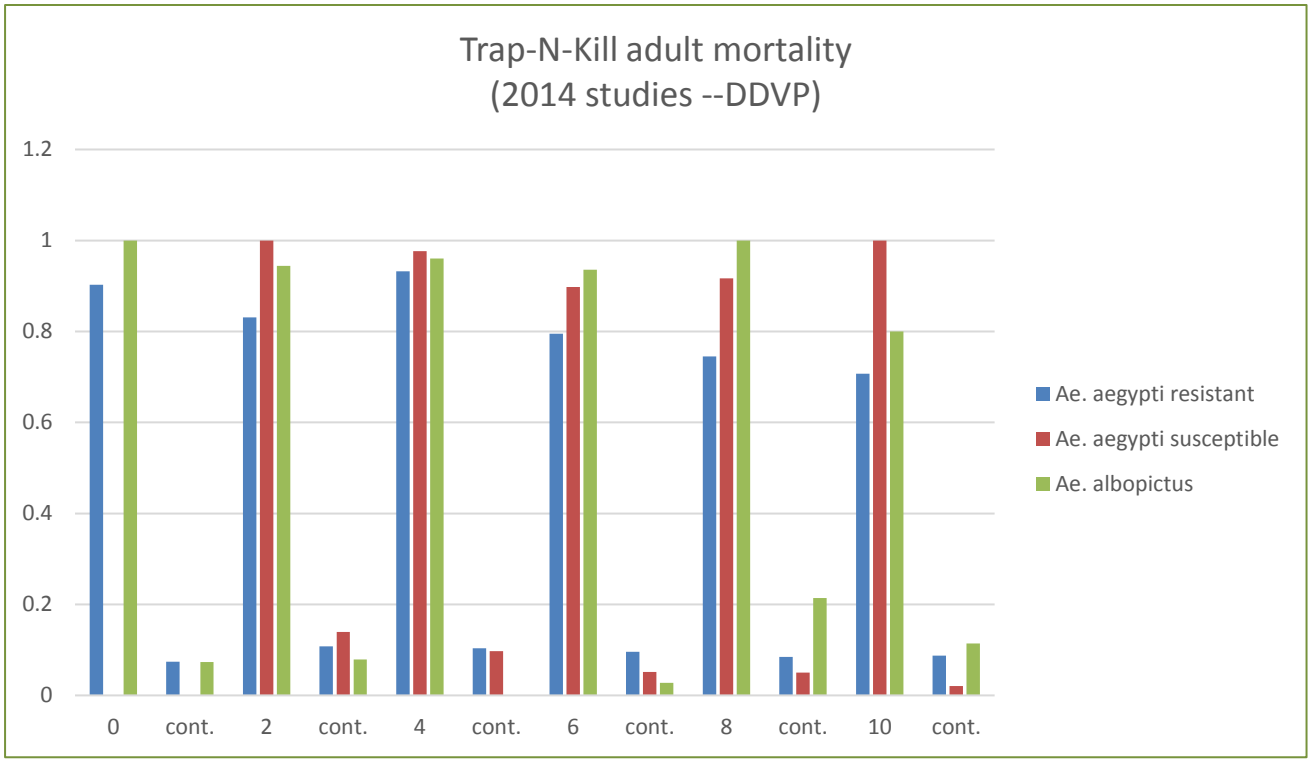

Figure 5. Adult Mortality for *Aedes aegypti* (resistant and susceptible strains) and *Aedes albopictus*. Semifield studies. Weeks 0-10, cont. = control treatment.

Use of Lethal Ovitraps for Dengue Control

The Queensland Dengue Action Response Team (DART) has used lethal ovitraps since 2004 with great success to combat dengue outbreaks and to prevent single cases from spreading. Through the targeted use of lethal ovitraps in a 200m radius around dengue focal points, DART has been able to virtually eliminate indoor residual spraying, fogging, and all other broadcast chemical methods of control for *Aedes aegypti*. A full version of their Dengue Management Plan is available online.[27]

SpringStar has adapted the Queensland plan to respond to individual cases of dengue. A complete outline of this plan is provided in Appendix C.

DDVP: Regulatory Status

The decision to use an organophosphate in the TNK was not taken lightly. Although DDVP had been reliably used worldwide since 1948 as an all-purpose insecticide, health risks related to organophosphates prompted the EPA to review the registration status of all organophosphates, including DDVP, in light of new data. At the time of the review in 1995, EPA proposed cancelling most of the current uses. The slow-release formulation for use in insect traps was one of the few uses not facing cancellation[28].

The EPA issued their final re-registration decision, after an extensive assessment of the possible health and ecological risks, and "determined that risks do no exceed levels of concern"[29]. In the interim, manufacturers had voluntarily withdrawn re-registration requests for larger pest strips and foggers, and removed the use patterns of lawn, turf, ornamental, and crack and crevice treatment from all labels. The labels were also amended to require use only in unoccupied areas. Remaining uses include bait granules, some ready-to-use sprays, and several sizes of resin-impregnated strips.

In 2004, the USDA, Forest Service also commissioned a Human Health and Ecological Risk Assessment for the use of DDVP as a control agent for gypsy moth[30]. SpringStar used this risk assessment in the decision making process when selecting their dichlorvos strip as a kill agent in the TNK, as the occupational exposure of Forest Service personnel would be similar to or greater than that encountered by professional mosquito control personnel using TNK. The Forest Service uses 1"x 4" strips of Hercon VaporTape II in milk carton traps for mass trapping of gypsy moths. The report concluded that most of the known toxic effects of DDVP are due to rapid exposure in high doses, and that the controlled-release formulation of the resin strips precluded that kind of exposure. Their risk characterization concluded that "in most cases, exposures to workers and general public should be negligible", and that only the most extreme worst-case scenarios would

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[27] http://www.health.qld.gov.au/dengue/documents/dengue-mgt-plan.pdf

[28] Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Dichlorvos*. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. 1997. p. 128

[29] Environmental Protection Agency (EPA), Office of Pesticide Programs. *Reregistration Eligibility Decision for Dichlorvos (DDVP).* US EPA, Washington DC. 2006. Pg 12 of 342.

[30] Durkin, PR and Follansbee, MH . *Control/Eradication Agents for the Gypsy Moth – Human Health and Ecological Risk Assessment for DDVP (Dichlorvos): Final Report*. Prepared for USDA, Forest Service, Forest Health Protection. GSA Contract No. GS-10F-0082F. Syracuse Environmental Research Associates, Inc, Fayetteville, NY. 2004.

exceed the level of concern. However, even those scenarios were estimated to be below acute observable toxicity levels.

Although DDVP is noted as a "potential carcinogen," real-life studies do not support that rating. A 2007 study was conducted by the National Cancer Institute, the National Institute of Environmental Health Sciences, the Yale School of Medicine and the University of Iowa[31]. Almost 50,000 pesticide applicators, including a group of over 4,600 DDVP users, were involved in the study. These applicators participated in the study for over 10 years. The study was conducted specifically to evaluate human health effects that might result from DDVP exposure to agricultural workers. This is the largest study to specifically evaluate DDVP use and cancer risk and the authors concluded that *DDVP exposure was not associated with human cancer*.

Trap-N-Kill®: Regulatory Status

SpringStar's Trap-N-Kill® with DDVP is registered by the US EPA, registration number 8730-50-66433. This registration was approved January 2014. State registrations have been issued for every state, including Puerto Rico and American Virgin Islands.

[31] Dichlorvos exposure and human cancer exposure risk: results from the Agricultural Health Study, Koutros, etal., DOI 10.1007/s10552-007-9070-0

Appendix A: EPA-approved Trap-N-Kill® label

Appendix B - Trap-N-Kill™ Mosquito Trap Assembly

The Trap-N-Kill™ Mosquito Trap avoids many of the pitfalls associated with other control techniques by taking advantage of certain aspects of *Ae. aegypti* and *Ae. albopictus* biology. Both these mosquito species lay their eggs in small, manmade water containers. They also practice skip oviposition, meaning that they lay small groups of eggs in multiple locations to maximize offspring survival. The Trap-N-Kill™ Mosquito Trap is designed to replace one of these oviposition sites. Female mosquitoes are lured into the trap by a combination of attractive visual and volatile cues. Upon entry, they are exposed to a concentrated DDVP vapor, which kills both the adult female and her offspring.

The Trap-N-Kill™ Mosquito Trap includes five components (Figure 7):

- The **reservoir** holds approximately 600 ml of water and has a hole on the side that facilitates mosquito entry and prevents the trap from overfilling.
- The **velour oviposition strip** provides a landing point for female mosquitoes.
- The **pesticide tab** contains 147.5 mg of DDVP encased in a slow-release resin.
- The reservoir **lid** concentrates the DDVP vapor to maximize its impact on mosquitoes. A refill hole in the center makes it easy to top off the water level in the traps during routine maintenance.
- A **zip tie** is provided to secure the lid to the reservoir and prevent tampering.

Setup. To prepare the trap for deployment, first staple the pesticide tab to the reservoir. Fold the velour landing strip over the lip of the reservoir and secure in place by twisting on the lid. Use the zip tie to attach the lid to the reservoir. Finally, fill the trap with water to the drainage hole. Organic material like grass clippings, oak leaves or dog kibble may be added to the water to increase the trap's attractiveness. See appendices for additional assembly instructions.

Deployment. The Trap-N-Kill™ Mosquito Trap is approved exclusively for outdoor use. Traps should be placed outside houses in shady areas where mosquitoes tend to congregate, e.g. in shrubbery and under decks. Traps may also be attached to posts, fences, etc., though they should be placed no more than three feet off the ground to maximize mosquito capture rate.

Trap-N-Kill™ Mosquito Traps should be deployed at a rate of four traps per home or approximately 30 traps per acre.

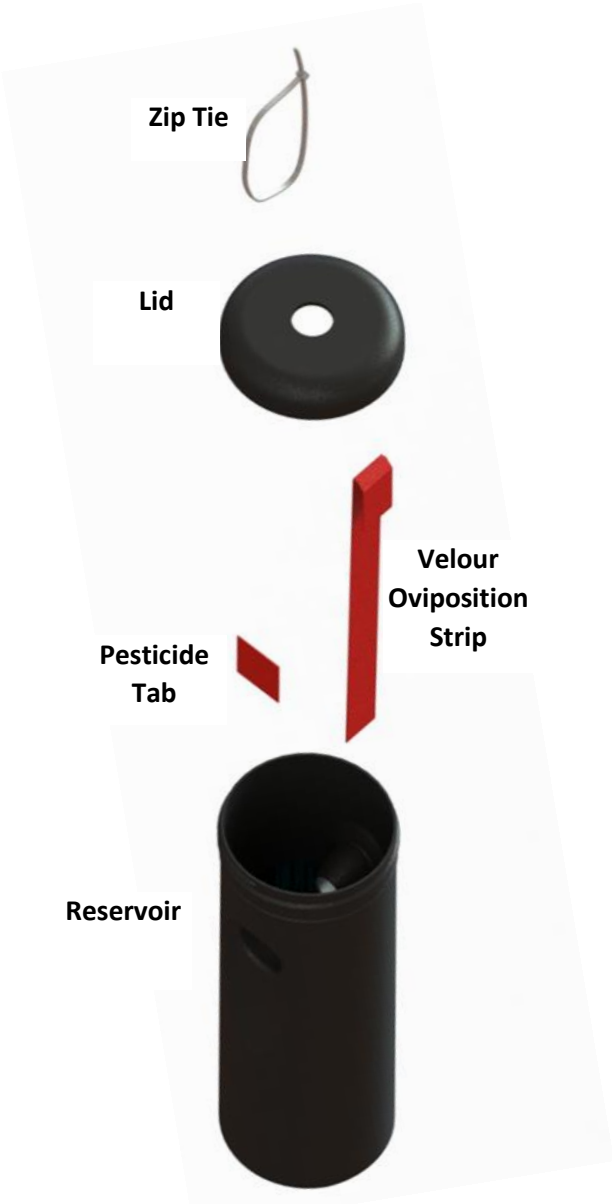


Figure 7. Trap-N-Kill Mosquito Trap components

The trap is most effective in areas where there are few alternative oviposition sites, so SpringStar recommends treating or eliminating nearby standing water in rain gutters, water containers, trash cans, etc. prior to trap deployment.

Maintenance. Replenish the water in the reservoir periodically to maintain trap's attractiveness. In areas with frequent rainfall and/or high humidity, traps may only require refilling once every couple of weeks. In drier areas, it may be necessary to refill the traps more frequently.

Replace the pesticide tab and velour landing strip every ten weeks. SpringStar offers replacement packs containing three tabs, strips, and zip ties; consult your supplier for details.

Evaluating efficacy. Eggs laid on the oviposition strip can be counted to determine if the trap is working (Figure 8). If a trap consistently contains a low number of eggs, it can be moved to another location on the property.

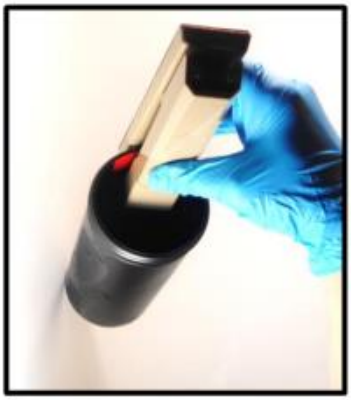
Figure 8. Aedes aegypti *eggs on trap landing strip*

Because adult mosquitoes often depart the trap before dying, the Trap-N-Kill™ Mosquito Trap is not an effective tool for monitoring adult population density. To assess the traps' impact on adult mosquitoes, use an independent monitoring device such as the AGO Mosquito Trap.

Assemble or Refill your Trap-N-Kill mosquito trap

1. Open pesticide tab pouch with scissors.

2. Remove tab from pouch.



3. Staple tab onto the wall of jar, above drain hole.

4. Fold velour landing strip to hook over side of jar.

5. Place landing strip in jar. Landing strip should not overlap pesticide tab.

6. Screw lid on to jar.

7. Use zip tie to secure lid to jar.

8. Fill trap with water to bottom of drain hole.

www.mosquitoscience.net SPRINGSTAR INC 1-800-769-1043

**Gloves are not required for Trap-N-Kill® assembly, but are recommended for use by professionals who handle large numbers of devices.*

Appendix C: MSDS

SpringStar Inc.
Safety Data Sheet

Section I: INDENTIFICATION AND COMPANY INFORMATION

PRODUCT NAME:	Mosquito Trap-N-Kill Target Insect: various Toxicant Strip for Outdoor insect traps
Recommended use:	Use in dry outdoor insect traps to kill target insect
Item Number:	TK71015, TK 71016
Date Issued:	April 4, 2014, Revised May 31, 2015
COMPANY:	SpringStar Inc. PO Box 2622 Woodinville, WA 98072 425-487-6011

For an emergency or more information call 717-764-1192 or
the National Pesticide Information, 800-858-7378

SECTION II. HAZARDOUS MATERIAL IDENTIFICATION

Component	Wt %	Hazard	OSHA PEL*	ACGIH TLV*
DDVP	10%	Poison	1.0mg/m3*	0.1 mg/m3 *
Related Compounds	0.75%			

*Exposure Limits 8 hrs. TWA (mg/m3)

EMERGENCY OVERRVIEW

DDVP is poisonous if swallowed, inhaled or absorbed through skin and eyes. Rapidly absorbed through skin. Repeated inhalation or skin contact may without symptoms, progressively increases susceptibility to DDVP poisoning.
Toxic to fish, birds and other wildlife. Do not contaminate bodies of water.

Potential Health Effects

ROUTES OF ENTRY: Ingestion, inhalation, and absorption through the eye or skin are all possible routes of entry for this active ingredient. Exposure by any of the routes may cause acute cholinesterase depression. Severe cholinesterase depression may be fatal.
SIGNS OF ACUTE OVEREXPOSURE: Acute Cholinesterase depression may be evidenced by headache, nausea, vomiting, diarrhea, abdominal cramps, excessive seating salivation and tearing, constricted pupils, blurred vision, tightness in chest weakness, muscle twitching and confusion; in extreme cases, unconsciousness, convulsions, severe respiratory depression and death may occur.

SIGNS OF CHRONIC OVEREXPOSURE: Repeated exposures to small doses of DDVP and other organophosphates may lower the cholinesterase to levels where the above symptoms of acute overexposure are observed.
CARCINOGENICITY: EPA under its 199 proposed Guidelines for Carcinogen Risk Assessment has classified DDVP as having “suggestive evidence of carcinogenicity, but

SECTION III. COMPOSITION & INFORMATION ON INGREDIENTS

not sufficient to assess human carcinogenic potential”. IARC lists DDVP (Dichlorvos) as being possibly carcinogenic to humans (Group 2B). CARE SHOULD BE EXERCISED IN HANDLING DDVP AND ITS FORMULATIONS.

ACTIVE

COMMON NAME:	DDVP, Dichlorvos
CHEMICAL NAME:	Dimethyl 2,2-vinylphosphate

CHEMICAL FAMILY of active ingredient: Organophosphate ester

C.A.S. NUMBER:	62-73-7
FORMULA:	$C_4H_7Cl_2O_4P$
CONSTRUCTION:	Laminated PVC controlled release dispenser EPA Reg. No. 8730-50, Canada Health PCP# 21222

SECTION IV. FIRST AID MEASURES

FIRST AID: ORGANOPHOSPHATE

IF SWALLOWED

- Call a poison control center or doctor immediately for treatment advice
- Have the person sip a glass of water if able to swallow
- Do not induce vomiting unless told to do so by the poison control center or doctor
- Do not give anything by mouth to an unconscious person

IF IN EYES

- Hold eye open and rinse slowly and gently with water for 15-20 minutes
- Remove contact lenses if present after the first 5 minutes then continue rinsing eye
- Call a physician immediately

IF ON SKIN OR CLOTHING

- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes
- Calla poison control center or doctor for treatment advice

IN INHALED

- Move person to fresh air
- If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth –to-mouth, if possible
- Call a poison control center or doctor for further treatment advice

Have the product container or label with you when calling a poison control center or doctor or going for treatment. NOTE TO PHYSICIAN: Atropine sulfate is the antidote of choice.
You may contact the National Pesticide Telecommunications Network at 1-800-858-7378

SECTION V. FIRE HAZARD INFORMATION

for emergency medical treatment information. Hours of operation are seven days a week, 6:30 am to 4:30 pm PST
FLASH POINT: N/A FLAMMABLE LIMITS in air: N/A
EXTINGUISHING MEDIA: Dry chemical, foam, water fog or spray Carbon dioxide, foam
SPECIAL FIRE FIGHTING PROCEDURES: If involved in fire, use air-supplied equipment. Do not inhale fumes. Wear full protective equipment and NIOSH approved pressure demand, self contained breathing apparatus UNUSUAL FIRE AND EXPLOSION HAZARDS: When burned the hazardous decomposition products that will result because of incomplete combustion include carbon monoxide, other unidentified products of hydrocarbon degradation, Nox, low level cyanides and hydrogen chloride.

SECTION VI. ACCIDENTAL RELEASE MEASURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED: Individual strips that are pouched, pick up and place in a sealed container. If strips are unpouched, and decontamination is warranted, decontaminate the area with dilute ammonia (less than 5% solution) and detergent. Flush the area with water. Absorb the rinse water using an absorbent such as, but not limited to clay, sawdust, straw or kitty litter and sweep into an open drum. Close the drum and dispose as hazardous waste. Do not reuse empty containers. Check with state and local authorities for disposal options.

SECTION VII. HANDLING AND STORAGE

GENERAL PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE: Store in sealed containers in a cool, dry place and away from open flames. To maintain product integrity protect from high temperatures. Keep container closed. Launder contaminated clothing before use. Wear protective equipment described above if exposure conditions warrant. Do not contaminate water sources, food or feed.

SECTION VIII. PERSONAL PROTECTION INFORMATION

RESPIRATORY PROTECTION: Usually none required.
EYE PROTECTION: Usually none required
VENTILATION: Good general ventilation should be sufficient.
PROTECTIVE GLOVES: None required but vinyl, latex or rubber gloves recommended for continuous handling.
OTHER PROTECTIVE EQUIPMENT: None under normal usage.
NOTE: Personal protection information shown above is based upon general information as to normal uses and conditions. Where special or unusual uses or conditions exist, it is suggested that the expert assistance of an industrial hygienist or other qualified

SECTION IX. PHYSICAL AND CHEMICAL PROPERTIES

professional be sought.

PHYSICAL PROPERTIES

BULK DENSITY:	N/A	SPECIFIC GRAVITY/250C:	0.9
MELTING POINT:	3000F	BOILING POINT:	N/A
FREEZING PT:	N/A	pH:	N/A
PERCENT VOLATILE by volume:		None specified	
ODOR DESCRIPTION:	Mild	VAPOR DENSITY (AIR = 1):	N/A

VAPOR PRESSURE (200C, mm HG): Not determined SOLUBILITY IN WATER: Insoluble
PERCENT ACTIVE IN PRODUCT: 10.0%

SECTION X: STABILITY AND REACTIVITY

PRODUCT STABILITY: UNSTABLE STABLE X
HAZARDOUS POLYMERIZATION: May Occur May Not Occur X
CONDITIONS TO AVOID: Do not store near easily ignited chemicals and materials or open flames. MATERIAL TO AVOID: Strong oxidizing agents HAZARDOUS DECOMPOSITION PRODUCTS: On combustion, the polymeric dispensers may produce CO, CO2, HCL and CL2.

SECTION XI TOXICOLOGICAL INFORMATION

ACTIVE INGREDIENT

INGESTION:	Oral LD_{50} (rat)	80/56 mg/kg (male/female)
INHALATION:	LC_{50} (rat)	>198 mg/m3 (4 hr, head only, vapor)
DERMAL:	Skin LD_{50} (rabbit)	205 mg/kg

Trap-N-Kill VAPORTAPE: Skin LD_{50} (Rats) >5050 mg/kg

IRRITATION:	Eye irritation:	Irritant
	Skin irritation	Irritant
SENSITIZATION:	Skin sensitization: (Guinea pig)	Possible Sensitizer

TERATOGENCITY: No evidence of teratogenicity in laboratory animals
MUTAGENCITY: No clear evidence of in vivo mutagenicity activity in mammalian assay systems.
CARCINOGENICITY: Two laboratory studies have shown a low incidence of fore stomach tumors in the mouse and mononuclear cell leukemia in the F344 rat. EPA under its 1999 proposed
evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential". IARC
lists DDVP (Dichlorvos) as being possibly carcinogenic to humans (group 2B). CARE SHOULD BE EXERCISED IN HANDLING DDVP AND ITS FORMULATIONS.
REPRODUCTIVE TOXICITY: Reproductive effects have only been seen at a dose level which

produced a generalized toxicity in the rat.
TOXICOLOGICALLY SYNERGISTIC PRODUCTS: No data are available.

SECTION XII: ECOLOGICAL INFORMATION

No adverse effects have been reported.
The ecotoxicological effects of this product have not been evaluated.
Chemical Fate Information No data available.

SECTION XIII: DISPOSAL CONSIDERATIONS

STORAGE: Store in a cool dry place.
PESTICIDE DISPOSAL: Do not reuse empty pouch or strip. Wrap strip in paper and discard in trash
CONTAINER DISPOSAL: Do not reuse empty pouch. Discard in trash.

SECTION XIV: TRANSPORTATION INFORMATION

SECTION XV: REGULATORY INFORMATION

Ground Transport (DOT) Class 60
US FEDERAL REULATIONS: This product is registered under EPA/FIFRA Regulations. It is a violation of Federal Law to use this product in any manner inconsistent with its labeling. Read and follow all label directions.
CANADIAN REGULATIONS: This product is registered under the Pest Control Product Act of Canada. It is a violation of Canadian Law to use this product in any manner inconsistent with its labeling. Read and follow all label directions. POISON,
SARA TITLE III DATA
Section 311 & 312 Hazard Categories
Immediate Health Hazard: Yes
Delayed Health Hazard: Yes
Fire Hazard: No
Reactive Hazard: No
Sudden Pressure Release Hazard: No
Section 302 Extremely Hazardous substances: DDVP (Dichlorvos, 62-73-7)
Section 313 Toxic Chemicals: DDVP (Dichlorvos, 62-73-7)
OSHA Classification: DDVP in the Vaportape formulation is hazardous
TSCA Status: Not listed on TSCA
CERCLA: Not subject to reporting requirements
RCRA: DDVP considered hazardous
Clean Air Act Ozone Depleting Chemical Substances: None
Clean Air Act Hazardous Air Pollutants: DDVP
Volatile Organic Compounds: None
USDA Status: EPA Reg. No. 8730-50- 66433
This product is not intended for use on food products

SECTION XVI OTHER INFORMATION

MSDS NUMBER: TK71015, TK 71016 DATE ISSUED: April4, 2014
BY: Michael Banfield DATE REVISED: May 31, 2015
TITLE: Product Manager

WARRANTY AND LIMITATION OF DAMAGES
SpringStar warrants that this product conforms to the chemical description on the label and is reasonably fit for the purposes stated on the label when used in accordance with the Directions for Use under normal conditions of use to the extent allowed by state law. SpringStar neither makes, not authorizes any agent or representative to make any other warranty of fitness or of merchantability, guarantee or representation, expressed or implied concerning this material except as stated above. This warranty does not extend to the use of this product contrary to the label instructions, or under abnormal use conditions, or under conditions not reasonably foreseeable to SpringStar. If this product is defective, Buyer's exclusive remedy shall be the replacement of the product, or if replacement is impracticable as determined by SpringStar, refund of the purchase price. To the extent allowable by law, SpringStar's maximum liability for breach of this warranty shall not exceed the purchase price of this product. In no case will SpringStar be liable for incidental, consequential or special damages resulting from handling, storage, use, misuse or abuse of this product.

Appendix D: Dengue/Zika Response Protocol

Protocol for Using Trap-N-Kill Lethal Ovitraps for Dengue/Zika Management

Recommended protocol, developed by SpringStar Inc, Trap-N-Kill® manufacturer. Modified from the Key West protocol (2010), which was approved for the Florida State Experimental Use Permit EUP No: FL10-EUP-01, with revisions adapted from the Queensland Dengue Management Plan (2011).

Executive Summary

Although there is some debate over all of the factors contributing to the increase of dengue incidence worldwide, it is clear that the four dengue viruses are spreading along with their mosquito vectors. The primary dengue vector, *Aedes aegypti*, is currently limited to warmer regions of the United States. It is primarily a domestic, day-biting mosquito that prefers to feed on humans and breed in artificial containers. The closely related, invasive *Aedes albopictus* is less choosey in terms of food sources of breeding sites, but is a more aggressive day-biter and is more widespread throughout the country. This mosquito has been indicated in US outbreaks of dengue, notably one in Hawaii in 2001-2002, and more recently in a locally-acquired case in Long Island, NY.

There is currently no vaccine to protect against any of the dengue strains, so prevention and management of the disease in an area is dependent on management of the mosquito vector and rapid communication between medical personnel and the vector control sector. This document will focus on the vector management aspect, specifically on how to incorporate the Trap-N-Kill (TNK) lethal oviposition traps into an established mosquito management plan.

Lethal ovitraps of multiple types have been successfully tested in a variety of countries and environments. The clear and rapid success rates in Queensland, Australia led to their adoption by Queensland's Dengue Action Response Team (DART). Other studies on these devices will be discussed, as they suggest alternate models by which this protocol may be adapted.

1 Background

1.1 Breeding and larval habitats

Ae. aegypti are peridomestic mosquitoes, preferring to live and around people's homes. They also prefer relatively clean water and artificial or small containers of water. Larvae can be found in: tires, flowerpots, garbage cans, pet water bowls, vases, discarded cans and bottles, the stems of plants like bromeliads, even in less than a Tbsp of water such as what collects in bottle caps or fallen leaves. Larger water containers, from roof gutters to rainwater or septic tanks can also be important breeding sites. *Ae. albopictus* is even more cosmopolitan, utilizing natural sites such as tree holes in addition to artificial containers. Neither of them are known to breed in marshes, ponds, lakes, or similar larger bodies of water

The Trap-N-Kill® provides an ideal-looking breeding site: dark, relatively clean water, and rough surface on which to lay her eggs. When the female mosquito visits TNK to oviposit, she receives a lethal dose of a fact-

acting insecticide, which kills her before she can take another blood meal and transmit the dengue virus. Any larvae that hatch from eggs will receive a toxic dose of the insecticide in the water and will not survive.

1.2 Adult mosquito behavior

Female *Aedes aegypti*, like virtually every other mosquito species, requires a sufficiently large meal of blood in order for her eggs to develop. This meal may require biting several hosts over a day or two in order to consume enough blood. Once she is satiated, she turns her focus towards oviposition, or laying her eggs. It takes 2-3 days from final blood meal until she is ready to lay her eggs. *Ae. aegypti* and *Ae. albopictus* do what is known as "skip oviposition", or laying a few eggs per cycle in each of up to 12 or more water containers. The advantage of literally 'not putting all your eggs in one basket' is clear: by laying in several breeding sites, the female maximizes the chance that at least one of the small water sources will not dry up

1.3 Disease transmission cycle

Dengue cannot be transmitted directly from person to person. Each part of the disease cycle takes a few days. A person sick with dengue is viremic – that is, they have high enough levels of the virus in their blood to transmit it – for up to 12 days after symptoms begin. If a local mosquito bites the infected person and picks up the virus, it goes through a period of viral replication – known as the Extrinsic Incubation Period (EIP) – before it can pass dengue on. This EIP is varies with temperature, but has been estimated to be an average of 15 days at 25°C and 6.5 days at 30°C[32]. This means that a mosquito cannot pass on the virus immediately, even if it bites a second person during the same feeding cycle. Once the mosquito does pass on the virus, it takes another 3-10 days for the virus to replicate in the second individual and for them to become ill and viremic, continuing the cycle. Some dengue infections manifest mildly, with symptoms similar to a bad flu, so the infected person may not visit a doctor. This is one way in which dengue becomes established in a population, when cases are not reported fast enough or at all, and the infected individual continues the transmission cycle unchecked.

1.4 Lifecycle target of lethal ovitraps

Lethal ovitraps target gravid female mosquitoes who may have picked up dengue, and kills them before the virus has had a chance to replicate, thus before they have had a chance to pass it on to another human. Because the female will visit multiple oviposition sites during one egg-laying cycle, the lethal ovitrap merely has to be one of the few that she visits to be effective.

1.5 History and use of lethal ovitraps

The lethal ovitrap was developed by US Army researchers Brian Zeichner and Michael Perich in the 1990s. It is a modification of (non-lethal) surveillance ovitraps, which have been used since the 1960s to monitor mosquito presence through the presence of eggs in the traps. The lethal ovitrap also upends conventional control methods; instead of delivering the insecticide to the mosquito (and the environment and non-target insects in the process), it draws the mosquito to a contained locus of insecticide. This drastically reduces environmental and non-target exposure to the insecticide. Pyrethroids have generally been the insecticide of choice, due to their rapid knockdown, contact mode of action, and high safety profile for humans and other vertebrates. However, other insecticide classes may be used in this method, to alleviate concerns about spreading pyrethroid resistance.

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[32] Chan, M. and Johansson M.A. 2012. The Incubation Periods of Dengue Viruses. PLoS ONE 7(11):e50972

Focused control with lethal ovitraps has significant precedence, based on many of the trials in Thailand and Brazil, as well as current use patterns in North Queensland, Australia. All field studies to date have been focused on *Ae. aegypti* in areas where *Ae. albopictus* is not or minimally present. In Australia, the standard rapid response to known dengue cases is to place LOTs in a 200m radius around houses with confirmed dengue cases; this focused use of LOTs in a relatively small area is sufficient to slow or even prevent outbreaks, if placed quickly enough after the onset of dengue symptoms.

For further reading on the history, development, and use of lethal ovitraps, we recommend:

- Hoel, DF, PJ OBenauer, M Clark, R Smith, TH Hughes, RT Larson, JW Diclaro, and SA Allan. 2011. Efficacy of ovitrap colors and patterns for attracting *Aedes albopictus* at suburban field sites in north-central Florida. *Journal of the American Mosquito Control Association* 27(3):245-251.[33]
- Queensland Dengue Management Plan for 2010-2015. Queensland Health, Queensland, Australia. Approved 11 Feb 2011.[34]
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2 When to Use Trap-N-Kill®

2.1 For prevention of dengue and general vector control

Lethal ovitraps are most effective when used at a ratio of no less than 1:10 with alternate breeding sites. Since determining the number of breeding sites in every household, yard, or discrete area is time consuming – if not impossible – it is easier to use a set number per household or per unit of area. Early trials tested up to 10 LOTs per house. Many of these early trials were performed in tropical, low-resource areas where there were many competing water sources: for example, in one Thailand study, there were an average of 162 potential breeding containers per house. The LOTs were not as effective in that trial. The Queensland Dengue Action Response Team (DART) has found that as few as 2 to 4 traps per household can be sufficient. SpringStar recommends using 4 TNK traps per yard, up to 10 for large or highly littered areas with many alternate breeding sites. In non-residential areas, such as junkyards, parks, cemeteries, and the like, we recommend applying the TNK traps at a rate of ~40 per acre.

Routine, widespread use of lethal ovitraps is not generally recommended, especially with non-biodegradable TNK traps. The amount of effort required to track and maintain the traps at an effective rate, while worthwhile during outbreak situations, may not be the best use of limited time, resources, and personnel in a non-emergency situation. Routine use may be appropriate in high-risk areas, such as around airports or other ports of entry for international travelers. As with any chemical pesticide, TNK traps should

[33] http://www.afpmb.org/sites/default/files/pubs/dwfp/publications/FY11/Hoel_Allan2011.pdf
[34] http://www.health.qld.gov.au/dengue/documents/dengue-mgt-plan.pdf
[35] http://mosquitoscience.net/pdfs/Ritchie-evolution-dengue-TPHU-ARA-05-ms.pdf
[36] http://www.mosquitoscience.net/pdfs/Williams-et-al-optimizing-ovitrap-use-JAMCA-ms-2006.pdf
[37] http://www.ncbi.nlm.nih.gov/pubmed/21805450

be used properly, in combination or rotation with other chemical types, in order to prevent pesticide resistance among the target insect.

2.2 In response to sporadic cases of dengue

When a case of dengue is suspected or has been identified, communication between health services and mosquito control agencies is vital. The goal is rapid elimination of mosquito vectors in areas of concern. Anywhere that the infected individual has spent significant daylight hours while viremic – e.g. home, work, etc – should be considered a potential dengue locus.

Once the potential dengue loci have been identified, mosquito control personnel should deploy TNK traps at the recommended rate in a 100-200m radius around each locus, combined with inspection, source reduction/larviciding, and any other measures that are considered standard practice by the mosquito control district. Since 2004, Queensland's DART has used this mass trapping method successfully, eliminating the need for most interior residual spraying. Traps should be left in place for at 4 weeks to ensure eradication of all potentially infected mosquitoes. After the 4 week period, all pesticide strips should be replaced or the traps collected, to prevent traps from breeding mosquitoes when the pesticide wears off.

Of course, this kind of rapid action requires well established and efficient communication between health and mosquito control personnel. Allowing even a 10 day delay between onset of the viremic period and the start of mosquito control methods can result in additional infections.

2.3 In response to outbreaks of dengue

When more than one case has been identified in an area, continue the containment response as much as is feasible. However, the reality of limited resources and personnel may require prioritizing some areas over others. Areas that should be of higher priority include:

- Clusters of multiple cases in a small geographic area, e.g. on one block or street
- Areas with the highest densities of mosquito populations
- Areas with significant amounts of people travelling in and out, e.g. amusement parks, hotel districts, transportation hubs, etc
- Areas that have not had identified cases of dengue before. Areas that have previously experienced dengue may already have circulating, sub-clinical infections. Focusing on areas with no history of dengue may help prevent more pockets of the disease from becoming established

If it is not part of standard practice already, it is important to conduct regular resistance testing among the mosquito populations, especially during outbreaks when it is vital for mosquito control to be maximally effective. Resistance monitoring will allow for early detection of developing resistance and a change of strategy and/or chemicals, before control methods completely cease to function.

2.4 Integration with other vector management practices

The Trap-N-Kill® method is most successful when combined with other vector control strategies, especially source reduction strategies. The higher the ratio of TNK traps to alternate breeding sites, the greater the likelihood that each female mosquito will visit a TNK during her egg-laying cycle. Although treating alternate breeding sites (with either chemical or biological controls) is helpful in overall mosquito control, it does not reduce site competition with the traps. When possible, alternate water sources should be emptied out or otherwise made inaccessible (i.e. mosquito-proof screening over permanent water storage jars).

Resistance management should be practiced with all adulticiding measures, using multiple action classes where possible. The current version of the TNK uses an organophosphate, so if aerial or other spraying, misting, or fogging is being regularly or concurrently done, a different action class should be used, such as a pyrethroid.

The TNK is compatible with all surveillance methods. However, it is not a surveillance method by itself. The insecticide used in the TNK has a rapid but not instantaneous knockdown rate, so not all affected mosquitoes die inside the TNK traps. The presence or absence of dead mosquitoes within the traps is not conclusive. They may, however, be modified into sticky ovitraps by applying a layer, strip, or removable panel to the upper inside portion of the trap. Sticky ovitraps allow for a complete record of all visitation, as well as for testing the trapped adults for the presence of the dengue virus. Due to greater deployment and maintenance effort required, sticky ovitraps are more appropriately used for surveillance before outbreaks occur rather than during large scale outbreaks.

3 Trap-N-Kill® Setup and Maintenance

3.1 TNK trap description

The Trap-N-Kill mosquito trap design is based on the lethal ovitrap technology which was described by Zeichner and Perich, evaluated in large scale field trials against *Ae. aegypti* in Brazil (2003) and Thailand (2003, 2008), and is in use as part of Standard Operating Procedures against dengue fever outbreaks in Queensland, Australia (2009-current). Although field trials have shown it to be effective on its own, the lethal ovitrap is intended to be used as part of an Integrated Pest Management system. The lethal ovitrap has been tested against *Ae. albopictus* in the laboratory (SpringStar, 2009-2013, unpublished) with promising results, but there have been no published field trials.

The current configuration of the Trap-N-Kill consists of a cylindrical, slim black polyethylene jar, ~8” high, with one conical entrance hold on the side that doubles as a drain hole. A dichlorvos-embedded polymer strip is stapled to the inside top rim of the jar, above the drain hole. An untreated red velour oviposition strip (1” x 9”) runs the height of the trap inside, secured by the lid. A lid with a 1” diameter hole in the center is screwed on the top, to minimize access by children or pets and to contain the pesticide vapor, while still allowing mosquitoes to enter. A zip tie can be threaded through the side hole and lid to prevent unauthorized removal of the lid, e.g. by a curious child. Once the trap is secured in place it is filled with water to provide an attractive oviposition environment for the mosquitoes. If desired, a small grass or hay pellet may be added to increase attractiveness. However, an added attractant is not necessary, as sufficient organic debris will fall into the trap, and *Aedes* mosquitoes prefer clean water.

3.2 Trap deployment

Each Trap-N-Kill® mosquito trap will be assembled in the field by trained personnel. Protective gloves must be worn when handling the insecticide strip. Deployment personnel will identify suitable placement sites such as low-traffic areas or cool, shady areas where the mosquitoes may prefer to rest. Traps should be attached to a sturdy substrate via zip ties, staple guns, wood screw, or other permanent attachment, and affixed at either ground level or within 1 meter of the ground. Traps should not be placed within 6ft of any children’s play structure. If homeowners request, due to presence of pets and/or children, the traps may be

placed higher according to the best judgment of the field staff[38]. After the traps are placed they will be filled with fresh water.

If present at the time of deployment, homeowners should receive a verbal introduction to the Trap-N-Kill® system and printed materials that explain the mosquito situation and how the Trap-N-Kill® fits into the dengue control efforts. The packets will also outline the safety features of the Trap-N-Kill® and ways to avoid potentially dangerous contact with the traps. These written materials can be given in either English or Spanish upon request. If homeowners are not present, the traps should be placed as described above, and printed materials left in both languages, which will include a phone number to call if they have additional questions.

Although *Ae. aegypti* and *Ae. albopictus* are known to skip-oviposit (that is, lay eggs in multiple locations during one egg-laying cycle), the TNK traps work best when there is minimal competition from alternate possible premises for competing breeding sites, and eliminate or larvicide all potential breeding sites that are located. Any water-holding container, both natural and artificial, including tree holes and roof rain gutters, should be examined. Breeding sites should be eliminated whenever possible (e.g. emptying and overturning buckets); if not, they should be treated according to the control agency's current best practices.

Traps should be deployed at a rate of 4 per household, or 20 per acre depending on the size of household plots. If lots are very small (as may be common in dense urban areas), 2 traps per household may be sufficient. If there are a large number of competing or alternative breeding sites (such as rain gutters, tree holes, trash cans, solid waste, plant trivets, or ponds) more traps may be deployed, up to 10 per household or 60 per acre. The optimum ratio of TNK traps to alternate breeding sites is at least 1 to 10; more than 4 TNKs should be placed at a house only when the number of competing breeding sites exceeds 40. TNK traps should be left in the field for 4 weeks and then retrieved. If dengue transmission continues in an area, the traps can be left another 4 weeks, with replacement of the insecticide strip.

3.3 Trap maintenance

As the current version of the trap is not biodegradable, traps must be monitored for retrieval. Otherwise they can eventually turn into mosquito breeding sites once the pesticide wears out. The status of every deployed Trap-N-Kill® should be monitored every two weeks. The insecticide strips must be replaced every four weeks with fresh ones. At the end of the treatment period, the Trap-N-Kill® units must be removed from the field. The jars and lids may be rinsed and saved for reuse; all other contents should be disposed of following label directions.

At each two-week interval, traps should be assessed for: 1) presence and integrity of trap; 2) water levels in the cup; 3) presence of eggs on the strip; and 4) presence of viable larvae or pupae in cups. If traps are missing or damaged (e.g. removed by homeowners, damaged by weedwhackers, etc) they should be replaced. If traps are dry, refill them to the side drain hole. The presence of eggs on the red velour strip indicates that mosquitoes have visited the traps; if no eggs are observed, consider moving the trap to another location. The insecticide strip must be replaced with a fresh one at four weeks. Should maintenance such as refilling the water be indicated prior to scheduled upkeep, based on observations made during other mosquito control activities, arrangements should be made to visit all traps and restore field performance as needed.

[38] *Ae. albopictus* oviposit within 1 meter of the ground a majority of the time, but have been found in breeding sites as high as 7 or even 15 meters above the ground. *Ae. aegypti* prefer ground-level breeding sites, but will visit traps placed up to ~2m off the ground.

Although viable larvae are not expected in the traps, as sufficient dichlorvos dissolves in the trap water to be lethal to larvae, the presence of larvae can indicate that resistance is developing in the local population. If this is observed, field personnel should immediately:

- Replace the pesticide strip in the trap
- Collect larvae from the trap for resistance testing in the laboratory
- A chemical larvicide such as methoprene may be added to the traps as backup if desired or if the traps will not be revisited soon due to limited personnel

4. Population measures

4.1 Resistance surveillance

Mosquito populations worldwide have been subjected to chemical pressures for several decades. As a result, many of the vector species have developed resistance to many of the commonly used chemicals and classes. Pyrethroid resistance is an unfortunate and growing problem in many populations. Therefore it is recommended to periodically monitor for resistance in any mosquito population on a regular basis, but especially before chemical control of any sort is undertaken. Kits for testing susceptibility of adult and larval mosquitoes can be obtained from the WHO, if resistance testing is not part of a mosquito control district's standard operating procedures.

Should any larvae be found alive in any of the TNK trap, they should be collected, along with water from the trap, for laboratory testing. Larval bioassays against known susceptible strains should be run, to compare mortality of collected vs susceptible strains in both the collected water and freshly treated water, to determine if larval survival is due to low pesticide levels, resistance, or both. If resistance is suspected, a larvicide from a different chemical class, such as methoprene, should be added to all deployed traps until resistance can be confirmed or ruled out.

4.2 Mosquito population monitoring

The TNK traps do not provide accurate adult population measures as the insecticide used, while fast-acting, does not instantly knock down the affected mosquitoes, allowing some of them time to exit the jar before perishing. Standard adult and larval population monitoring should be maintained per the mosquito control agency's standard protocols, depending on the season and level of dengue activity. Use of the CDC AGO Trap or the BGS trap is recommended.

If sticky ovitraps are used, two measures may be calculated, although values of concern have not been definitively established. The Sticky Ovitrap Index (SOI), or the mean number of female *Ae. aegypti* per ovitrap per week, greater than 1 is suggestive of a risk for dengue transmission. Alternately, if more than 50% of sticky ovitraps are positive for *Ae. aegypti*, especially during containment of a dengue case, then the risk for ongoing transmission is high.

House index (% of premises infested with *Ae. aegypti* larvae and/or pupae), Container index (% of water-holding containers infested), or Breteau index (number of positive containers per 100 premises) are all useful larvae indices for risk assessment. These indices were developed by the WHO for yellow fever control[39] and, as they measure the same vector species, can be applied to dengue control. Adults may be

[39] See Queensland Dengue Management Plan, pg 45, for a summarized risk assessment table based on the WHO (1972) guidelines for yellow fever control. http://www.health.qld.gov.au/dengue/documents/dengue-mgt-plan.pdf

collected using aspirators or host-seeking adult traps such as BGS traps, however, larval and/or pupal indices are considered better measures of population density. We recommend, in general, weekly monitoring of adult populations and bi-weekly or monthly larval/pupal surveillance.

END

FOR FURTHER INFORMATION

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