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of the Florida Mosquito Control Association

An Official Publication of the AMCA THE AMERICAN MOSQUITO CONTROL ASSOCIATION

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About the Cover: The cover image was provided by Sandy Gross, trap truck supervisor, Lee County Mosquito Control District. While ID'ing mosquitoes in July 2011 she "came across these two *(Culex nigripalpus)* in exactly the same position as in the pic … and thought it was pretty neat how they were positioned. While trying to adjust the colors … this is what happened." She submitted the photo at the suggestion of co-workers, to the approval of the *Wing Beats* Editors.

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Evaluation of Three Strategies for Aedes Control
by Christopher R Lesser and Mark D Latham

INTRODUCTION

In recent years, *Aedes albopictus* and *Ae aegypti* populations have become more problematic for mosquito control districts due to expanding ranges (*Ae albopictus* especially), increasing population size, and the threat of disease transmission. These factors have resulted in increased demand for public-agency mosquito control services. As a result, some mosquito control districts now spend over half of their resources and control efforts targeting peridomestic *Aedes* mosquito populations. This places strain on these agencies, since until quite recently, no mosquito control effort was devoted to these species. Furthermore, very few mosquito control districts have developed an effective plan for addressing public complaints/service requests. Traditional Integrated Mosquito Management (IMM) techniques developed for salt marsh or other freshwater but rural *Aedes* species are generally ineffective when applied to peridomestic, container inhabiting mosquitoes. Source reduction and public education, while effective for many other mosquito species, have been proven to be largely ineffective against *Ae aegypti* and *Ae albopictus* since some individuals [*Homo sapiens*] within the community often have indifferent opinions on sanitation. Larvicide applications can be very effective but extraordinarily expensive and time consuming since an inspector/sprayman must generally hand-apply larvicides throughout an entire community in order to be effective. Additionally, a single property owner can harbor hundreds of individual mosquito-breeding containers turning a small backyard into a miniature Mosquito-Hilton. Also, hand-applied larvicides are ineffective unless repeat treatments are timely. Lastly, ULV applied adulticides have proven to be ineffective since these applications are typically made in the later evening hours to take advantage of favorable thermal-inversions and a decrease in outdoor activities within the urban environment. Unfortunately *Ae aegypti* and *Ae albopictus* are primarily diurnal and crepuscular with little flight activity taking place nocturnally. Previous research has clearly shown that ULV adulticides are ineffective when target species are in periods of rest or hiding.

While *Ae aegypti* and *Ae albopictus* are primarily a nuisance pest in the US, the re-emergence of dengue in south Florida (see sidebar, below) in 2009/2010 and introduction of West Nile virus to the US in 1999 has caused many to re-evaluate the significance of these mosquito species as public health threats and has further underscored the need for the development of improved control methods.

The purpose of our research is to evaluate the efficacy of three different and novel strategies for controlling peridomestic mosquito populations. To be effective, each strategy must be proven to be: 1) cost effective, 2) acceptable by the public, 3) legal, 4) not labor intensive, and 5) implemented by most mosquito control districts.

DENGUE FEVER

Dengue is the most prevalent mosquito borne viral disease in the world affecting 50 to 100 million individuals annually and resulting in the death of approximately 25,000. Dengue has historically been endemic to US Southeast region from Florida to Texas but from 1946 to 1980 dengue was eradicated from the continental United States. From 1980 through 1999, only a handful of dengue cases were reported in the US, with these cases isolated to Texas border towns where large outbreaks of dengue were occurring in Mexico. This changed in 2009 with 27 locally-acquired human cases of dengue in Key West, FL; approximately 70 cases were again documented in Key West in 2010 and several additional human cases of dengue were locally-acquired in Broward and Dade Counties in 2010. Serology studies by the Centers for Disease Control and Prevention indicate that approximately 5% of Key West residents have been infected with dengue. As such, dengue is re-establishing itself in Florida and the need to find an effective control strategy for domestic mosquitoes is highly important.

Figure 1: Ovitraps used in this study: (left) 20 oz black plastic cup with seed germination paper and (right) standard 1 pint Mason jar with masonite board.

Research aimed at the development of better IMM for peridomestic mosquitoes has received increased attention in recent years with a large amount of attention focused on ground-based application of ULV larvicides (methoprene and Bti) in controlled field tests using sentinel larvae as a measure of efficacy. This research conducted in Manatee County, FL (2010), Delaware (2008 and 2009), New Jersey (2010) and Virginia (2006) has demonstrated that such delivery mechanisms can be highly effective. Research has demonstrated that Bti and methoprene, applied neat or diluted through gasoline-powered ULV foggers often resulted in 80 – 100% larval mortality in these tests. But it must be noted that these are controlled tests and no research has yet evaluated the efficacy of ULV larviciding in controlling area-wide mosquito populations. The objective of our research is to evaluate the area-wide effectiveness of: 1) ground ULV-larviciding, 2) ground ULV adulticiding and 3) combination of ground ULV larviciding and adulticiding in reducing populations of domestic *Aedes* mosquitoes.

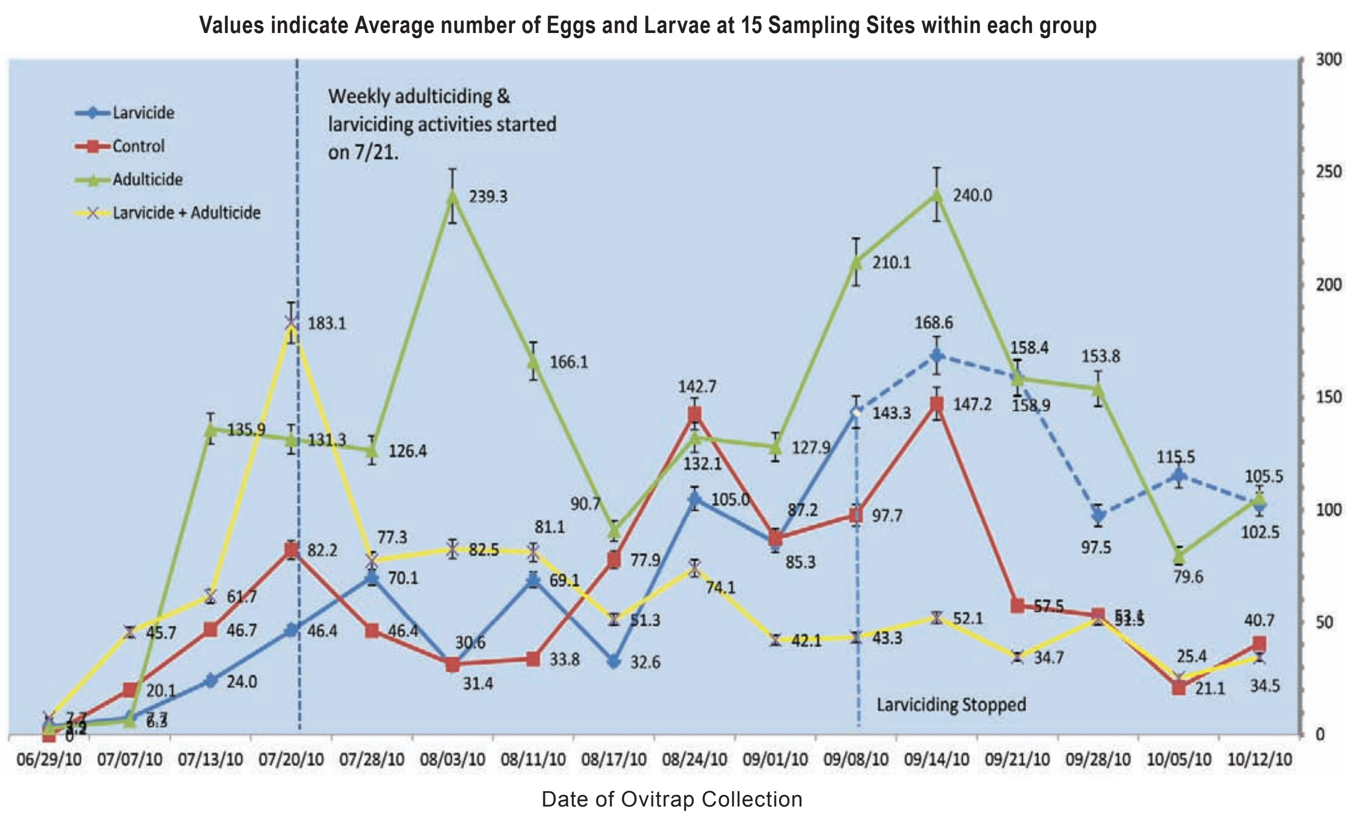


Figure 2: Efficacy of three techniques for control of *Aedes aegypti* and *Ae albopictus* populations.

METHODOLOGIES

Three test sites and one control were selected in residential neighborhoods of Bradenton and Palmetto, FL all with a history of significant *Ae aegypti* and *Ae albopictus* populations. Sites ranged from 145 to 413 acres. Each site was designated as a: 1) larvicide (only), 2) adulticide (only), 3) larvicide and adulticide, 4) control. Each site was treated weekly. Baseline population data was collected for 4 consecutive weeks starting in late June 2010 using 2 styles of ovitraps. A total of 30 ovitraps were placed in each of the 4 study sites; 15 standard "Little Black Jar" (LBJ) ovitraps consisting of a 1 pint Mason glass jar painted black and 15 Florida Medical Entomological Laboratory-designed (FMEL) ovitraps; see Figure 1. Standard LBJ ovitraps used a 1 x 6 inch Masonite board as an egg-lying medium while the FMEL ovitrap used a sheet of Nasco seed germination paper. Eggs were collected and counted weekly. Mosquito larvae in the ovitraps were filtered and counted weekly and included in the overall population assessment.

Altosid 5% was applied neat at 4.0 fl oz/acre over a 300 ft swath using a VecTec Grizzly ULV gasoline-powered fogger. Droplet VMD was determined to be 42 microns at 3 psi as measured on a Magnesium-oxide slide positioned 10 ft behind the spray head. Larvicide applications were generally made 1 hour prior to sunset when vertical air thermals were favorable and wind activity was reasonably strong (at least 2 mph) in order to facilitate chemical movement throughout the treatment area. Omega Mist-Mac 30:30 (permethrin/PBO) was applied at the rate of 0.007 lb ai/acre using a London Fog

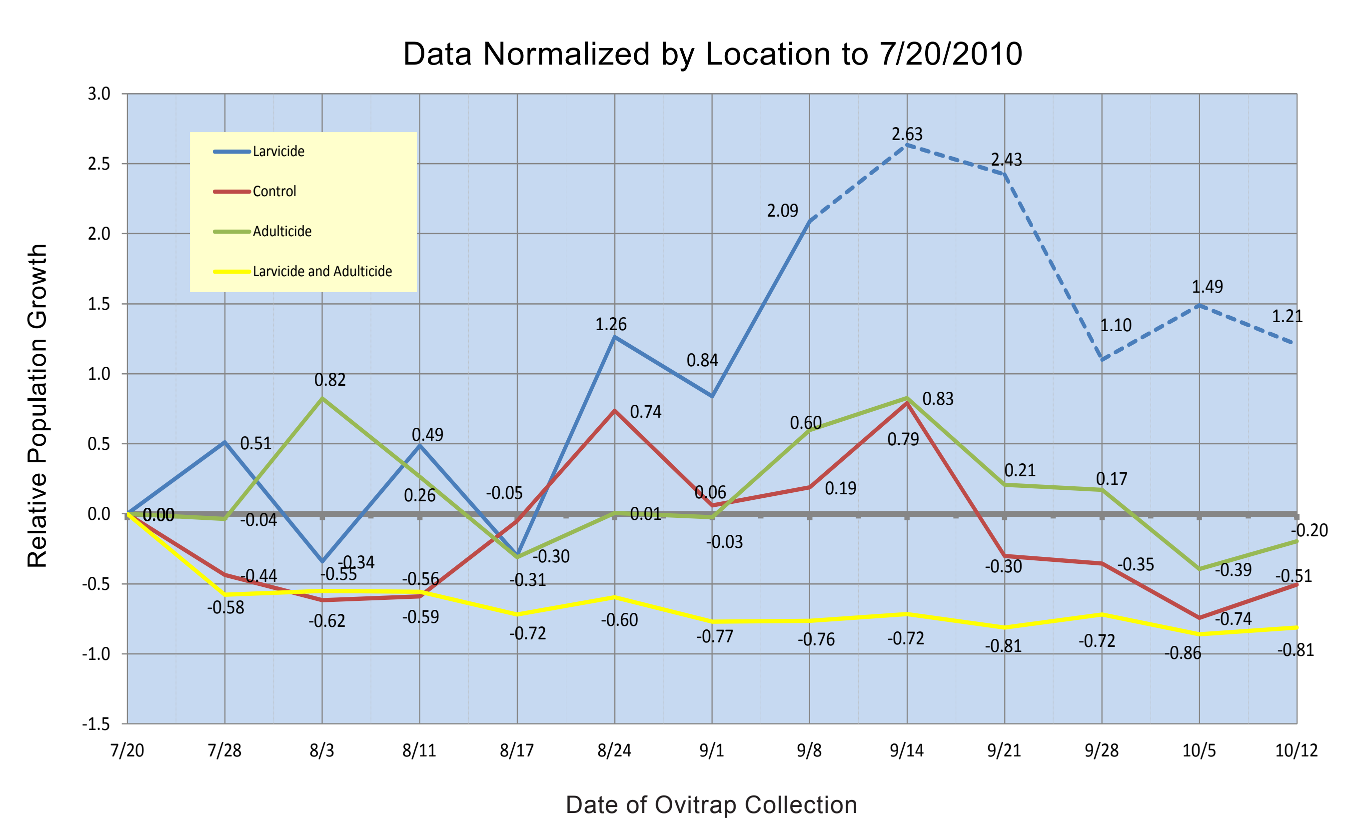


Figure 3: Efficacy of three techniques for control of *Aedes aegypti* and *Ae albopictus* populations.

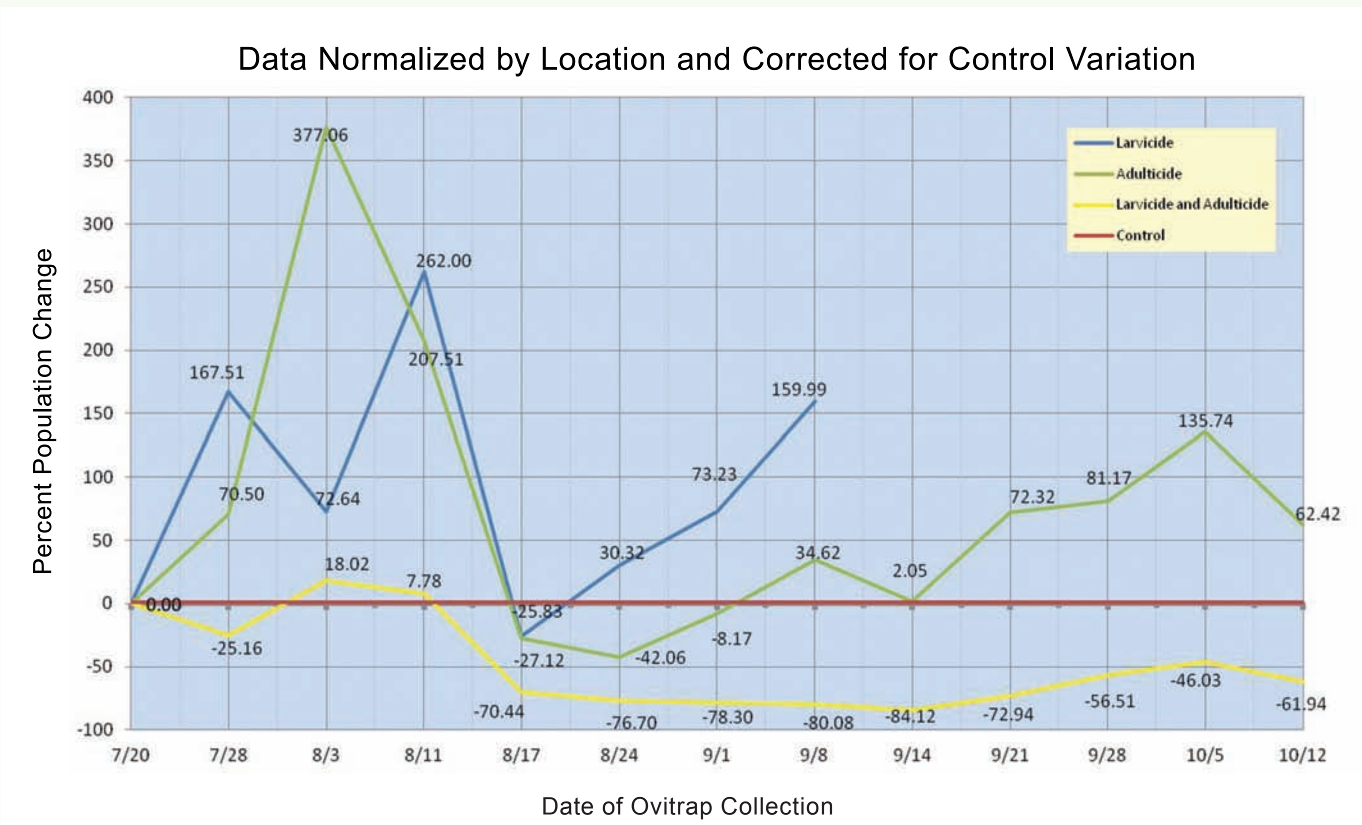


Figure 4: Efficacy of three techniques for control of *Aedes aegypti* and *Ae albopictus* populations.

18-20 at 6 psi. Droplet VMD was 15 microns as measured on a Teflon slide. Adulticide applications were made at about 1 hour before sunset and terminated within 30 minutes of twilight to coincide with typical *Ae aegypti/ albopictus* flight activity.

RESULTS

Collection of baseline population data (pre-treatment) started on June 29, 2010. Weekly chemical applications started July 21, 2010. Figure 2 shows population change over the 15-week study. Mosquito populations peaked during the week of September 14 in the control, larvicide and adulticide plots (although it should be noted that larvicide-only treatments were stopped during the week of September 8) and on July 20 in the larvicide and adulticide plots indicating that ULV-larviciding (only) and ULV adulticiding (only) had no effect on the mosquito population. The combination of ULV larviciding and adulticiding had a very significant negative effect on mosquito population size.

Figure 3 displays relative population growth normalized to the pre-treatment population size within each study area as observed on July 20. Relative change in the control plot are also indicated. Overall a net population decline in the combined "larvicide and adulticide" plot (yellow line) was observed; the overall population decline with this treatment schedule was immediate with a 58% reduction during 1st week post treatment and up to 86% reduction in subsequent weeks as compared to pre-treatment population size. The larvicide (only), adulticide (only) and control plots were characterized by variability above and below the neutral growth line ("zero") but neither the

larvicide or adulticide treatments indicated a negative population trend over the duration of the study as expected. Overall, neither treatment would be effective independently to control these *Aedes* populations.

Figure 4 displays relative population changes within the 3 treatments plots and normalized according to pre-treatment population size - same as Figure 3 - and is also normalized to "zero" the control population thereby mitigating the effects of weather patterns and/or other physical, environmental or biological factors that cause mosquito population to naturally oscillate. As such, Figure 4 most accurately represents treatment effects. This graph indicates an overall increase in population size with larvicide-only and adulticide-only treatments while the overall population trend within the combined larvicide and adulticide treatment plots was negative. The larvicide-only treatment area observed a 262% increase in mosquito populations 3 weeks after the initial chemical applications while the adulticide-only treatment area observed a 377% increase in *Ae aegypti/albopictus* population size 2 weeks following the first weekly chemical application. We do not feel that either is a "cause-effect" relationship but rather a natural spike in mosquito populations that was not abated through ULV-spraying to any measurable or acceptable standard. It should be noted that mosquito populations within the combined larvicide and adulticide test area also observed a slight increase in mosquito population size during this same 2-3 week period but rising only 18 and 7% each respective week. Significant population trends appear on 8/17 and continue for the next 8 weeks through the end of the research study period. Populations within the combined larvicide & adulticide treatment areas dropped to 70-80% below pretreatment baseline for much of the study period and rising to just a 46% reduction in early October which corresponded to a similar population increase in the adulticide-only treatment area. Recall that this normalized data is based on the control population dynamics; in late September and October the control population observed an unexplained and abrupt population decrease. The larvicide-only and adulticide-only treatments resulted in a general increase in population size from 8/17 onward and increased above pre-treatment baselines by 8/24 (larvicide-only) and 9/8 (adulticide-only).

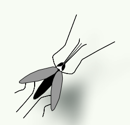
DISCUSSION

The results of this study indicate that up to 84% reduction of domestic *Aedes* mosquito populations is possible by weekly application of combined larvicide and adulticide applications made with ground ULV methods. Given this result, it becomes confusing when we observe no population mitigating effects of larviciding-only and adulticiding-only treatments. Study site habitats are similar, treatment times were uniform and weather conditions were virtually identical among all 4 study areas. In the 2011 calendar year, the role that site selection and site variables will be evaluated in a similar research program.

It should be noted that the number of public service requests were reduced within all 3 treatment areas during the study period but it is also noteworthy that the number of observed adult mosquitoes (ie, landing rate counts) in the larvicide-only and adulticide-only treatment areas did not appear to be reduced below control sites or historical numbers typically seen at each site. The reduction in public service requests in these areas was likely a result of the Manatee County Mosquito Control District's high profile presence in the study areas on weekly basis leading to a public perception that: *"The Mosquito Control District is already out here twice a week – so why call?"* The number of public service requests within the Control site remained high.

It had been generally assumed that approximately 50% of all domestic *Aedes* mosquitoes were *Ae albopictus,* but this research determined that approximately 95% of all eggs collected and reared to adult were *Ae aegypti*. This also corresponded to those observations made in the field within these 4 study areas.

Future research will continue to evaluate ground-based ULV delivery mechanisms for the control of domestic mosquitoes. Additional research will focus upon aerial delivery of larvicide, characterization of the spray cloud and control efficacy of this delivery.



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The Demise of Small Mosquito Control Programs
by Rosemarie Kelly

For the most part, small programs do a very limited amount of mosquito control. Their budgets don't allow aerial application of mosquito control products and usually don't include mosquito surveillance. And these are often the first programs to be cut when counties face budget shortfalls.

Does it matter?

The Georgia Division of Public Health's (GDPH) arboviral surveillance program, which began in 2000 when West Nile virus (WNV) was spreading south from New York, includes mosquito surveillance and testing components. Within this program, most of the surveillance is done in urban areas where the risk of WNV transmission is highest.

Clayton County is a small, densely populated county located just south of Atlanta; see Figure 1. Their mosquito control program evolved from a spray-only program, which started in 1988, to a scheduled adulticide program, and then to a complaint-driven adulticide and larvicide program.

When WNV arrived in Georgia in 2001, Clayton County was the only one of nine metro Atlanta counties with a county-funded mosquito control program in place. The mosquito control staff worked closely with the local public and environmental health departments to reduce the risk of West Nile fever/encephalitis in Clayton County. When arboviral surveillance began in the county in 2002, mosquito control responded to every report of increased *Culex* mosquitoes with both adulticiding and larviciding.

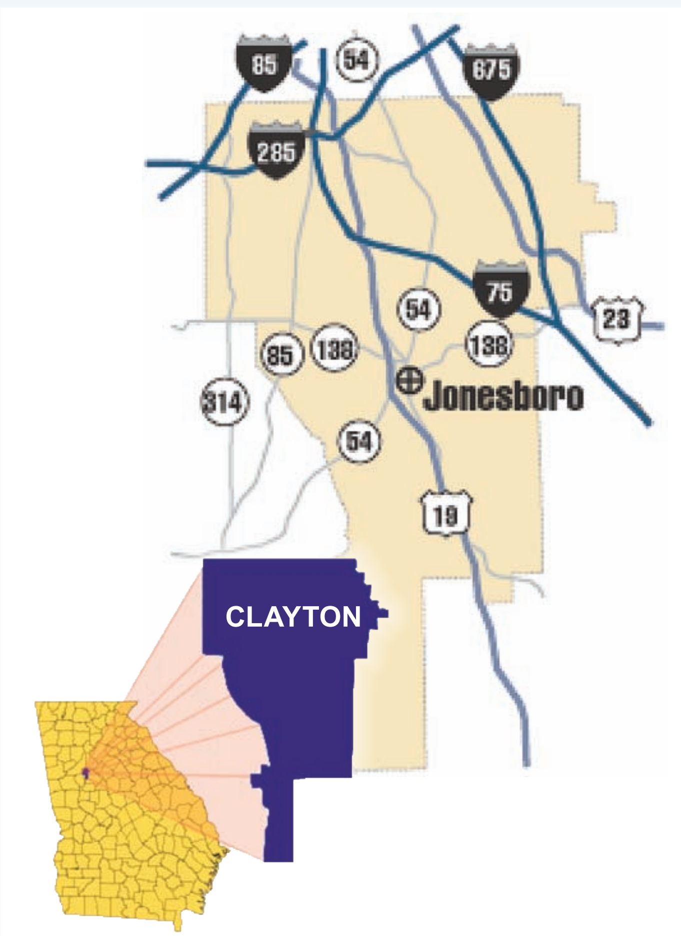

Figure 1: Clayton County, Georgia.

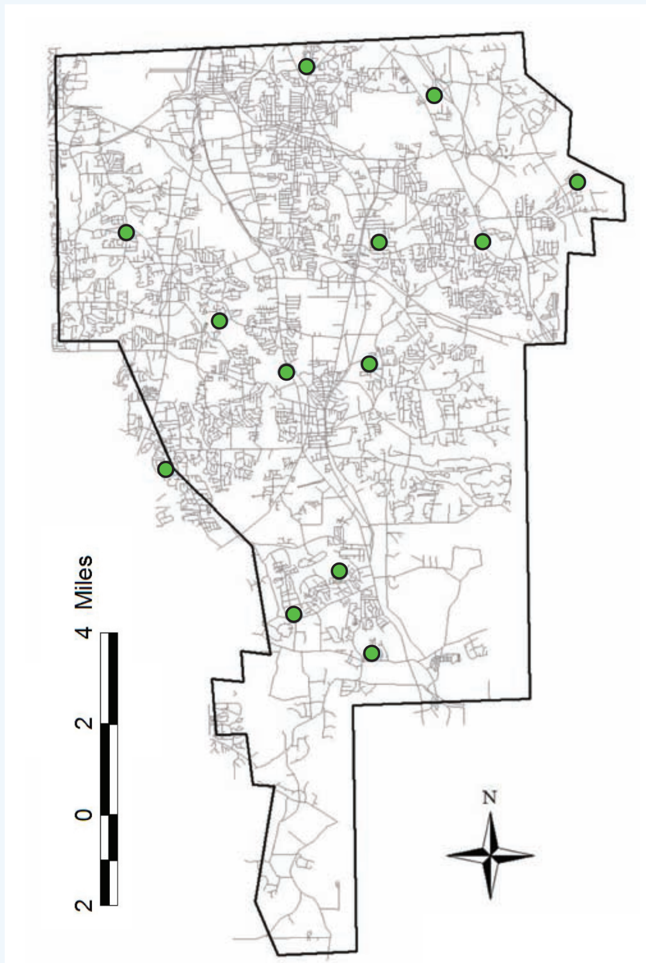

Figure 2: Clayton County surveillance sites.

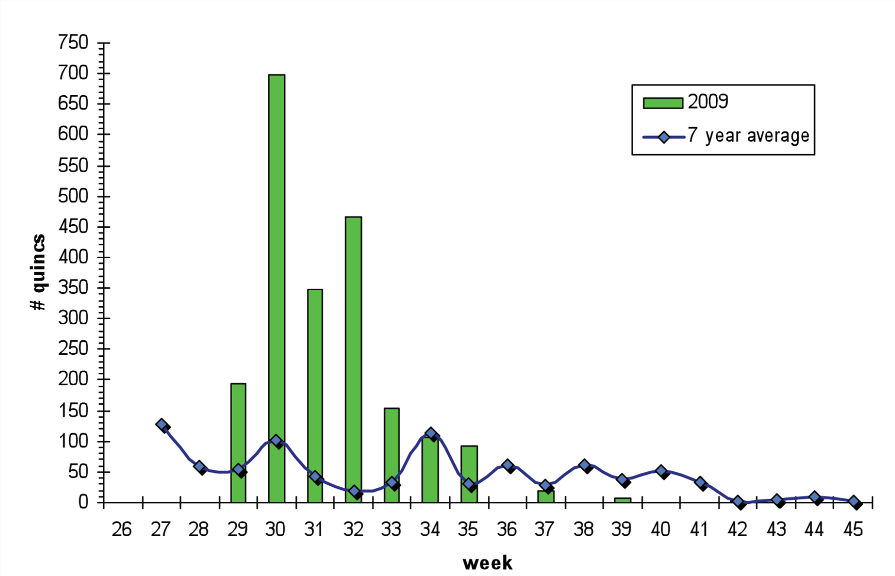

Figure 3: 2009 Clayton County *Culex quinquefasciatus* surveillance data.

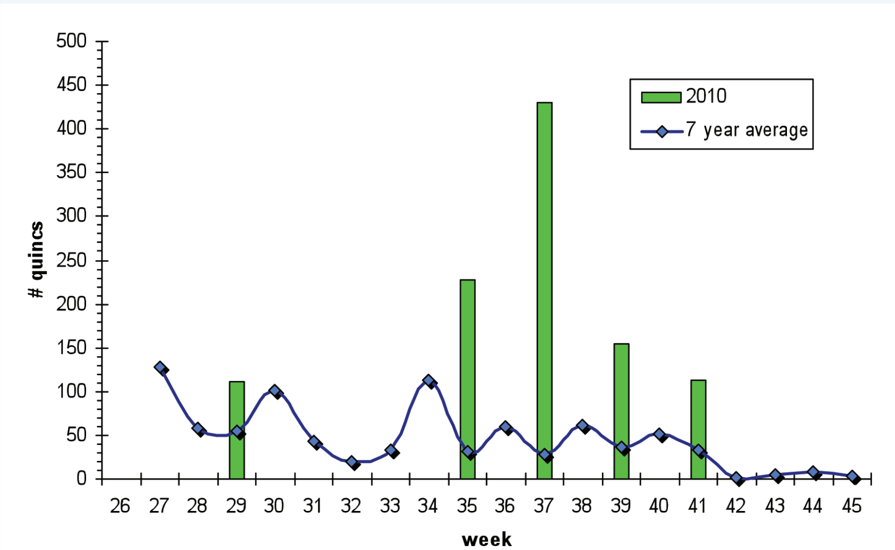

Figure 4: 2010 Clayton County *Culex quinquefasciatus* surveillance data.

Mosquito surveillance based on gravid traps has continued in Clayton County since 2002. Low human case numbers are due in part to educational efforts by the local health departments and by the efforts of mosquito control. Initially, surveillance was done at 18 sites. Additional surveillance was done in 2004 in response to an increase in human disease in areas where no surveillance was being done. Since the local mosquito control staff did not have resources that allowed for routine, in-house surveillance, they depended on complaints and on the arboviral surveillance provided by the GDPH to target areas of higher West Nile fever/encephalitis risk.

In 2005, a sentinel surveillance program was set up in Clayton County; see Figure 2. There were yearly meetings between mosquito control, local public and environmental health departments, and the GDPH entomologist. Mosquito Control remained responsive to all potential WNV problem areas. The Mosquito Control director worked hard to keep Clayton County's program as up-to-date as possible given their limited resources. Surveillance continued, as did the relationship between Mosquito Control, the GDPH, and the local public and environmental health departments. Clayton County had a good mosquito control program that used Integrated Mosquito Management (IMM) techniques to help reduce both nuisance and vector mosquito species.

Everything changed in 2009 when the mosquito control program was discontinued in Clayton County. Although there were no human cases of West Nile fever/encephalitis that year, the number of vector mosquitoes collected was high. Without recourse for mosquito control efforts, the worry was that this would increase the risk of human disease in 2010.

In 2009 there was a considerable increase in the numbers of *Culex quinquefasciatus* over the previous 7 years when mosquito control was available; see Figure 3. In late March of 2010, Clayton County became the first county in the US to have a West Nile fever/encephalitis case reported.

Culex quinquefasciatus numbers remained higher than normal in the county throughout 2010; see Figure 4. Without mosquito control, the local health departments were able to provide only outreach and limited larviciding. Larviciding efforts were limited because resources for larger scale larviciding were simply not available. Additionally, because of resource limitations, education and control measures were only done following a report of human disease.

A number of published reports suggest that mosquito control programs, and especially those using Integrated Mosquito Management techniques, are needed to reduce the risk of arboviral transmission at the local level.

A study from Michigan indicated that people in communities with no mosquito control program had a tenfold greater risk of West Nile fever/encephalitis than those in areas where mosquitoes were controlled (http://www.cdc.gov/ncidod/dvbid/westnile/conf/pdf/Walker_6_04.pdf).

A Chicago area study suggested that mosquito control programs made a difference in WNV infection rates. The Des Plaines Valley District, with an intensive program to kill mosquito larvae, had four West Nile fever/encephalitis cases per 100,000 people, while the North Shore District, with a less ambitious program, had 51 cases per 100,000. This study showed that the program with the most mosquito surveillance and best documented larviciding and adulticiding operations had the fewest number of West Nile fever/encephalitis cases (Tedesco, Ruiz and McLafferty 2010).

This is not new information. The efficacy of aerial insecticide applications to reduce the transmission of Saint Louis Encephalitis (SLE) virus was shown during an epidemic in Dallas, TX in 1966. This study presented evidence that infection rate is reduced as a consequence of anti-mosquito measures. Before aerial spraying there was an SLE virus infection rate of 1 in 167 mosquitoes tested. After aerial control operations the SLE virus infection rate was 1 in 28,639 mosquitoes (Hopkins et al. 1975)

So, are small programs important?

There was a documented increase in vector populations after the demise of Clayton County's mosquito control program. Concurrently, there was an apparent increase in the risk of West Nile fever/encephalitis based on the presence of increased numbers of vector species and the detection of an early human case of West Nile fever/encephalitis in 2010. There was also a suspected increase in nuisance species and mosquito complaints, although these data were not collected.

Since the size of mosquito populations is crucial to disease transmission, it is important to reduce these populations below transmission thresholds. Even small programs can provide a reduction in vector populations and reduce the risk of vector-borne disease transmission.

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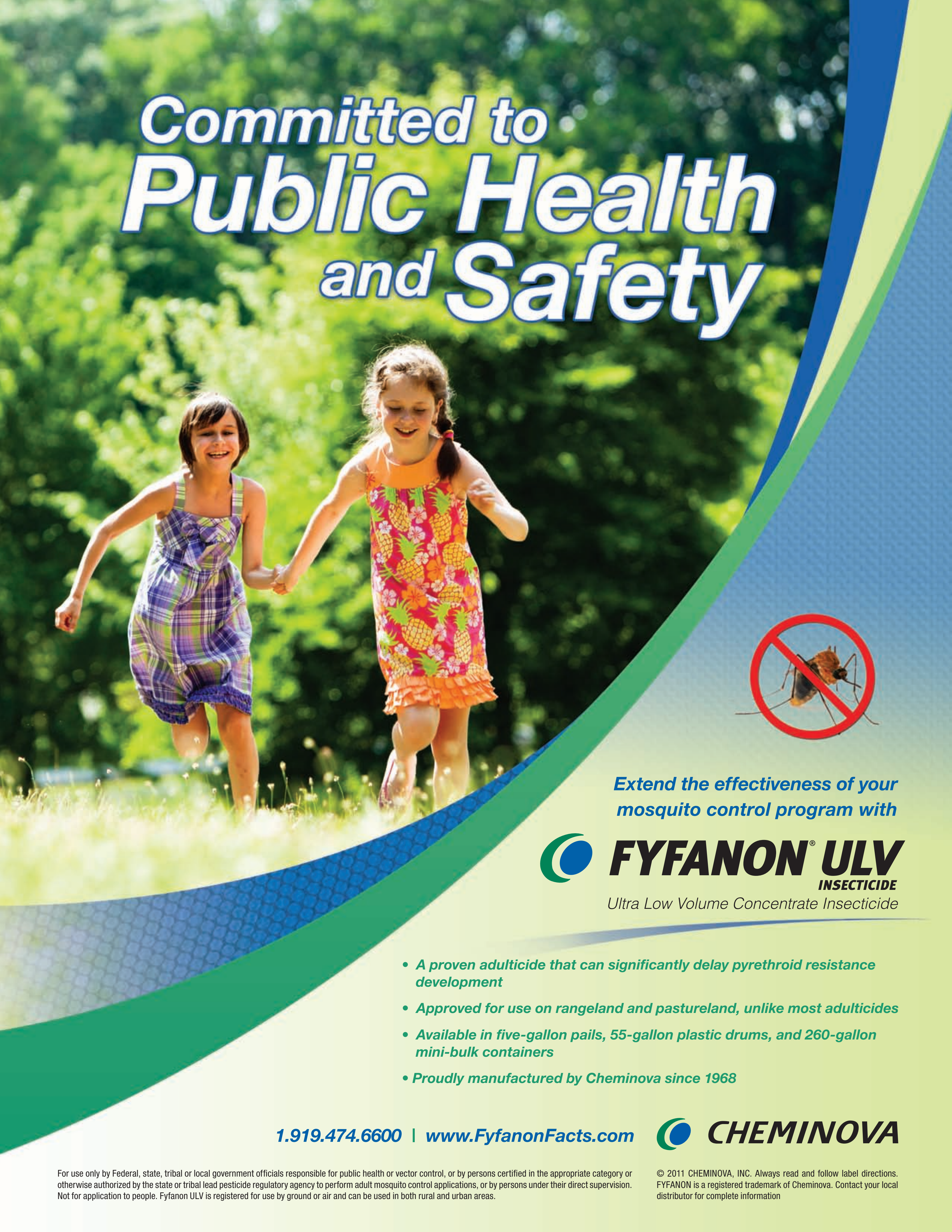
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Vector Control: Thinkers vs Doers
by Manuel F Lluberas

There are no words to describe the level of devastation left in and around Port au Prince, Haiti in the aftermath of the violent earthquake that shook the country during fifteen or so seconds, January 2010. Entire neighborhoods lay in ruins. Hospitals, churches, offices and homes lay intertwined in unrecognizable piles of broken cement and twisted metal. Homes that were once shelters against the intense tropical sun and torrential rains became death traps for large numbers of families, many of which were killed quickly by the tons of debris falling on them. An untold number of others were unable to extricate themselves from their collapsed homes and eventually died of their wounds, associated sequelae, dehydration or simply perished because the meager rescue resources were overwhelmed by the magnitude of the disaster or were also victims of it.

The condition in which Haiti finds herself after the earthquake, combined with the lack of infrastructure before the disaster presents the real possibility of simultaneous outbreaks of malaria and dengue fever while the country struggles to deal with the litany of other pre-existing conditions and a myriad of other issues related to poor sanitation and hygiene. Then came cholera.

In less than a minute, Haiti went from the poorest country in the Western Hemisphere to perhaps one of the most devastated countries in the world with a long list of experts and non-governmental organizations providing their professional opinions on how to proceed. This is a very common phenomenon in the wake of emergencies and disasters, especially when dealing with public health issues, but particularly with regards to vector-borne disease control. Disasters seem to generate a flurry of activity from public health luminaries from prominent organizations to provide expert opinions.

While it is certainly true that we live in a world based on knowledge, the real question is what type of knowledge is most valuable?

As a shrinking world tries to solve larger and more complex problems, public health professionals must not stray from basic science and field observations. Public health, especially as it relates to vector-borne disease control in the aftermath of emergencies and disasters seems to have lost the time-honored value of real experience and close observation on the ground where dusty clay or cement dust meets Gore-Tex® boots. We need to return to the real world of applied knowledge and real experiences like those gathered from field entomologists. In the Panama Canal Zone, the combined leadership and efforts of William C Gorgas, Ronald Ross, Joseph A LePrince and Samuel T Darling led to the elimination of yellow fever and a marked reduction in malaria incidence through an integrated mosquito control program. In Europe, Dr Giovanni Batista Grassi and others did their part in Italy. Perhaps one of the greatest success stories in public health entomology comes from Brazil where Fred Soper, a man of legendary energy endowed with great common sense, campaigned vigorously for the eradication of *Anopheles gambiae*. In just 22 months, he was able to eradicate the mosquito from an area in Brazil of about 18,000 square miles, an area larger than the State of Maryland (http://www.theus50.com/area.php). His success was considered a great public health achievement long before the introduction of DDT in anti-malaria programs (http://www.malariasite.com/malaria/history_control.htm) and earned him many medals and citations. His campaign was so successful that *An gambiae* is still absent from Brazil.

"Knowing in the abstract is not the same as doing in the practice, but doing, as it turns out, can open up whole new ways of knowing" (Webber 2009). The world loves experts, but the problem in public health seems to be that the culture of knowers has overwhelmed the culture of doers and the doers have been relegated to "secondary thinkers" and not always taken seriously by those in the higher centers of knowing.

When it comes to emergency vector control, it seems that thinking has replaced doing and theory has trumped practice.

While in Haiti, for instance, the brief discussions related to which would be the appropriate course of action in response to the impending vector-borne disease crisis on top of the disaster crystallized this never-ending struggle between thinkers and doers so eloquently described by Alan M Webber in his book titled ***Rules of Thumb*** (which should be placed in the list of required reading for public health schools and professionals).

Shortly after the dust began to settle in and around Port au Prince, some of those experts with impressive titles and letters attached to their names left their offices in prominent institutions and travelled to Haiti to add their names and opinions to the relief effort. After a couple of quick meetings with some of the few remaining and available members of the Ministry of Health and a handful of experts from non-governmental organizations and institutions of higher thinking, the participants arrived at the conclusion that implementing an indoor residual spray (IRS) campaign for malaria vector control and introducing mass distribution of long-lasting insecticide-treated mosquito nets would be an appropriate intervention for the prevention of malaria and other mosquito-borne diseases in Haiti. While this combination has been adopted in most of sub-Saharan Africa and proven effective in a number of countries against *Anopheles gambiae* and a handful of other malaria vectors, the reader with basic entomological knowledge might question the logic behind the implementation of these tools against malaria vectors in Haiti. Simply put, IRS requires a structure on which to spray the residual insecticide and nets must be installed and used when the vector is actively seeking a blood meal. Neither of these conditions was present in Haiti, where a large portion of the population is sleeping in tents or outdoors and the main malaria vectors and a number of other mosquito species are actively seeking a host outdoors and/or during times when nets are completely ineffective. More telling perhaps was the plan to augment these interventions with wide scale aerial spraying of Port au Prince. Moreover, selection of these interventions was agreed upon without any discussion related to entomological surveillance, a simple correlation between the number and location of the confirmed cases and actual mosquito populations, identification of the areas actually generating the mosquitoes, entomological inoculation rates, source reduction and larviciding or any other vector control-related tool, indicator or consideration, including costs.

Vector control deliberations in the aftermath of the disaster in Haiti are a clear example of the struggle between the two basic ways of knowing. The first way is from the head. It is the kind of knowing that comes from reading and thinking – the type in which knowers or theorizing experts excel. The other kind of knowledge comes from doing. Unlike the first type of knowing that starts in the head and stays there, this kind of knowing starts in the hands, moves up to the head and then back down to the hands in a knowing-doing loop.

The situation in Haiti seems to point out that a large number of organizations once tasked with providing leadership regarding the world's public health issues and had accepted those challenges with very positive and tangible results in the past seem to be driven today by theoreticians who derive their ideas from ideology and not necessarily from real experience. Many of these organizations employ highly-paid staff advisors and consultants with impressive résumés many of whom have seldom walked on cement dust after an earthquake or the salty mud left by a tsunami, seen the inside of an African or South American hut while conducting IRS operations, witnessed the struggle for life of a child in malaria's or dengue hemorrhagic fever's death grip, tried to console one of the mothers burying these children, or sat with a microscopist trying to locate an elusive plasmodium or filarial worm from hundreds of microscope slides using dirty microscopes and earning measly wages. Moreover, when it comes to vector-borne disease control, much of the advice given is in the treat-the-patient concept while ignoring the fact that millions will continue to be killed and hundreds of millions more will be affected as long as the vectors remain well entrenched in the affected areas.

Many public health entomologists know first-hand the "knowers know better" syndrome. During the course of the last couple of decades, for example, getting funding for most public health entomology projects has required proposals to be presented by "thinkers" with advanced degrees, impressive résumés and a long list of letters after their names. Field public health workers, entomologists in particular, who see the problems firsthand and can devise practical solutions virtually on the spot might be useful in implementing what the thinkers might have prepared, but the actual preparation and meeting of the minds leading to the development of any plan is usually left to the thinking experts and consultants.

The real question is, as someone who might be placed in a position to come up with a plan to help in a situation like the one the people of Haiti are going through, who do you ask, the thinkers or the doers? The people who need the most help must have access to the kind of knowledge that comes from doing, not necessarily the knowledge generated from thinking. The on-site doer usually knows the most about the enemy and what lets him win or lose.

Success in public health, especially that related to vector control comes from finding what works and why. It is most often reached through empirical evidence, not theory. Success comes from having answers you can trust and believe in. To get these answers, we must go beyond simply knowing. We must obtain them through doing.

Thinkers can contribute, but they must accept that there is no substitute for experience and thus incorporate the experience of the doers in their contributions and not just their own theories. Doers, on the other hand, must occasionally take a break to become thinkers and carefully analyze their chronicles and those of other doers. Individuals with meaningful field experience in vector control should be included as decision-makers in the cadre of consultants/advisors selected by governments and international organizations.

We will be able to prevent or control malaria and other vector-borne diseases only when thinkers base their analysis on the accomplishments of doers, doers re-examine their own accomplishments, and both sides work together.

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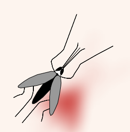

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New Partnership Bears First Fruit: Experimental Use Permit for Pyriproxyfen

by Randy Gaugler

Insecticides are indispensable tools for mosquito management. Yet the number of available agents has declined because of the increasing cost to maintain their registration. New active ingredients are needed, but new chemistry development for mosquitoes is crippled by the need for manufacturers to recoup the massive costs of developing a new insecticide. Mosquito control is a minor market at best. The default strategy is for existing actives developed for agricultural markets to be redeployed for mosquito control. Pyriproxyfen represents one such opportunity.

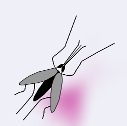
Pyriproxyfen is an insect growth regulator that acts as a juvenile hormone mimic, resulting in a lethal disruption of insect development. This 'reduced-risk pesticide' is non-toxic to birds or animals and is neither carcinogenic or genotoxic. The compound is labeled in the US as NyGuard® (MGK Corporation) for use against a wide range of insects. The effectiveness of pyriproxyfen as a mosquito larvicide is promising, yet this chemical has seen virtually no use against mosquitoes, in large part due to label restrictions against vehicle-mounted sprayers and area-wide mosquito control.

An extraordinary alliance that includes federal (US Department of Agriculture's Agricultural Research Service (USDA-ARS) in Beltsville and Gainesville), university (Rutgers, IR-4 Project), military (Navy Entomology Center of Excellence), industry (MGK, Sumitomo), state (New Jersey, Florida), and county (Anastasia Mosquito Control District, Mercer County Mosquito Control, Monmouth County Mosquito Commission) elements has resulted in the US Environmental Protection Agency (EPA) issuing an Experimental Use Permit (EUP) for pyriproxyfen, the first step in adding this compound to an otherwise depleted mosquito control weapons arsenal.

The goal is to develop practical application strategies by testing a commercially available product via vehicle-mounted spray equipment in area-wide treatments delivered by local mosquito control personnel. In addition to broadcast applications using pyriproxyfen as a conventional mosquito larvicide, the autodissemination strategy pioneered by Takaaki Itoh and Greg Devine will be tested operationally on an area-wide basis under the EUP. The study is aimed at managing urban container-inhabiting mosquitoes, most notably the highly invasive Asian tiger mosquito, *Aedes albopictus*. Risk assessment, including residue analyses, will be a key component of the study. MGK Corporation, in addition to contributing critically needed data for the EUP submission, is donating product for all treatments. The experiments are supported by grants to the Rutgers Center for Vector Biology from USDA-ARS and the Deployed War-Fighter Protection (DWFP) Research Program of the US Armed Forces Pest Management Board.

In an additional development, the Rutgers Center for Vector Biology has recently been designated and certified by federal, state and university regulators as a "Pesticide-Producing and Device-Producing Facility." This unique capability enhances the Center's ability to develop and test novel pesticides and formulations on-site.

The EUP initiative was led by the IR-4's new Public Health Pesticides Program. The program was created to help fill and maintain the toolbox of toxicants, repellents, attractants, and other chemical tools used to manage mosquitoes, ticks, sand flies, and other arthropods that transmit human or animal disease. Major IR-4 partners include the DWFP and the USDA-ARS. For more information about the Program, contact Program Manager Karl Malamud-Roam at kmr@aesop.rutgers.edu or 732-932-9575 x 4628.



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New Tool for Mosquito Surveillance: Development of a Chemical CO_2 Generation Device

by Frank H Cornine III and John D Prohaska

In this article we describe our recent work on the development and field demonstration of a new type of carbon dioxide baiting source for mosquito surveillance. Everyone involved in mosquito control is aware of the close relationship between carbon dioxide and mosquito attraction. Mosquitoes use carbon dioxide plumes to find host animals for blood feeding. It is well documented that the use of carbon dioxide greatly enhances mosquito collection using the standard Center for Disease Control (CDC) trap. Effective mosquito and disease vector surveillance depends on collecting a sufficient number of specimens to assess public health risks. The overall goal is to develop more efficient baiting methods and devices that increase collection efficiency, improving the accuracy of public health risk assessments.

The suspected link between carbon dioxide and mosquito attraction dates back to perhaps sometime in the late 19th century, but it wasn't until 1922 when Willem Rudolfs first showed that CO_2 affects mosquito behavior (Rudolfs 1922). Since then, there have been many studies documenting the enhanced collection efficiency when using carbon dioxide in mosquito trapping and its influence on mosquito feeding behavior (Gillies 1980). There are other chemicals, such as lactic acid and octenol that are attractive to mosquitoes. When combined with carbon dioxide, these act to effectively attract mosquitoes at a distance. But given all the science regarding the role of CO_2 as an attractant, it is clear from a practical point of view that carbon dioxide when used with a CDC trap is an effective method to sample mosquito populations.

In the United States and Canada there are hundreds of public mosquito control districts that manage the health risk from mosquito populations, yet many of them do not use carbon dioxide as an attractant during their collections. Of course the needs of each district vary depending on its geographical and human population characteristics, so one would not expect every district to have an extensive mosquito population monitoring program. However, it is somewhat surprising that not more of the mosquito collection programs use carbon dioxide as an attractant since the benefit of using carbon dioxide during mosquito surveillance is well documented. Given this, one is tempted to ask why? Does carbon dioxide cost too much? Is the process too complicated? Are the associated safety issues an impediment?

In general for mosquito surveillance, one wants a consistent and controllable CO_2 flow rate between 200-700 ml/min. There are several methods to generate carbon dioxide for mosquito baiting. These include dry ice (solid CO_2), compressed gas cylinders, and hydrocarbon fuel combustion. Dry ice is a

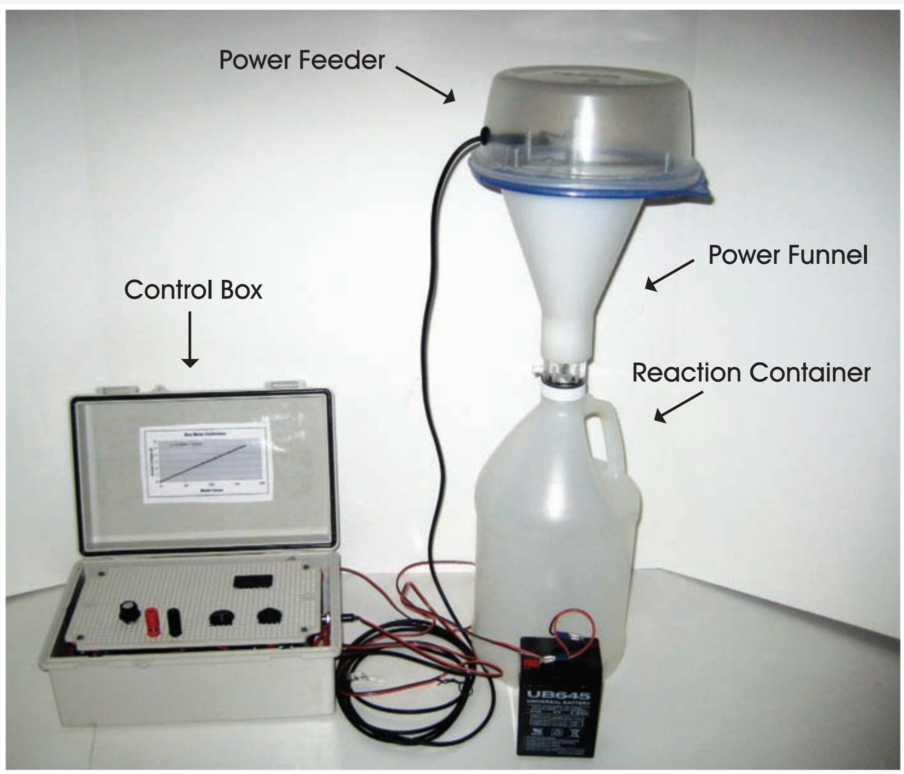
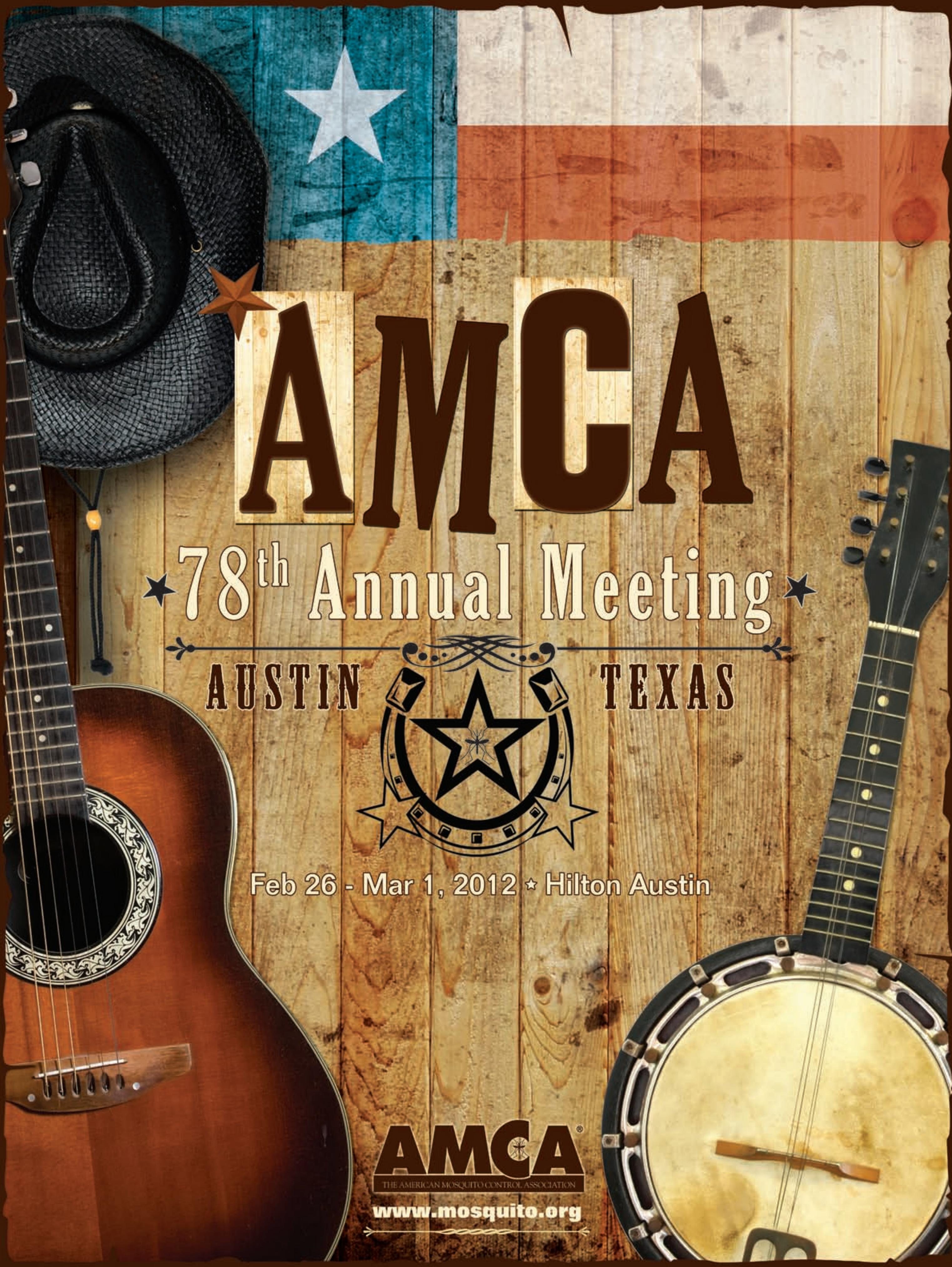


Figure 1: The Blackstone Photonics CO_2 baiting source.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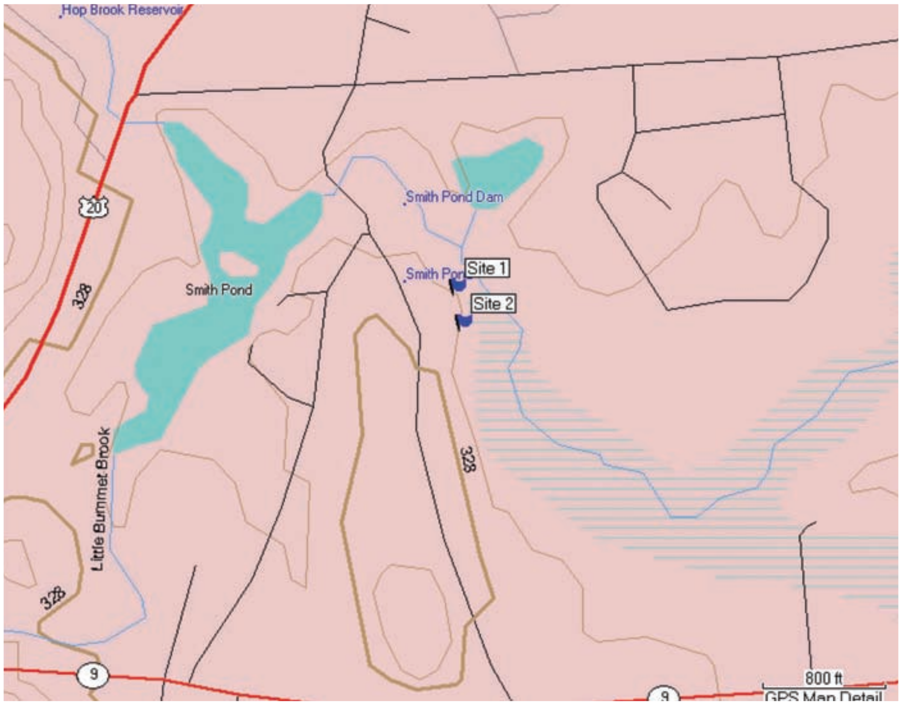

Figure 2: Mosquito monitoring test sites in Northborough, MA.

very cold material (-109°F) and requires specialized handling, refrigeration, thermoses, and insulated gloves. It sublimes at a rate of 5-10 lbs per 24 hours in a typical thermos container. This sublimation rate corresponds to a CO_2 flow of 800-1600 ml/min. The exact flow rate is difficult to control because it depends on thermal insulation factors, ambient weather conditions, and the amount of dry ice used. Weight is not much of an issue since common thermoses weigh only 1-2 pounds, but the storage and handling of CO_2 are challenges for this method. Compressed gas cylinders provide very accurate gas flow control and can readily satisfy mosquito monitoring requirements, but they are relatively heavy. A full 5 lbs gas cylinder weighs either 17.5 or 12.5 lbs for a steel or aluminum cylinder, respectively. The pressure regulator weighs another 2-4 lbs. In addition they present a safety hazard. When mishandled they can explode and become a projectile that can be quite destructive. Hydrocarbon fuel, such as propane, can be used to generate CO_2 through combustion. The primary issue with any fuel is that it creates substantial amount of heat associated with combustion which presents a fire hazard for unattended collection stations.

Blackstone Photonics of Millbury, Massachusetts has been developing a new carbon dioxide source for mosquito collecting. It is based on a novel chemical reactor design and specially modified alkali bicarbonate chemistry. We have applied our experience in meso-scale materials developed for optical chemical sensing to develop this technology. The chemical reactor is made of light weight plastic and is electronically controlled, powered by a battery; see Figure 1. Including the reaction raw materials the entire unit weighs less than 7 lbs, making it very convenient to transport to remote monitoring sites. The chemistry is ecologically friendly and is composed of items one might find in a kitchen cupboard, presenting no hazard to the operator or the environment. The reactor allows us to electronically control the rate of reaction and therefore the carbon dioxide flow rate between 0-800 ml/min. The device is easy to set-up and use. Set-up consists of pouring a dry powder into one container and mixing water with another powder in a second container which can be done before heading into the field. The unit can also be programmed to turn on and off at a specific time.

In early 2010 Blackstone Photonics approached the Central Massachusetts Mosquito Control Project (CMMCP) in Northborough, MA for advice in mosquito collection techniques and began a collaboration which bore the results discussed in this article. Together we planned a study comparing the collection efficiency of the new Blackstone CO_2 source with a standard gas cylinder source. A convenient site was identified for the study that we thought would yield good numbers of various species (predominately *Coquillettidia perturbans*). The site was located in Northborough, MA near local wetlands; see Figure 2. Two collection stations were used; one with the standard gas cylinder and the other with Blackstone source. They were separated by about 100 ft to minimize competition between the traps and switched between trap nights. CO_2 flow rates were adjusted to 250 ml/min. A collection bottle rotator (model 1512, John W Hock Co) with eight containers was used to gather information about how the collections proceeded through the night. Each trap was mounted on a 4 x 4 inch post; see Figures 3 and 4. Collection began each day three hours before sunset and the time interval for each collection cup was 90 minutes. After each night of collections we would bring the specimens and equipment back

Figure 3: Standard gas cylinder baited trap setup in the field.

Figure 4: Blackstone CO_2 generation source baited trap setup in the field.

Figure 5: Retrieving mosquito specimens from both CO_2 traps.

to the shop to identify and count mosquitoes; see Figures 5 – 7. The Blackstone baiting source would be prepared sometime during the day of the collection and would take only a few minutes. The collection period was about a month and a half from June 23 to August 5, 2010. Over this time we were able to obtain nine good collections for comparisons. During the study the Blackstone CO_2 source was exposed to a variety of weather conditions ranging from hot and humid New England summer conditions to down pours from severe thunder storms. The unit performed quite well in all conditions without any significant problems.

Based on our results we can make a number of observations and comments. Overall, we see that there is a significant fluctuation in the number of mosquitoes collected from night to night and also from CO_2 source to source; see Figures 8 and 9. Night-to-night fluctuations are expected given variations in the evening temperature, wind speed, and humidity. In addition we see a general trend of the total mosquito count decreasing as the season progresses, which is also expected given the extended drought that coincided with collections; see Figure 8. This general trend essentially forced us to stop the study prematurely. In examining source to source variability for a given night's collection we observed, in some

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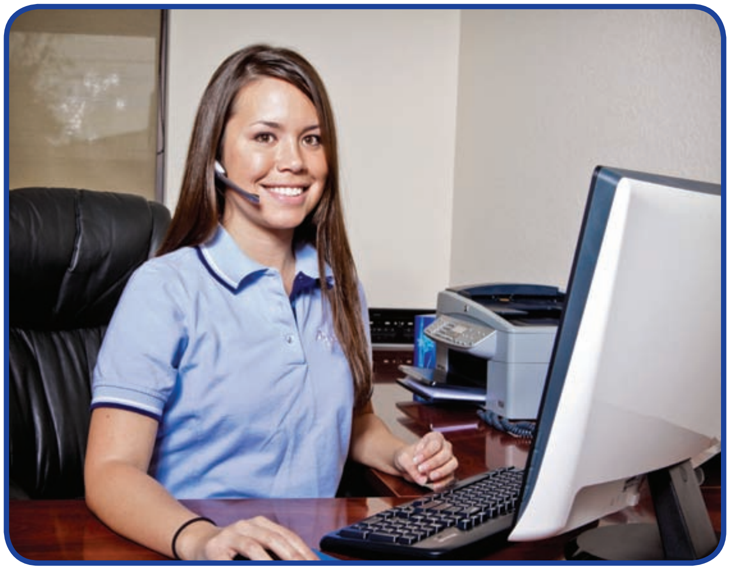

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Figure 6: Mosquito samples from a collection bottle ready to be identified.

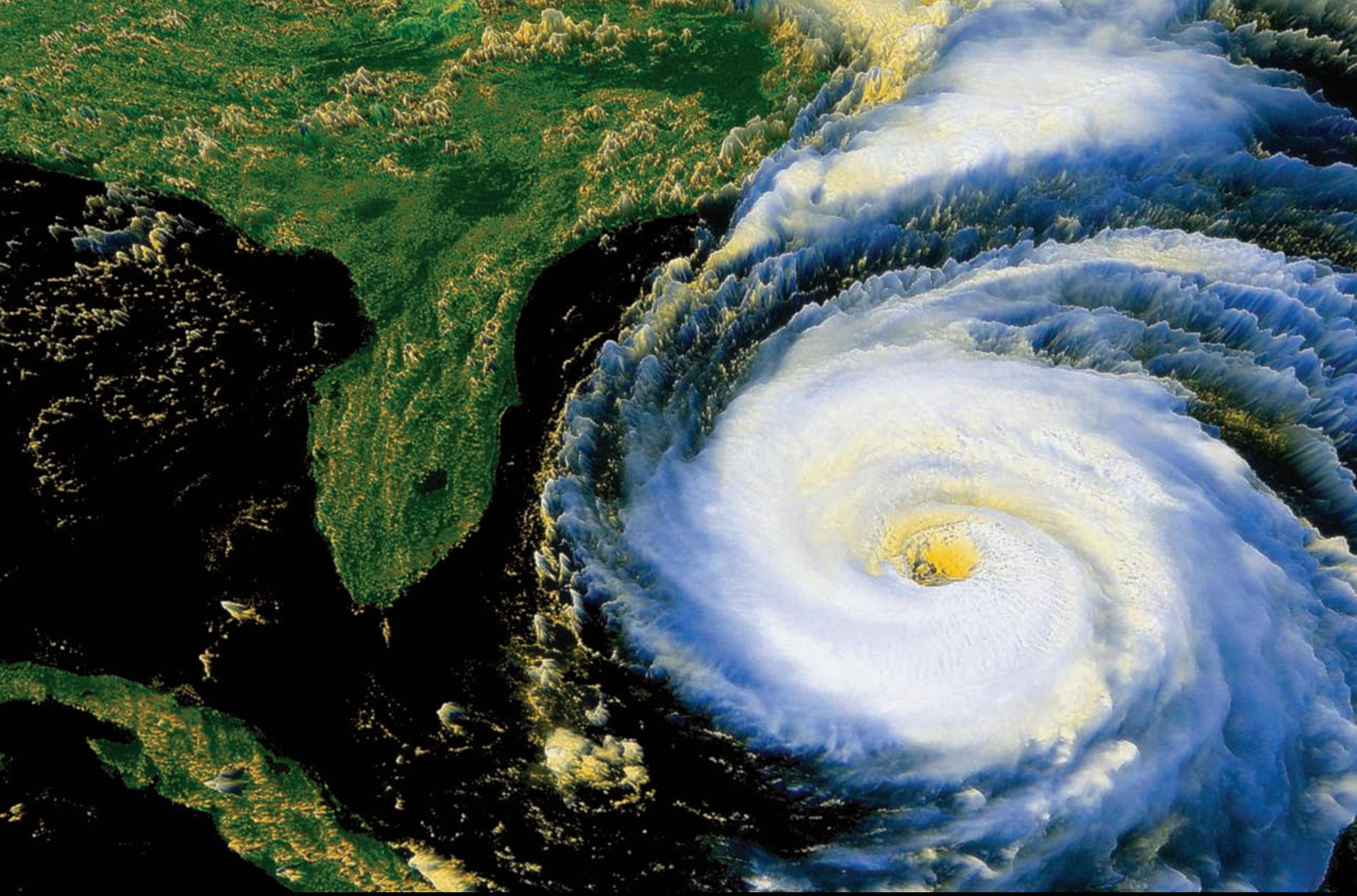
cases, a substantial variation and in others virtually none. If we look at the cases more closely we see that two trials show significantly greater counts for the gas cylinder (numbers 3 & 6) and two other trials show significantly greater counts for the Blackstone source (numbers 2 & 4). For the remaining trials, the collection numbers are essentially equal; see Figure 8.

Since the total number of collections represents a relatively small sample, it is difficult to determine precisely the origin of (and to assess the statistical significance) the variation we observe. The origin of the variation may be instrumental and or environmental in nature. In an effort to minimize any instrumentally induced error, we have performed extensive calibration testing of the Blackstone CO_2 source with a reference gas cylinder. These calibration tests show good agreement between the two sources. Local environmental variations could be another possible explanation. The local geography of the sites varied somewhat which could lead to microclimate differences that affected the mosquito counts for those specific cases. Either way, on average these variations may not be significant. The total number of mosquitoes collected over the entire period using the standard gas cylinder and the Blackstone source as bait was 408 and 564, respectively.

When examining the data for the number of mosquitoes collected in 90 minute intervals after sunset for two collection nights (7/8, 7/20) we made a number of interesting observations; see Figure 9. First, traps using both CO_2 sources exhibited a similar time collection response. We also observed that the majority of mosquitoes were collected during the first 180 minutes after sunset for both carbon dioxide sources with little activity outside this period and that there is a peak occurring in the first 90 minutes after sunset. These observations are consistent with previous host-seeking activity studies for similar mosquito species (Schmidt 2003).

In conclusion, Blackstone Photonics and the CMMCP compared the effectiveness of a new carbon dioxide source compared with a standard gas cylinder using a CDC mosquito trap. The number

Figure 7: Counting and identifying mosquito specimens from trap collection.



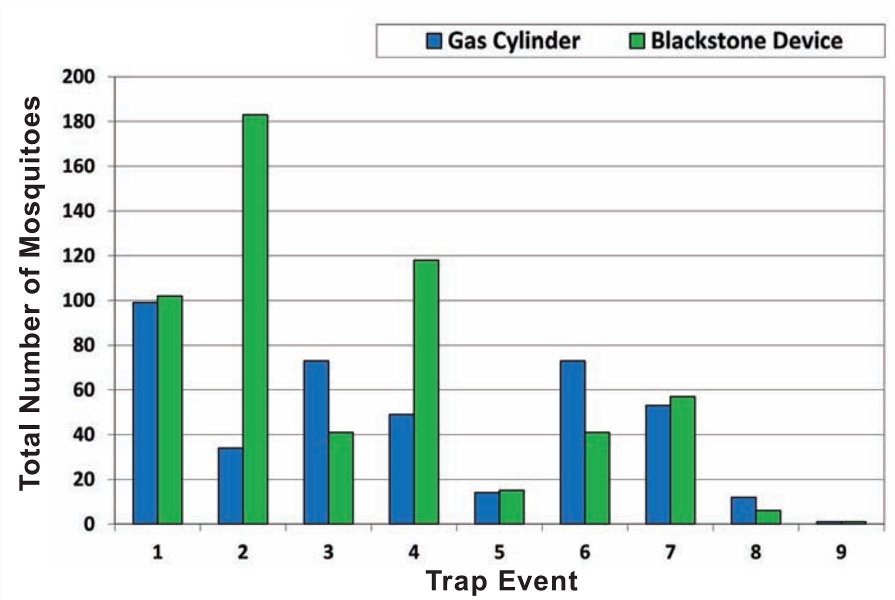


Figure 8: Collection night mosquito totals for both gas cylinder and Blackstone CO_2 baited traps.

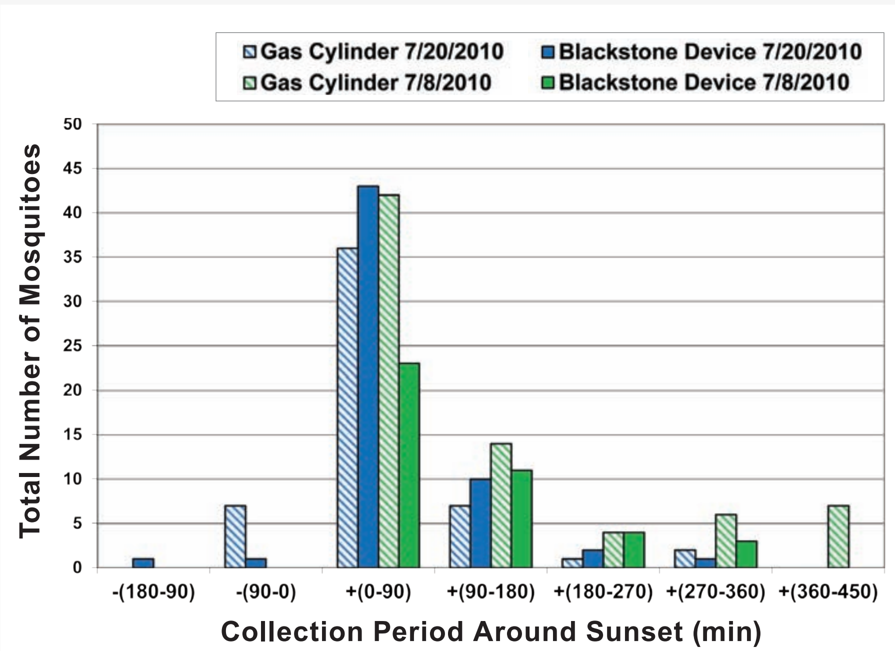


Figure 9: Mosquito collection numbers in 90 minute intervals around sunset for select trap nights of both gas cylinder and Blackstone CO_2 baited traps.

of mosquitoes collected during this study show a good correlation between the two gas sources indicating the Blackstone generator showed significant promise as a potential new carbon dioxide source for field mosquito surveillance. The new carbon dioxide source was exposed to a range of weather conditions and continued to function without any major problems. The new Blackstone Photonics carbon dioxide source offers significant benefits over existing gas sources for mosquito baiting. These advantages include; light-weight, contains environmentally friendly materials, gas flow is controlled electronically, and is convenient and safe to use. It is our belief that one day this new carbon dioxide source will become an effective tool augmenting mosquito surveillance programs. Using this CO_2 source would make it easier for some mosquito control districts to employ carbon dioxide baiting as part of their standard surveillance procedures and improve their ability to make accurate risk assessment of public health risks from mosquitoes.

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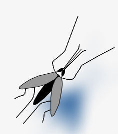

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From Where I Sit: Notes from the AMCA Technical Advisor by Joe Conlon

Things are happening on several different fronts regarding regulations potentially affecting mosquito control operations.

NPDES

As I had noted in the last AMCA Newsletter, Senators Barbara Boxer (D-CA) and Benjamin Cardin (D-MD) have put Senate review of HR 872, the so-called "Reducing Regulatory Burdens Act of 2011" on hold. Determining the reasoning behind this move has been difficult, but largely involved jurisdictional problems associated with the bill going through the Agriculture Committee and bypassing the Environment and Public Works Committee and its subcommittees, in whose jurisdiction Clean Water Act issues fall. It has not been known whether the Senators actually had significant disagreements with the contents of the bill.

Recently, however, Senator Cardin provided an explanation of his opposition to HR 872. In remarks on the Senate floor, Cardin said, "HR 872 is based on the notion that the law governing the licensing of pesticides provides all the environmental safeguards that are necessary. In proponents' view, obtaining a Clean Water Act permit would be duplicative. That is incorrect." He further noted that, "Approval of a pesticide under FIFRA only requires that the chemical 'will not generally cause unreasonable adverse effects on the environment.' Clean Water Act permits, on the other hand, are approved based on a pesticide's impact on a specific waterway." "...To exempt pesticides from comprehensive regulation would unreasonably compromise the quality of our waterways." Therein lies their primary rationale for opposition, with the term "comprehensive regulation" being the operative and "unreasonably compromise" providing a vague and subjective criterion that can be interpreted as the regulators and politicians see fit. This is what passes for Transparency, Sound Science, and the Rule of Law nowadays.

To further the irony, Senator Cardin said, "HR 872 simply goes too far, providing blanket exemptions and ignoring the real water quality problems that pesticides are causing in America's waters today." This while stating that backyard applicators and local lawn care companies should be exempt from the permit, and agricultural applications to land should continue to be exempt. He does, however, admit that emergency applications to control disease carrying insects and other pest infestations should be exempt. So there's that. But he can't help begging the question. "Clearly, the nation has a problem with pesticide pollution in our waters that needs to be addressed." Are the pesticides in question the result of the mosquito control applications that the bill would exempt? If not, then his statement assumes as truth that which has not been proven.

Yet, he also noted that "Providing targeted exemptions of *de minimis* users of pesticides makes good sense." Does this not include mosquito control? Based on what factual evidence and data is he evidently convinced that we're the problem, requiring ever more comprehensive regulation? Does the fact that the studies to which he refers are speaking almost exclusively about legacy pesticide residues hold any sway with him, or is he merely playing to environmental interests in Chesapeake Bay and we're the most visible (and vulnerable) means to scoring political points?

Someone in the Senate needs to pin him down on specifics and refuse to let him get away with gauzy generalizations about pesticides and the health of our waterways. He, and others of the same ideological persuasion need to be forced to clarify their assertions and their potentially devastating economic impact on public health in a public forum so that cost/benefits can be fully acknowledged. He tacitly cedes this point when he states, "Permits should be easy to obtain and impose minimal monitoring, recordkeeping and reporting requirements." As expected, the irony of imposing this massive and costly system because our waterways are, in his words, at significant risk from pesticides, while exempting those applicators most likely to be the cause of that risk, seems to have eluded him.

The Senate hold on HR 872, while unfortunate, does not necessarily spell the end of our legislative

fix for the Clean Water Act problem. The House Committee on Appropriations recently approved legislation (HR 2584) on July 12th that would restrict EPA's ability to institute the NPDES program requirements mandated by the 6th Circuit Court of Appeals. In essence, the provisions of HR 872 were added as a rider to the Fiscal 2012 Interior and Environment Appropriations bill, which cuts about $1.5 billion from EPA compared to Fiscal 2011 enacted levels. This opens up an entirely new can of political worms for all involved and has already been demagogued by activists as a sneaky, end-around ploy - which it was, business as usual in politics. Indeed, Representative Timothy Bishop (D-NY) said he plans to offer an amendment that would strip Title V (the pesticide exemption wording from HR 872) from the bill. I'm not sure if this will come to pass, as the full House has yet to vote on HR 2584 as of this writing.

Further complicating the process is the recent budget/debt limit debate in the Senate. With FY 2011 expiring at the end of September, the various separate appropriations bills may end up being amalgamated into an omnibus package or continuing resolution for FY 2012. So it seems that getting our legislative fix through ridership on "must pass" legislation may be in trouble, particularly if they go the continuing resolution route.

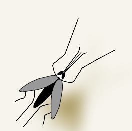
We'll keep you posted on developments. In the meantime, we still need to contact our Senators to ensure their support for HR 872. There are surely a number of legislators whose minds are made up and can't be made to see the situation for what it clearly is. Nonetheless, we must keep up the pressure using information and established scientific data to support our position. In these turbulent times, one can never know when the tipping point for overregulation in the face of shrinking budgets has been reached. Imposition of unnecessary unfunded federal mandates must be met with a resolve to force the regulatory parties and those legislators supporting them to publicly lay their cards on the table for the taxpayer to see, so that a fully informed electorate can exert their will either for or against burdensome regulation.

ENDOCRINE DISRUPTORS

Legislation targeting so-called "endocrine disruptors" was introduced in both the House and Senate recently. HR 2521, titled "The Endocrine-Disrupting Chemicals Exposure Elimination Act of 2011" was introduced by Representative James Moran (D-VA) and 12 co-sponsors, while Senator John Kerry (D-MA) introduced a similarly titled bill in the Senate. These bills are meant to regulate or eliminate the use of chemicals found to exert effects on male and female developmental and reproductive disorders; brain and neurobehavioral disorders; metabolic syndrome, pre-diabetes, diabetes, improper glucose and fat metabolism, obesity, and cardiovascular disorders; hypothalamus, hippocampus, thyroid, adrenal, immune, bone, cardiovascular, and other endocrine organs and systems throughout all life stages; hormonally driven cancer; and other related effects unless exposure is mitigated. They further require EPA and other agencies to propose actions, including new regulations and orders, if research finds "a minimal level of concern associated with a chemical's potential to disrupt the human endocrine system." Alarmingly, these bills create authority for citizens to sue agencies "to compel" a response to research findings, and requires agencies to report to Congress on their proposed courses of action, but at least it does not require agencies to promulgate new rules. The bill also stresses that it does not provide agencies with any new authority to "regulate a chemical or the route or source of human exposure to a chemical." Yet.

In another classic example of the begging the question fallacy, Representative Moran states "With instances of developmental disorders such as autism and attention deficit disorder skyrocketing, it is evident something is seriously wrong with our environment. There is a great need to expedite federal research into the over 80,000 chemicals in our water and air and how they may be linked to this disturbing trend. The [bill] will let science, not politics, determine what effect these chemicals may be having on our children." Instances of these disorders may indeed be skyrocketing, but evolving diagnostic criteria and liberalized case definitions of autism and ADD that have become a cottage industry in and of themselves may be the cause – not chemicals.

Yeah, sure. No politics here. Move along…



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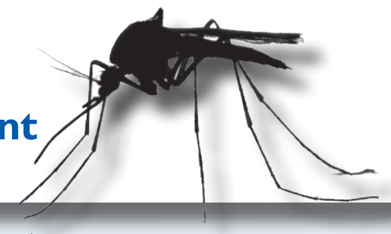

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