Persistence of aerially applied mosquito-pesticide, naled, in fresh and marine waters

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Abstract

Naled, an organophosphate pesticide, received considerable attention during 2016 as it was applied aerially to control the first mosquito-borne Zika virus outbreak in the continental United States. Stakeholders living in affected areas raised concerns about its environmental impacts. One factor influencing environmental impacts is the persistence of the chemical applied. The objective of this study was to evaluate the persistence of naled – and its degradation bi-product, dichlorvos – in natural waters. Initial naled concentrations were measured at ground level after full-scale aerial spray activities. Laboratory experiments were designed to evaluate factors (fresh versus marine water chemistry, temperature, and sunlight) that may promote the degradation of naled and dichlorvos in the environment. Results show that natural fresh and marine water chemistry promoted naled degradation as experiments with de-ionized water resulted in half-lives greater than 6 days. The half-life in natural waters without light ranged from 5 to 20 hours with lower half lives at higher temperatures. Under light exposure, degradation was accelerated and yielded more dichlorvos. Detectable levels (0.05 µM for naled and 0.10 µM for dichlorvos) were measured in water samples collected from the field during aerial spray events. Results can be used in risk assessments that consider both naled and dichlorvos to better understand ecological impacts and to develop improved public health recommendations.

Keywords: naled, dichlorvos, aerial spray, water, Zika

1.0 Introduction

On August 1, 2016, the first local (mosquito-to-human) transmission of Zika virus was documented in the continental U.S. within Miami-Dade County, Florida. Since then, efforts have been mounted to control the vector, mosquitos such as *Aedes egypti* and *Aedes albopictus*. One large aspect of these efforts includes aerial insecticide sprays of the organophosphate pesticide, naled. The sprays during 2016 were controversial among affected communities due to a lack of

consistent information concerning the fate of the insecticide and the subsequent ecological and human health impacts (Miami Herald 2016, Daily News 2015).

Naled (dimethyl 1,2-dibromo-2,2 dichloroethyl phosphate) is an organophosphate pesticide that is generally used to control adult mosquitoes' during disease outbreaks and for the control of nuisance mosquitos. Naled decomposes by debromination primarily to dichlorvos (dimethyl 2,2-dichlorovinyl phosphate), another organophosphate pesticide, which also has been used historically to control mosquitos (US EPA 1995). Both organophosphates inactivate mosquitos by interfering with the activities of cholinesterase, an enzyme that is essential for the proper functioning of the nervous system (PMEP 1993). Although the primary targets of naled application are mosquitos, non-target organisms such as pollinators, aquatic invertebrates, and humans can be affected.

Pollinators, including honeybees (*Apis mellifera*), butterflies, birds, beetles and midges (Ollerton et al. 2011) can be impacted by naled at concentrations applied for mosquito control purposes (Rinkevich et al. 2015, Dulin et al. 2012, Hoang et al. 2011). For instance, studies have found that direct contact of naled increased bee mortality by 5 times above the baseline rates immediately after a single spray of naled with a flat-fan nozzle system at an altitude of 30 meters at a deposition rate of 6.2 ± 0.7 mg/m² (Zhong et al 2003, Dukes et al 2004). Furthermore, in another study focusing on five species of butterfly native to Florida (Hoang et al. 2011), lethal doses (LD_{50}) ranging from 0.02 to 0.3 mg/m^2 and 0.1 to 2.3 mg/m^2 were documented when in contact with the thorax and when in contact with the forewings, respectively. In toxicological studies using aquatic invertebrates of the planktonic shrimp *Mysidopsis bahia*, the 96 hour LC_{50}, or acute toxicity was determined to be 8.8 ug/L naled and 19 ug/L dichlorvos (Johnson and Finley 1980).

Human exposure to naled can cause a variety of acute symptoms including headache, nausea, dizziness, confusion and/or disorientation (U.S. EPA 2002). Exposures during pregnancy have been linked to impaired child neurodevelopment (Silver et al. 2017, Hertz-Picciotto et al. 2018). The decomposition product of naled, dichlorvos, has been linked to prostate cancer. A study in California assessed the number of prostate cancers in farmers over a period of 10 years. The study concluded that more cases of cancer were reported amongst farmers who used dichlorvos compared to farmers who did not (Mills and Yang 2003). Dichlorvos has since been phased-out from use as a pesticide (U.S EPA 1995). In order to protect the public from the unintended effects of naled sprays, including the potential exposure to dichlorvos, research is needed to evaluate the rate at which these pesticides remain in the environment to minimize inadvertent human exposures.

Very few studies exist that evaluate the environmental persistence and degradation of naled. To our knowledge, studies do not exist that evaluate comprehensively different water types along with environmentally relevant environmental conditions of sunlight and temperature, especially in time series. None are available that compare field data from aerial spray activities against laboratory data for both naled and dichlorvos. During the field studies, water samples were maintained in experimental trays, which provided a consistent surface area of exposure per unit volume. Thus, the variable dilution that occurs within in natural water bodies was controlled. To the best of our knowledge, no studies have measured naled and dichlorvos field deposition in water under controlled dilution. One study measured unintentional pesticide drift from aerial application in the Atlantic Ocean and Florida Bay. Though naled recovery at sample locations in these water bodies was limited; after one aerial application, 50% of sample sites tested positive for dichlorvos (Pierce et al. 2005). Although ecological (USDA 2018, Davis et al. 2007, Schleier et al. 2008, 2010a) and human health risk assessments exist (Peterson et al. 2006, Schleier et

91 al. 2009), none consider the impacts of naled and dichlovos together especially given limitations
92 in field measurements that consider both (Schleier et al. 2010b).

93 In terms of information that is available, more is available on the fate of dichlorvos. In air, the half-
94 life of dichlorvos is estimated to be less than 3 days (Howard 1991) as it reacts with moisture in
95 the air (ATSDR 1997). For water, the degradation of dichlorvos is also dominated by hydrolysis,
96 with higher temperature and pH associated with faster degradation (ATSDR 1996, Tietze et al.
97 1996, Latif et al. 1984). Naled is sensitive to sunlight (Tietze et al. 1996) and thus distributed in
98 amber bottles to protect it from degradation (NPIC 1996). In contrast, degradation of dichlorvos
99 directly by sunlight is not anticipated because it does not readily absorb UV light (Gore et al. 1971,
100 Howard 1991). Naled is also susceptible to degradation by sulfur compounds under anaerobic
101 conditions (Gan et al. 2006a,b). The reactions with sulfur compounds under anaerobic conditions
102 are particularly relevant in terms of potential transformation pathways in anoxic coastal
103 environments – such as mangrove marshes where anoxic conditions can occur in sediments. In
104 urban areas where naled was used during the 2016 Zika outbreak, surface waters were generally
105 aerobic. Studies are needed to investigate the degradation of naled and dichlorvos in natural
106 waters under ambient conditions. Lack of field data on the initial concentrations of naled and
107 dichlorvos was considered as a data gap in the development of ecological and human health risk
108 assessments, along with a lack of understanding of their degradation rates simultaneously.

109 ***Naled Degradation Process****.* Degradation of naled begins with the oxidation of the end bromine
110 in water resulting in the loss of both bromines to convert naled to dichlorvos (Gan et al. 2006)
111 (Figure S1). Dichlorvos is then split into dichloro-acetaldehyde and dimethyl phosphate .These
112 two degradation products of dichlorvos are believed to be of low toxicity and the existing data do
113 not show adverse human health impacts (ATSDR 1997).

Although the overall mechanism of degradation has been documented in laboratory controlled conditions, the rate of degradation in the natural environment is difficult to predict as the degradation is dependent upon several factors, including temperature, sunlight, and the natural background chemistry of the water (whether fresh or marine). Coupled with field measurements at ground level, degradation rates can be used to assist with the development of guidelines for the duration of hive coverage (U.S. EPA 2016) and the period that people should remain indoors after a spray event. If sunlight is a major factor influencing the degradation of naled, the pesticide application time (night versus daylight hours) could be carefully selected to minimize impacts.

Because of public health concerns raised by the community at the time of the 2016 Zika outbreak, both field and laboratory experiments were conducted. The purpose of the field measurements was to detect the pesticide and measure its concentration at near ground level. The laboratory experiments were conducted to document the rates at which the pesticides degrade. The combined results from the field and laboratory data can be used to estimate initial concentrations at ground level (from field experiments) and subsequent concentrations under different environmental conditions (from laboratory experiments that evaluate fresh versus marine waters, temperature, and sunlight). These combined results provide the exposure concentrations for naled and dichlorvos from the time it reaches ground level exposure zones until it dissipates. Such results can be used in future studies that focus on comprehensive risk assessments that consider realistic ground level concentrations and the the time course of naled and dichlorvos dissipation simultaneously.

2.0 Methods

After characterization of various waters used, subsequent studies included laboratory-contolled experiments to evaluate degradation rates of naled and dichlorvos, and two field efforts were undertaken to evaluate concentrations at ground level after full-scale aerial sprays. A summary of these experiments is provided in Table 1.

2.1 Water Sample Collection and Pre-Characterization

The waters utilized for experimentation included freshwater from a lake and marine water from the Atlantic Ocean. The freshwater sample was collected from a floating dock that extended 15 meters from the lake's edge (Tropical Park, 7900 SW 40th St, Miami, FL 33155, latitude and longitude: 25˚43'25"N, 80˚19'21"W) and the marine water was collected by wading in 0.5 m of water at the coast (South Beach, 599 Ocean Dr., Miami Beach, Fl. 33139, latitude and longitude: 25˚46'50"N, 80˚07'48"W). Water samples were collected in five liter pre-cleaned carboys that were rinsed with the sample water three times prior to the collection of the sample.

The water samples were then analyzed for basic physico-chemical parameters of pH (Orion Star A211 Benchtop calibrated to pH of 4 and 10), turbidity (Turner Designs TD-40 nephelometer calibrated with 2 and 20 NTU formazin standards), and salinity (YSI Model 650 calibrated to 35.5 psu ocean water standard). Samples were analyzed for major ions (Na^{+}, Mg^{+2}, K^{+}, and Ca^{+2}) by atomic absorption spectroscopy with flame atomization (Perkin Elmer AA800, with corresponding standard solutions). Samples were also analyzed gravimetrically for total dissolved solids and total suspended solids. The combustion catalytic oxidation method with nondispersive infrared detection was used for total and non-purgeable organic carbon analysis (Shimadzu TOC-L analyzer). Details about the natural water characteristics are provided in Table S1 in the supplement.

2.2 Materials

Standards of naled (CAS Number 300-76-5, 97.1%) and dichlorvos (CAS Number 62-73-7, 98.3%) used to create calibration curves were purchased from Chem Service Inc. (West Chester, PA). The naled used for experimentation was provided by AMVAC (Commerce, CA, CAS Number 300-76-5, Batch KB87-41-4, 99.6%). The solubility of the AMVAC experimental standard in water was 5200 μM (AMVAC 2015, U.S. EPA 2020). Ethyl acetate (CAS Number 141-78-6, $C_4H_8O_2$) was purchased from Alfa Aesar (Ward Hill, MA, 99%). The internal standard, tetrachloro-m-xylene (TCMX, CAS Number 877-09-8) was purchased from Accustandard (New Haven, CT). De-ionized water came from a Millipore system with a resistivity of 18.2 MΩ·cm (at 25°C).

2.3 Consistent Processing Steps for Organophosphate Analyses

The pre-processing of laboratory samples was consistent for all calibration standards and for all laboratory experiments. Laboratory samples were generated by adding a predetermined mass of naled (AMVAC) to the water sample (1 mg to create a 50 μM solution in de-ionized water), vortexing the solution for 5 minutes, and allowing the reaction to take place under the experimental conditions. For each laboratory-generated sample, 500 μL of sample were extracted and added to an amber 2-mL vial containing 500 μL of ethyl acetate to stop the reaction (as recommended by Hall et al. 1997 and Hengel and Lee 2014). The 2-mL vials were vortexed for 15 minutes to attain equilibrium extraction. One hundred microliters of the samples were added to a separate amber 2-mL vial containing a 200 μL glass insert. The internal standard, TCMX, was added to each glass insert as 10 μL of a 41 μM (10 mg/L) solution in ethyl acetate. Naled and dichlorvos were then analyzed using gas chromatography coupled with mass spectroscopy (GC-MS) (Agilent 5975C GC-MS, Santa Clara, CA fitted with a HP TG-5MS column using a 5 μL injection volume). The column oven was programmed from an initial value of 50 ºC and held for 4 minutes,

then ramped at 25 ºC/min up to 100 ºC, then ramped at 5 ºC/min up to 190 ºC for a total run time of 24 minutes. The temperature at the injector was 250 ºC and for the detector was 300 ºC. The retention time for dichlorvos was 11.8 minutes, for TCMX was 20.4 minutes, and for naled 21.2 minutes.

2.4 Laboratory Experiments

The laboratory experiments conducted as part of this study can be broadly separated into those conducted under simulated sunlight irradiation or in the dark. The effect of two different temperatures (23 and 30 ˚C) was explored to assess the impact of variable conditions of south Florida during the summer night and early morning hours (when aerial sprays typically occur). The 23 ˚C corresponded to the measured room temperature and the 30 ˚C was maintained under incubator conditions to simulate typical environmental conditions in south Florida. Although temperature is controlled under room conditions it is recognized that variations may occur on the order of ± 1 ˚C. Amber vials were used to prepare samples for all experiments. For the set with light, samples were transferred to quartz beakers to allow for light exposure (Newport Model 66921 equipped with a 350-watt xenon lamp producing 1000 W/m^2). Both sets of experiments, with light and in the dark, were conducted using de-ionized water, as well as with freshwater and marine water.

For the de-ionized and natural water experiments conducted under dark conditions, 50 µM solutions of naled were prepared in triplicate within 60 mL amber vials. Aliquots of 500 µL were taken at various time intervals over periods of 28 and 7 days, respectively. All water types were evaluated at room temperature (23 °C). At 30 °C, only fresh and marine natural waters were evaluated to simulate the effect of elevated temperature in the natural conditions of south Florida.

206 Experiments conducted under light irradiation were similar except that the reaction durations were
207 300 and 120 minutes for de-ionized water and natural waters, respectively, as determined through
208 preliminary experimentation. Two sets of triplicate experiments were conducted at room
209 temperature, one with freshwater and another with marine water. One experiment also was
210 conducted with de-ionized water. Outlier analysis (Q test) was used to reject experimental runs
211 using a 90% degree of confidence (Skoog et al. 1992).

212 For each experiment, naled and dichlorvos concentrations were normalized by the initial naled
213 concentration in molar units. Error bars on graphs correspond to the standard deviation of the
214 replicated experiments. Rate constants and half-lives were computed by fitting a best fit line to
215 the natural logarithm of the normalized molar concentration for the corresponding chemical (naled
216 or dichlorvos) versus time. The rate constant was determined from the slope. The half-life was
217 computed as the time corresponding to the natural logarithm of one-half of the initial normalized
218 concentration. The supplemental text includes additional details concerning the computation of
219 the rate constants.

220

221 *2.5 Field Sample Collection During Aerial Spray*

222 Water samples were exposed to aerial sprays on two dates (Table 1). These sprays focused on
223 dosing for nuisance mosquitos such as the flood water species of *Culex* and *Psorophera*, and
224 some salt marsh mosquitoes (*Aedes taeniorhynchus*), as these sites were not considered at risk
225 for Zika transmission at the time of field experimentation. The first date (June 29, 2017) was aimed
226 at dosing the Southwest Ranches area of the Town of Davie in Broward County, Florida (GPS
227 coordinates: 26˚ 2’ 48” N, 80˚ 23’ 20” W; Air temperature of 25 ˚C, 85% humidity, winds at 1.0 m/s

228 NNW) during the hours of 4:00 to 6:30 am. The target dose was 5.8 kg of active ingredient per
229 square kilometer.

230 The second date corresponded to August 28, 2018 during an aerial spray mission aimed at dosing
231 East Naples in Collier County, Florida (GPS coordinates: 26˚ 2' 47.9"N, 81˚ 40' 39.6"W; Air
232 temperature 25 ˚C, 95% humidity, winds at 2.7 m/s ENE) for nuisance mosquitos, again as the
233 site was not considered at risk for Zika transmission. According to flight records, the aircraft left
234 the airport at 9:45 pm and landed at 11:10 pm; treatment was applied within that window. The
235 target dose was 5.3 kg/km^2. Spray systems were calibrated to produce a volume median droplet
236 size (VMD) of 24 to 30 microns, as per label requirements, for both missions. The sample
237 collection location was set near the center of the spray area for both missions, which is presumed
238 to receive the full dose from the aerial spray. More details about each flight mission including
239 airplane characteristics and airplane tracks relative to the sampling locations are provided in the
240 supplemental text. The set up for water sample collection was very similar between both flight
241 missions. However, only fresh and marine waters were tested during the June 29, 2017 mission.
242 Also, these samples were collected at sunrise - so they were exposed to some solar radiation
243 prior to collection. For the August 28, 2018 mission, three water types were tested, which included
244 both fresh and marine waters as well as de-ionized water. Also, during the August 28, 2018
245 mission, multiple water samples were collected after the aerial spray whereas only one was
246 collected for each water type after the June 29, 2017 mission (Table 1). The samples collected
247 during August 28 were collected at night and were thus not subject to solar radiation prior to
248 collection.

249 Water samples were exposed to the aerial spray using glass trays (3-L volume prewashed with
250 ethanol and having a surface area 23.50 x 33.75 cm) placed on a table (0.8 m above ground level
251 corresponding to the human inhalation zone and near ground). Two and a half liters of water

252 samples were then placed into each tray resulting in a water depth of 3.2 cm. Samples were
253 transferred from the trays into 1-L amber bottles using a dedicated stainless steel spoon (ethanol-
254 washed). For the June 29 mission, because only two samples were collected (one before aerial
255 spray and one after), one tray was used for each water type: one filled with freshwater and one
256 filled with marine water. For the August 28 mission – because 4 samples were to be collected
257 over time – two trays were used for each water type: two filled with freshwater, two filled with
258 marine water and two filled with de-ionized water.

259 For each water type, a background sample was collected shortly prior to the airplane flight take
260 off. Subsequent samples were collected 30 minutes after the airplane was seen overhead. Thirty
261 minutes was chosen to allow for the spray to migrate from the airplane down towards the ground.
262 The collection of water samples consisted of transferring 900 ml of water sample to the amber
263 bottle and then adding 60 mL of ethyl acetate and gently shaking the bottle upside-down to
264 promote contact of the ethyl acetate with the water.

265 Samples were then placed in a cooler containing freezer packs and were transported to the
266 laboratory (Florida Spectrum Environmental Services, Ft. Lauderdale, FL) for analysis within 12
267 hours of collection. At Florida Spectrum, the samples were analyzed by GC-MS (Agilent GC 6890/
268 MS 5973) according to EPA Method 8270 (U.S. EPA 2015) using a 30m x 0.25 mm x 0.25 µm
269 (RXI-5 Sil-MS) column and a 1 µL injection volume. Prior to injection, the samples were subject
270 to three consecutive extractions, which included sonication and liquid/liquid separation into
271 methylene chloride (EPA Method 8270) utilizing a separatory funnel (EPA Method 3510C) with
272 evaporation to 1 ml to achieve lower detection limits. Detection limits of the method were 1.0 x
273 10^{-4} µM for naled and 3.0 x 10^{-4} µM for dichlorvos.

274

3.0 Results and Discussion

3.1 Laboratory Analysis of Naled Degradation

3.1.1 Experiments Without Light

Degradation of naled and dichlorvos in de-ionized water without light was relatively slow (Figure 1a) with half-lives on the order of hundreds of hours and rate constants of 0.003 h^{-1} (with a standard deviation of ± 0.0002 h^{-1}) (Table 2). Seventy-seven percent of the initial naled concentration was present in de-ionized water after 24 hours. Dichlovos was present at the outset of the experiment and at 24 hours it represented 84% of the initial naled concentration. After 28 days, naled and dichlorvos were present at 15% of the initial naled concentration. Of interest is that initial proportions of dichlorvos were variable within the first few hours. Initially, dichlorvos represented 70% of the naled molar concentration. This proportion increased to 84% at 24 hours presumably due to the conversion of naled to dichlorvos. After 3 days, both the naled and dichlorvos continued to decrease at a similar rate.

Other studies evaluating the degradation of dichlorvos in de-ionized water report similar half-lives. Lartiges and Garrigues (1995) found that dichlorvos persisted for 6 months in ultrapure water at a temperature of 6 °C and 81 days at 22 °C. In the current study, dichlorvos was quantified in de-ionized water at the end of a 28-day experiment run at 23 °C. Using the rate constant from the 28 day experiment to extrapolate to 81 days, the resultant concentration would have been 0.3 µM and consistent with the results of Lartiges and Garrigues (1995).

In comparison, more rapid degradation of naled and dichlorvos was noticed in fresh and marine waters (Figure 2a). These results suggest that natural waters (as opposed to de-ionized water) promote the degradation of naled and dichlorvos. The degradation of naled was found to be faster

than that of dichlorvos. At room temperature, for naled, the half-lives were 20 hours for fresh water and 12 hours for marine water. For dichlorvos half-lives were 166 hours for fresh water and 128 hours for marine water. Furthermore, temperature was found to impact the degradation of naled and dichlorvos. By increasing the solution temperature to 30 ˚C naled and dichlorvos displayed faster degradation in both fresh and marine waters, which is consistent with previous reports (Faust and Suffet 1966, Gan et al. 2006b). The rate constants of naled at 30 ºC was 0.065 h^{-1} (± 0.013 h^{-1}) for fresh water and 0.11 h^{-1} (± 0.054 h^{-1}) for marine water. For dichlorvos the rate constants were 0.038 h^{-1} (± 0.030 h^{-1}) and 0.081 h^{-1} (± 0.038 h^{-1}) for fresh and marine waters, respectively. Overall naled and dichlorvos under dark conditions persists on the order of days.

3.1.2 Effect of Light

Simulated sunlight was chosen to mimic the effect of daylight on the persistence of naled and dichlorvos in various waters. First, de-ionized water solutions of naled were explored to eliminate any other effect. These solutions demonstrated rapid degradation (k= 2.3 h^{-1} for naled) under simulated sunlight (Table 2). Over one-half of the naled had degraded in de-ionized water after 30 minutes and, after 150 minutes, was non-detectable (Figure 1b). However, elevated concentrations of dichlorvos were noticed in the first 150 minutes. At 300 minutes, the dichlorvos decreased to one-third of its initial concentration. This is consistent with the results of the degradation of dichlorvos in DI water under light irradiation (Figure S2). When the natural water solutions were exposed to the same conditions, the degradation rates of naled were again much faster (k= 2.6 to 2.9 h^{-1} for naled), with half-lives of 15 and 17 minutes for fresh water and marine water, respectively. However, the profile of dichlorvos production as a result of the degradation of naled under simulated sunlight irradiation is distinctively different for various conditions. For instance, as the experiments proceeded under light conditions for natural waters, naled was converted to dichlorvos, reaching a point where the molar concentration of dichlorvos exceeded

naled at times greater than 20 minutes. This level of conversion was not observed when the samples were incubated under dark conditions. The degradation of naled was also considered rapid when compared to dark conditions. When considering the degradation of both naled and dichlorvos together in natural waters under light irradiation, the overall (sum of naled and dichlorvos) half-lives were still comparatively short, 67 to 70 minutes for fresh and marine waters. These results are consistent with those of Tietze et al. 1996 who evaluated degradation of naled and dichlorvos on filter paper in dark versus exposed to direct sunlight conditions. Tietze found that in direct sunlight, the half-life of naled was 1.4 hours and, under dark conditions, ranged from 4.8 to 8.2 hours. In the current study, we found the half-lives were shorter, less than 20 minutes under simulate sunlight conditions. This might be attributed to the synergistic effects of photolysis and degradation in the natural water samples.

Degradation in natural waters is believed to be driven by TOC. No TOC was detected in DI water. However, high concentrations of TOC were detected in freshwater (523 mg/L) and marine water (283 mg/L). Furthermore, the concentration of non-purgeable organic carbon, which is mostly composed of humic acid was found to be 520 mg/L for freshwater and 206 mg/L for marine water. In this case, the humic acid compounds act as nucleophiles to enhance the rate of degradation of naled and dichlorvos. This is consistent with the results of Fontaine et al. (2013), which showed that humic acid catalyze the degradation of 2,4-dichlorophenol under light conditions. This explanation is further supported by the increase in dichlorvos formation under light conditions. The presence of humic acid compounds is known to increase the rate of nucleophilic substitution of the naled bromines (Sadowsky et al. 2014, Park et al. 2000). This increase over time was not reported in the filter paper experiment by Tietze et al. However, this finding is consistent with the results of Gan et al. 2006 who observed increases in dichlorvos in anoxic waters immediately after naled was introduced.

Overall, these results suggest that photolysis is the most efficient mechanism for the degradation of naled. Simulated sunlight in the laboratory setting was applied to fresh and marine water solutions of naled. In both instances, naled was degraded to below detection within 3 and 2 hours of irradiation for de-ionized and natural waters, respectively. However, during light degradation experiments, dichlorvos was formed and remained at detectable levels after a period of two hours. On the other hand, in dark, the production of dichlorvos was more limited – or lacking – as dichlorvos degraded exponentially in a similar fashion as naled.

The second most significant mechanism for the degradation of naled appeared to be through dehalogenation via nucleophilic substitution catalyzed by the high level of humic acid compounds found in natural fresh and marine waters. Degradation of naled was relatively slow in de-ionized water resulting in dichlorvos production, which was subsequently degraded at a similar rate as naled. Additionally, the rate of degradation was further accelerated in natural waters. The naled remained at detectable levels in de-ionized water for over a month, whereas in natural fresh and marin waters naled concentrations decreased to below detection limits in a few days. The slower rate of degradation in de-ionized water relative to the natural waters supports the effect of humic acid componds in natural fresh and marine waters.

Lastly, temperature within the anticipated ranges in the natural environment also appears to influence the rate of degradation. At the slightly higher temperatures of 30 °C, degradation of naled was higher than at room temperature of 23 °C. The influence of temperature within this range was small relative to the influence of hydrolysis, natural water chemistry and photo-degradation.

368 *3.2 Field Measurement Results*

369 Results from field aerial sprays showed detectable levels of naled (from 0.028 to 0.064 μM) and
370 dichlorvos (from 0.004 to 0.101 μM) in the de-ionized water and in the natural waters (Table 3).
371 Considering the application rate of naled (5.8 kg/km^2 for June 29 and 5.3 kg/km^2 for August 28),
372 the amount of naled observed at ground level in the water samples represented between 7% and
373 10% of the target amount applied for the June 29th event and for the August 28 event. Overall the
374 90+% loss at 30 minutes is high relative to the half-lives which were on the order of hours for dark
375 conditions observed in the laboratory component of this study.

376 Sunlight is believed to not have affected the field samples. The August 28 samples were collected
377 in the darkness of night. On June 29, at the time of sampling (7:00 am which was 30 minutes after
378 sunrise), the sunlight was at very low intensity. The intensity of the simulated sunlight during
379 laboratory experimentation was much stronger, corresponding to 12 noon. In the laboratory
380 experiments, the proportion of naled to dichlorvos at a time of 30 minutes exposed to sunlight was
381 measured at 1.0 : 1.6 for fresh water and 1.0 : 2.0 for marine water. The proportions observed in
382 the field sample (8.5 : 1.0 and 7.0 : 1.0 for fresh and marine water, respectively) were more
383 consistent with the proportions associated with dark conditions. Thus photolysis in air is not a
384 likely explanation for the lower levels of naled observed at ground level.

385 The reason for the discrepancy between the application rates and the amount observed in the
386 water at ground level could be due to the interaction with atmospheric particulate matter and
387 moisture resulting in the degradation of naled during the fall from the airplane. Dichlorvos is known
388 to remain in the atmosphere, which can possibly explain the high loss rate of the pesticides at
389 ground level. Additionally dichlorvos is known to be volatile and can return back to the air phase
390 after deposition. For instance, in Tulare County, California, for an agricultural application with

391 higher doses (185 kg/km^2 naled), the maximum naled concentrations (6.30 µg/m^3) was measured
392 in the air within 5 h of the spray, while the maximum dichlorvos in air (0.99 µg/m^3) was measured
393 within 24-48 h of the spray (Hall et al. 1997). This suggests the presence of an atmospheric source
394 of dichlorvos after the spray event, possibly due to its volatilization after deposition. For both
395 events, the sampling sites were surrounded by farms and farm animals; thus, it is possible that
396 the atmosphere contained sufficient particulates that could facilitate the degradation of the
397 pesticides and possible volatilization of dichlorvos.

398 For the August 28 event, concentrations were observed to increase slightly from 11:10 to 12:20
399 pm – perhaps due to the time it takes for the pesticide to fall to ground level from its release point
400 (91 m above ground) (Figure 3). According to the manufacturer's recommendations, it takes
401 approximately 30-90 minutes for the targeted droplet size to reach ground level when sprayed
402 from an altitude of 60 m to 91 m above ground. Pesticide concentrations leveled off 12:45 pm, 70
403 minutes after the airplane completed its mission. This leveling off likely represents the end of the
404 accumulation of the pesticide and the start of the degradation under dark conditions. The
405 degradation rates was found to follow the order de-ionized water < freshwater < marine water.
406 Although these results are consistent with the order observed in the lab experiments, a larger
407 difference in the degradation rate would have been anticipated between the de-ionized water
408 samples and the natural waters. In the laboratory experiments, the half-life of naled in de-ionized
409 water was 131 hours, whereas the half-lives of the natural waters varied between 12 and 20
410 hours. It is possible that the de-ionized water exposed to the atmosphere could have been
411 impacted by incidental particulate matter, thereby accelerating degradation under field conditions
412 that were not observed under laboratory scale conditions.

413 The ratios of naled and dichlorvos also were not consistent between the two spray missions. For
414 the June 29th event, dichlorvos was a fraction (11-14%) of the naled concentration in the water

samples. For August 28, there was more dichlorvos in the water samples than naled, which was unexpected given the dark conditions under which the samples were collected. The differences in the proportions of naled to dichlorvos may be due to differences in spray atmospheric conditions, airplane characteristics or age of the Dibrom® solution. During both spray missions, humidity was high, at 85% for the June 29 mission and at 95% for the August 28 mission. Tietze et al. (1996) reports higher degradation rates during higher humidity; thus, the differences in the proportions of naled to dichlorvos for the two field spray events could have been influenced by differences in humidity.

Application rates in the current study (5.3 to 5.8 mg/m^2) are at levels that can be toxic to honey bees, butterflies (Zhong et al. 2003), and mysid shrimp (Pierce et al. 2005). Precautions can include covering commercial hives during spray activities and avoiding sprays over ecologically sensitive water bodies. Human health risk assessments are recommended in conjunction with knowledge about degradation rates to develop strategies to minimize exposures. Risk assessments should consider the conversion of naled to dichlorvos and consider the risks from both collectively. Risk assessments can be conducted using target naled levels from aerial spray activities coupled with theoretical degradation rates as a conservative method to evaluate impacts. Given the lower levels of naled measured relative to the target dose, this assessment should also utilize actual measured levels of naled and dichlorvos at ground level which in the current study were found to be at about 10% of the target amount. Results from a risk assessment can be used to provide the public with advice in terms of the time to remain indoors after a spray if a hazard is determined. Given the accelerated degradation under sunlight conditions, precautionary guidance can include, for example, recommending that communities remain indoors until after sunrise.

Although efforts were taken to process samples immediately for extraction within ethyl acetate, it is possible that some of the conversion of naled to dichlorvos observed in the current study may have been influenced by sample processing. Ideally future studies should also focus on the degradation of dichlorvos without the presence of naled to evaluate the behavior of dichlorvos degradation without the new inputs from naled degradation. Additionally, extending the duration of the field measurements towards times of full daylight would be useful to confirm the results from laboratory experimentation conducted under light conditions.

4.0 Conclusion

Regardless of the condition, including sunlight, water characteristics, and temperature, results showed that naled undergoes some degradation to dichlorvos. Sunlight was found to play the most significant role in accelerating the degradation of naled. When combining sunlight with fresh or marine water, rapid degradation of naled was observed. Field collection of aerial naled sprays confirmed an undeniable degradation of naled to dichlorvos before it could be recovered at ground level. Of interest would be to evaluate the concentration distribution of naled and dichlorvos in the vertical air column to evaluate whether the conversion occurs as the droplets fall towards the ground.

Although dichlorvos has been banned from aerial spray activities, greater conversion of naled to dichlorvos would be expected during daylight hours which may result in various toxicity levels to exposed humans and non-target organisms. For this, results suggest that if the conversion of naled to dichlorvos is to be minimized, it should be applied during the night time as opposed to spraying during daylight hours. However, spraying during daylight hours would result in a shorter-lived presence of these pesticides. Therefore, a balance should be maintained between the

efficacy concerning mosquito control where the presence of naled is desired versus the rate of conversion of naled to dichlorvos to other degradation products.

A logical next step from the current study is to utilize the results in ecological and human health risk assessments recognizing the high propensity for naled to convert to dichlorvos. Risk assessments should consider the combined impacts from both of these active ingredients and should also integrate uncertainty in measurements using statistical approaches. With risk assessments that consider both naled and dichlorvos guidance can be developed to minimize environmental and public health impacts.

Acknowledgments: This study was funded by NSF CBET-1709939. We thank the Miami-Dade, Collier, and Broward County mosquito control districts for sharing information about their local spray activities. We are also appreciative of Miami-Dade County Department of Health for guidance on public outreach. EMZ acknowledges support from Ball State University.

References

Agency for Toxic Substances and Disease Registry (ATSDR), 1997. Toxicological Profile for Dichlorvos. U.S. Dept of Health and Human Services, ATSDR, Division of Toxicology and Toxicology Information Branch. Atlanta, GA.

AMVAC Chemical Corporation, 2015. Safety Data Sheet, Dibrom® Concentrate. Los Angeles, CA.

Code of Federal Regulations (CFR), 2007. Dichlorvos (DDVP); Termination of Certain Uses and Label Amendments, 72 FR 35235, pages 35235-35236, Docket Number: EPA-HQ-OPP-2002-0302.

Daily News, 2016. Miami fights Zika with Chemical that could cause Birth Defects, by Chris Sommerfeldt (September 7, 2016). http://www.nydailynews.com/news/national/miami-fights-zika-chemical-birth-defects-article-1.2780795

Davis, R.S., Peterson, R.K., Macedo, P.A., 2007. An ecological risk assessment for insecticides used in adult mosquito management. *Integrated environmental assessment and management*, 3 (3), pp. 373-382.

Dukes, James, He Zhong, Mike Greer, Phil Hester, Donald Hogan, and Jane A S Barber. 2004. A Comparison of two ultra-low-volume spray nozzle systems by using a multiple swath scenario for the aerial application of fenthion against adult mosquitoes. *Journal of the American Mosquito Control Association.* 20.1, 36-44.

Dulin, F., Halm-Lemeille, M.-P., Lozano, S., Lepailleur, A., Sopkova-de Oliveira Santos, J., Rault, S., Bureau, R., 2012. Interpretation of honeybees contact toxicity associated to acetylcholinesterase inhibitors. *Ecotoxicol. Environ. Saf.* 79, 13-21.

Fontaine, B., Nuzzo, A., Spaccini, R., Piccolo, A., 2013. Degradation of 2,4-dichlorophenol and coupling into humic matter by oxidative biomimetic catalysis with iron-porphyrin. *Journal of Geochemical Exploration*, 129, 28-33.

Gan, Q., Singh, R.M., Jans, U., 2006a. Degradation of naled and dichlorvos promoted by reduced sulfur species in well-defined anoxic aqueous solutions. *Environ. Sci. Technol.* 40, 778-783.

Gan, Q., Singh, R.M., Wu, T., Jans, U., 2006b. Kinetics and mechanism of degradation of dichlorvos in aqueous solutions containing reduced sulfur species. *Environ. Sci. Technol.* 40, 5717-5723.

Gore, R.C., Hannah, R.W., Pattacini, S.C., Porro, T.J., 1971. Infrared and ultraviolet spectra of seventy-six pesticides. *J. Assoc.Offic. Anal. Chem.* 54, 1040-1081.

Hall, G.L., Mourer, C.R., Shibamoto, T., Fitzell, D., 1997. Development and Validation of an Analytical Method for Naled and Dichlorvos in Air. *Journal of Agricultural and Food Chemistry*, 45, 145-148.

Hengel, M., Lee, P., 2014. Community air monitoring for pesticides—part 2: multiresidue determination of pesticides in air by gas chromatography, gas chromatography–mass spectrometry, and liquid chromatography–mass spectrometry. *Environ Monit Assess*, 186, 1343–1353.

515 Hertz-Picciotto, I., Sass, J.B., Engel, S., Bennett, D.H., Bradman, A., Eskenazi, B., Lanphear, B.,
516 Whyatt, R., 2018. Organophosphate exposures during pregnancy and child neurodevelopment:
517 Recommendations for essential policy reforms. *PLoS Medicine*, 15(10), art. no. e1002671.

518 Hoang, T.C., Pryor, R.L., Rand, G.M., Frakes, R.A., 2011. Use of butterflies as nontarget insect
519 test species and the acute toxicity and hazard of mosquito control insecticides. *Environmental*
520 *Toxicology and Chemistry*, 30, 997-1005.

521 Howard, P.H., 1991. Handbook of Environmental Fate and Exposure Data for Organic Chemicals.
522 Lewis Publishers, Chelsea, MI.

523 Johnson, W.W., Finley, M.T., 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic
524 Invertebrates. Resource Publication 137. U.S. Department of Interior, Fish and Wildlife Service,
525 Washington, DC.

526 Lamoreaux, R.F., and Newland, L.W., 1978. The fate of dichlorvos in soil. *Chemosphere*, 10, 807-
527 814.

528 Lieberman M.T., Alexander M., 1983. Microbial and nonenzymatic steps in the decomposition of
529 dichlorvos (2,2-Dichlorovinyl O,O-Dimethyl Phosphate). *J. Agric. Food Chem.* 31, 265-267.

530 Miami Herald, 2016. Tempers flare as public protests spraying for Zika mosquitos in South Beach,
531 by Joey Flechas (September 14, 2016). http://www.miamiherald.com/news/health-
532 care/article101823792.html

533 Mills, P.K., Yang R. 2003. Prostate cancer risk in California farm workers. *Journal of Occupational*
534 *and Environ. Med*. 45, 249–58.

National Pesticide Information Center (NPIC), 1996. Extoxnet Extension Toxicology Network, Pesticide Information Profiles, Naled. http://extoxnet.orst.edu/pips/naled.htm

Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? Oikos Synthesising Ecology, 120, 321-326.

Park, J.-W., Dec, J., Kim, J.-E., Bollag, J.-M., 2000. Dehalogenation of Xenobiotics as a Consequence of Binding to Humic Materials. *Arch. Environ. Contam. Toxicol.* 38, 405–410.

Peterson, R.K.D., Macedo, P.A., Davis, R.S., 2006. A human-health risk assessment for West Nile virus and insecticides used in mosquito management. *Environmental Health Perspectives*, 114 (3), 366-372.

Pierce, R.H., Henry, M.S., Blum, T.C., Mueller, E.M., 2005. Aerial and tidal transport of mosquito control pesticides into the Florida Keys National Marine Sanctuary. *Revista de Biología Tropical*. 53, 117-125.

Rinkevich, F.D., Margotta, J.W., Pittman, J.M., Danka, R.G., Tarver, M.R., Ottea, J.A., Healy, K.B., 2015. Genetics, synergists, and age affect insecticide sensitivity of the honey bee, Apis mellifera. *PLoS ONE*, 10(10), art. no. e0139841.

Sadowsky, D., McNeill, K., Cramer, C.J., 2014. Dehalogenation of Aromatics by Nucleophilic Aromatic Substitution. *Environmental Science & Technology*, 48(18), 10904-10911

Schleier III, J.J., Peterson, R.K.D., 2010a. Toxicity and risk of permethrin and naled to non-target insects after adult mosquito management. *Ecotoxicology*, 19(6), 1140-1146.

554 Schleier III, J.J., Peterson, R.K., 2010b. Deposition and air concentrations of permethrin and
555 naled used for adult mosquito management. *Archives of Environmental Contamination and*
556 *Toxicology*, 58(1), 105-111.

557 Schleier III, J.J., Macedo, P.A., Davis, R.S., Shama, L.M., Peterson, R.K.D., 2009. A two-
558 dimensional probabilistic acute human-health risk assessment of insecticide exposure after adult
559 mosquito management. *Stochastic Environmental Research and Risk Assessment*, 23 (5), 555-
560 563.

561 Schleier III, J.J., Davis, R.S., Shama, L.M., MacEdo, P.A., Peterson, R.K.D., 2008. Equine risk
562 assessment for insecticides used in adult mosquito management. *Human and Ecological Risk*
563 *Assessment*, 14 (2), pp. 392-407.

564 Silver, M. K., Shao, J., Zhu, B., Chen, M., Xia, Y., Kaciroti, N., Lozoff, B., Meeker, J.D., 2017.
565 Prenatal Naled and Chlorpyrifos Exposure Is Associated with Deficits in Infant Motor Function in
566 a Cohort of Chinese Infants*. Environ. Intern.* 106, 248–256.

567 Skoog, D.A., West, D.M., 1992. Analytical Chemistry: An Introduction, Fourth Edition. Saunders
568 College Publishing, New York, pp. 50-52.

569 Tietze, N.S., Shaffer, K.R., Hester, P.G., 1996. Half-life of naled under three test scenarios. *J.*
570 *Am. Mosquito. Contr.* 12, 251-254.

571 U.S. Department of Agriculture (USDA), 2018. Draft Human Health and Ecological Risk
572 Assessment for Naled in Exotic Fruit Fly Applications. Animal and Plant Health Inspection Service,
573 USDA, Raleigh, NC.

U.S. Environmental Protection Agency (U.S. EPA), 1995. Dichlorvos (DDVP): Deletion of Certain Uses and Directions". U.S. Environmental Protection Agency: Office of Pesticide Programs. April 19, 1995. pp. 19580–19581. Docket Control Number OPP-38511.

U.S. Environmental Protection Agency, 2002. Naled Facts. EPA 738-F-02-007. https://archive.epa.gov/pesticides/reregistration/web/html/naled_fs.html. U.S. Environmental Protection Agency. SW-846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods; Office of Resource Conservation and Recovery: Washington, DC, USA, 2015. http://www3.epa.gov/epawaste/hazard/testmethods/sw846/online/index.htm (accessed on March 10, 2019).

U.S. Environmental Protection Agency, 2016. Naled for Mosquito Control. https://www.epa.gov/mosquitocontrol/naled-mosquito-control,

U.S. Environmental Protection Agency, 2020. Naled, 300-76-5 | DTXSID1024209. https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID1024209 - properties

Zhong H., Latham M., Hester P.G., Frommer R.L., Brock C. (2003). Impact of naled on honey bee *Apis mellifera* L. survival and productivity: Areal ULV application using a flat-fan nozzle system. *Arch. Environ. Cont. Tox.* 45, 216-220.

Table 1: Summary of: a) laboratory experiments used to evaluate degradation of naled and dichlorvos under different environmental conditions and b) field experiments to evaluate levels of naled and dichlorvos at ground level after full-scale areal spray activities.

Experiment Type		Water Type		
Lab or Field Experiment	Experimental Conditions	Freshwater	Marine water	De-ionized water
Laboratory	Dark at 23 °C	3	3	3
Laboratory	Dark at 30 °C	3	3	N/A
Laboratory	Simulated Sunlight at 23 °C	3	3	1
Field	June 29, 2017[a] at 25 °C	1	1	N/A
Field	August 28, 2018[a] at 25 °C	4[b]	4[b]	3[b]

N/A = Not Applicable

[a] Field samples included a sample collected before aerial spray at time zero, and a number of sample(s) as indicated by the number indicated after the spray. The first sample after each spray was collected 30 minutes after the airplane was observed overhead.

[b] For August 28, 2018 the "after" samples were collected at time 30, 60, 100, and 125 minutes after the airplane was observed overhead. The three samples of deionized water corresponded to 30, 60, and 100 minutes.

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610

611 Table 2: Rate constants and half-lives of naled and dichlorvos in de-ionized, fresh, and marine
612 waters without sunlight at room temperature (23 °C) and at 30 °C and under simulated sunlight.
613 Standard deviations of the rate constants are provided below the rate constants in parenthesis.

614

	Without Light				With Light
Water Type	Room Temperature		Temperature at 30 °C		Room Temperature
	Naled	Dichlorvos	Naled	Dichlorvos	Naled
Rate Constants (h^{-1})					
De-ionized	2.8×10^{-3} (1.8×10^{-4})	2.5×10^{-3} (5.3×10^{-5})			2.3×10^{0}
Fresh	3.6×10^{-2} (2.7×10^{-3})	1.4×10^{-2} (4.3×10^{-3})	6.5×10^{-2} (1.3×10^{-2})	3.8×10^{-2} (3.0×10^{-2})	2.9×10^{0} (1.2×10^{0})
Marine	6.3×10^{-2} (2.2×10^{-2})	1.5×10^{-2} (1.0×10^{-2})	1.1×10^{-1} (5.4×10^{-2})	8.1×10^{-2} (3.8×10^{-2})	2.6×10^{0} (3.6×10^{-1})
Half Lives (h)					
De-ionized	131	344			0.31
Fresh	20	166	11	112	0.25
Marine	12	128	8.6	26	0.29

615

616

617 Table 3: Results from field naled exposure of sample waters on June 29, 2017 and August 28,
618 2018 and normalized on a per surface area basis. The samples collected prior to the exposure to
619 the spray were below detection limits. The detection limits of the method used to analyze naled
620 and dichlorvos were 0.0001 µM for naled and 0.0003 µM for dichlorvos.

621
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Sample ID	De-Ionized Water (µM)	Freshwater (µM)	Marine Water (µM)	De-Ionized Water (kg/km^2)	Freshwater (kg/km^2)	Marine Water (kg/km^2)
June 29, 2017 Sampling Date						
(Before) 3:50 AM	N/A[a]	ND[b]	ND	5.8[c]	5.8[c]	5.8[c]
Naled, 7:10 AM	N/A	0.034	0.049	N/A	0.41	0.59
Dichlorvos, 7:10 AM	N/A	0.004	0.007	N/A	0.03	0.05
August 28, 2018 Sampling Date						
Naled						
(Before) 9:30 PM	ND	ND	ND	5.3[c]	5.3[c]	5.3[c]
11:10 PM	0.033	0.032	0.028	0.39	0.38	0.34
11:40 PM	0.046	0.041	0.041	0.56	0.49	0.49
12:20 AM	0.064	0.048	0.046	0.77	0.58	0.55
12:45 AM	N/A	0.051	0.044	N/A	0.61	0.53
Dichlorvos						
(Before) 9:30 PM	ND	ND	ND	0[c]	0[c]	0[c]
11:10 PM	0.073	0.062	0.054	0.51	0.43	0.38
11:40 PM	0.092	0.086	0.067	0.64	0.60	0.47
12:20 AM	0.101	0.091	0.070	0.71	0.63	0.49
12:45 AM	N/A	0.091	0.078	N/A	0.64	0.55

623 [a] N/A: Not applicable, [b] ND: Not detected, [c]Correspond to target levels of naled from airplane as planned by the mosquito district.
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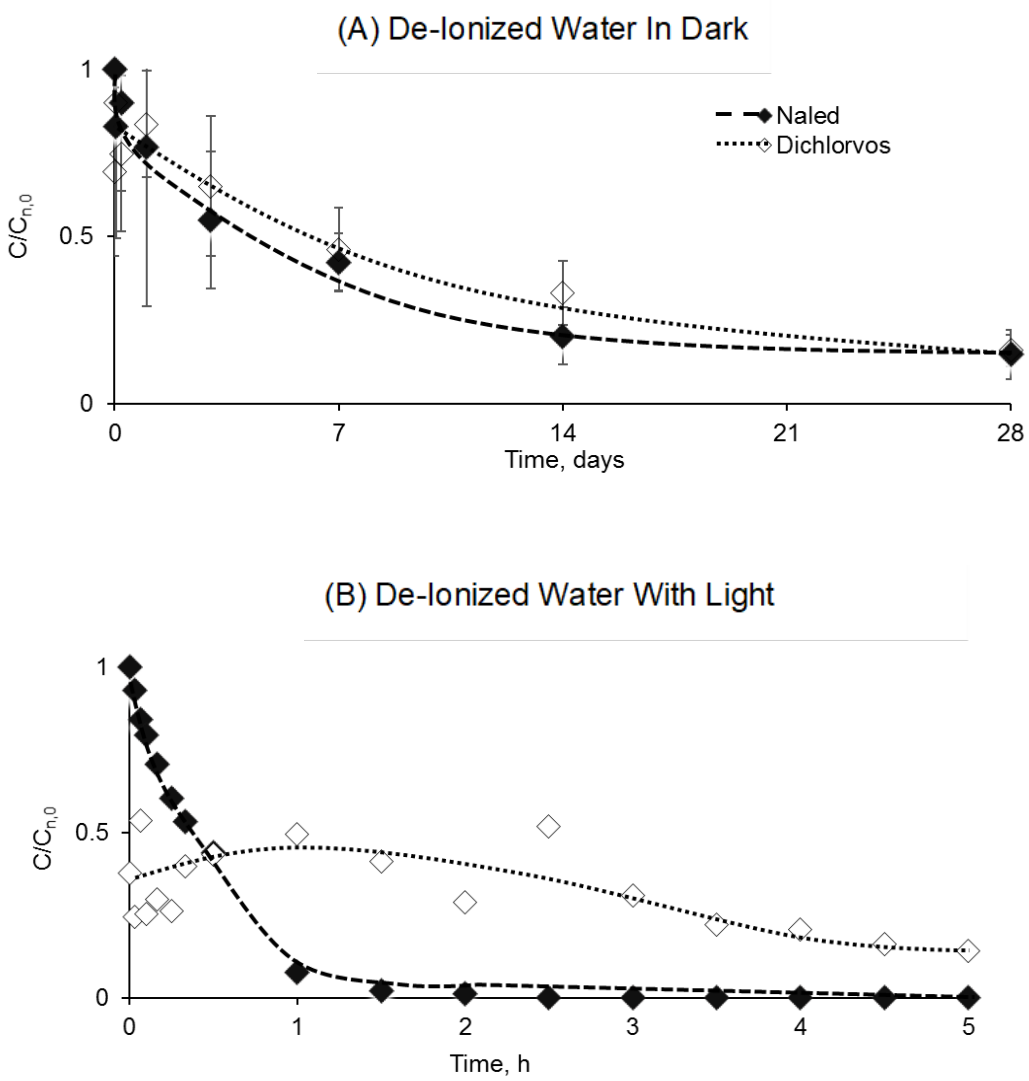


Figure 1: Concentrations of naled and dichlorvos, C, in de-ionized water solution at room temperature normalized to the initial naled concentration, $C_{n,0}$, (A) corresponds to experiments in dark and (B) corresponds to experiments under light. Note the difference in units and scale for the horizontal axis.

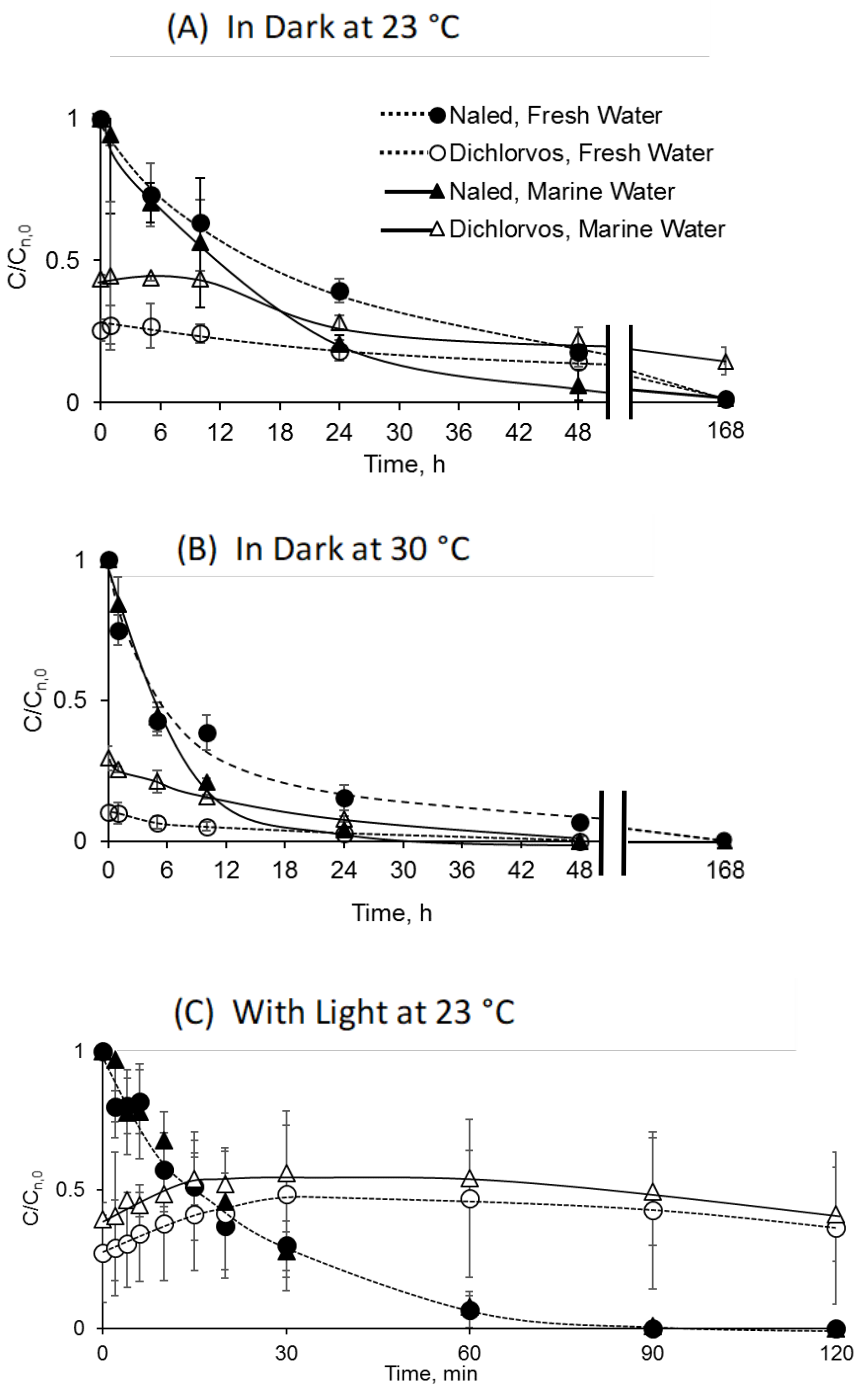


Figure 2: Concentrations of naled and dichlorvos, C, in natural freshwater and marine water, normalized to the initial naled concentration, $C_{n,0}$. (A) Correspond to experiments run at room temperature (23 °C) in dark, (B) at 30 °C in dark, and (C) under simulated light at 23 °C.

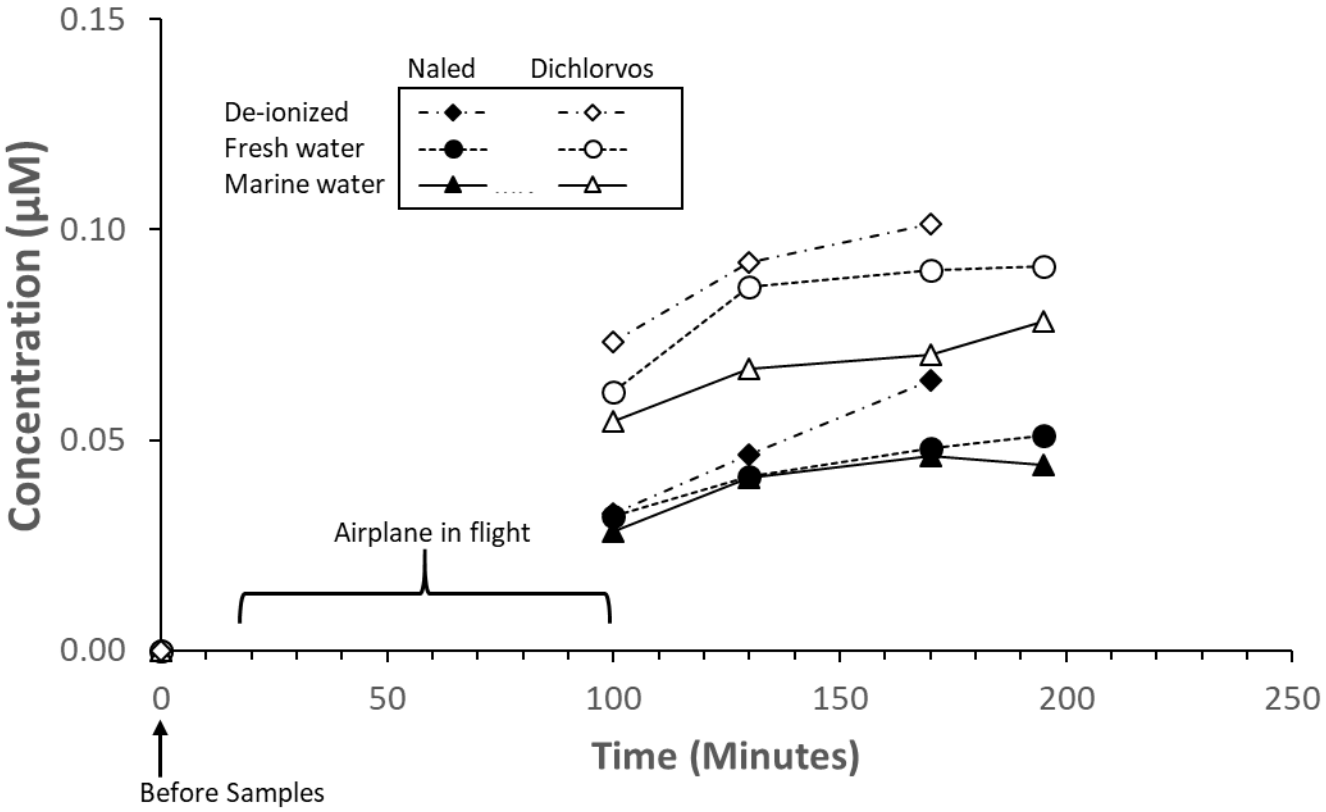
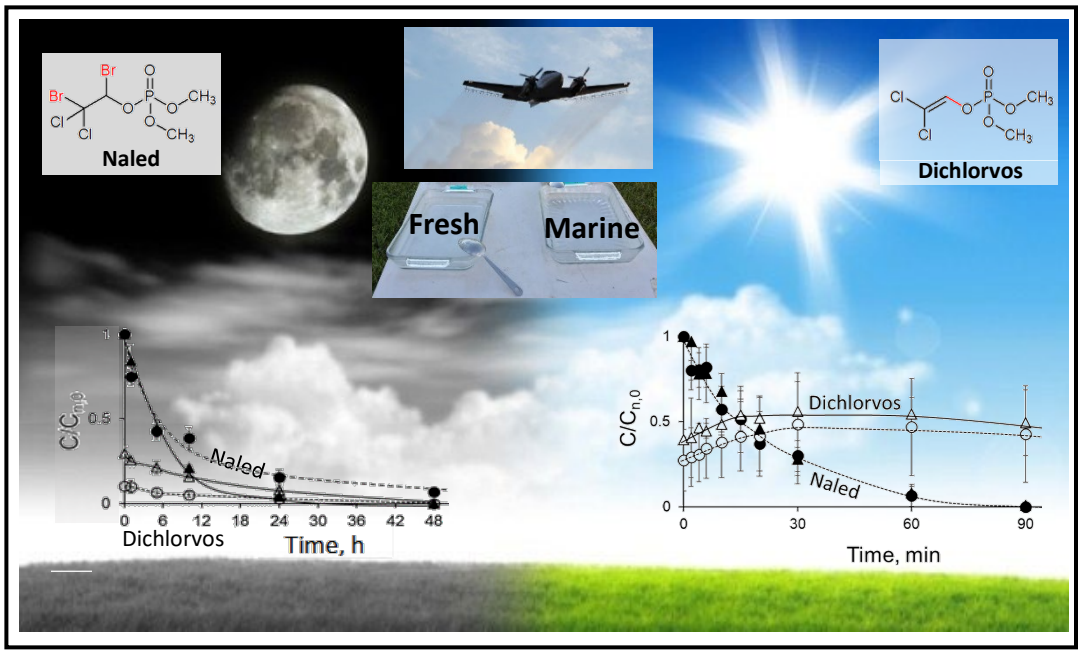


Figure 3: Persistence of naled and dichlorvos in de-ionized, fresh, and marine water immediately after the aerial spray mission held on August 28, 2018. Samples collected prior to the aerial sprays were below detection limits. T = 0 corresponds to the beginning of the aerial mission

Graphical Abstract



Highlights

- Field measurements provided levels of naled and dichlorvos at ground level.
- A 90% loss of active ingredients were observed at ground level.
- Naled exhibited rapid degradation in natural waters.
- The rate of degradation was enhanced by temperature and sunlight.

Supplemental text for:

Persistence of aerially applied mosquito-pesticide, naled, in fresh and marine waters

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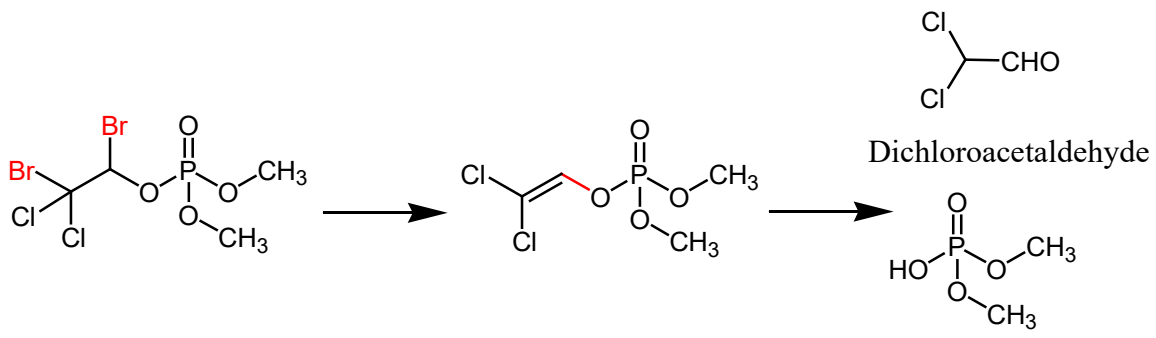
Table S1: Basic physico-chemical characteristics of natural waters utilized in this study. TSS, TDS, TOC, and NPOC represent total suspended solids, total dissolved solids, total organic carbon, and non-purgeable organic carbon. respectively. De-ionized water utilized in this study corresponded to a resistivity of 18.2 MΩ·cm (at 25°C).

Natural Water Type	pH	Turbidity (NTU)	Conductivity (mS/cm)	TSS (mg/L)	TDS (mg/L)	TOC (mg/L)	NPOC (mg/L)	Major Cations (mg/L)			
								K^{+}	Ca^{+2}	Mg^{+2}	Na^{+}
Lake Freshwater	8.26	0.61	0.2	7.7	329	523	520	2.2	26.4	1.9	8.8
Marine Water	8.02	0.43	47.5	3.8	41,400	283	206	--	411	1220	11,015



Naled Dichlorvos Dichloroacetaldehyde Dimethyl phosphate

Figure S1: Degradation pathway of naled (modified from Gan et al. 2006 and after Lieberman and Alexander 1983)

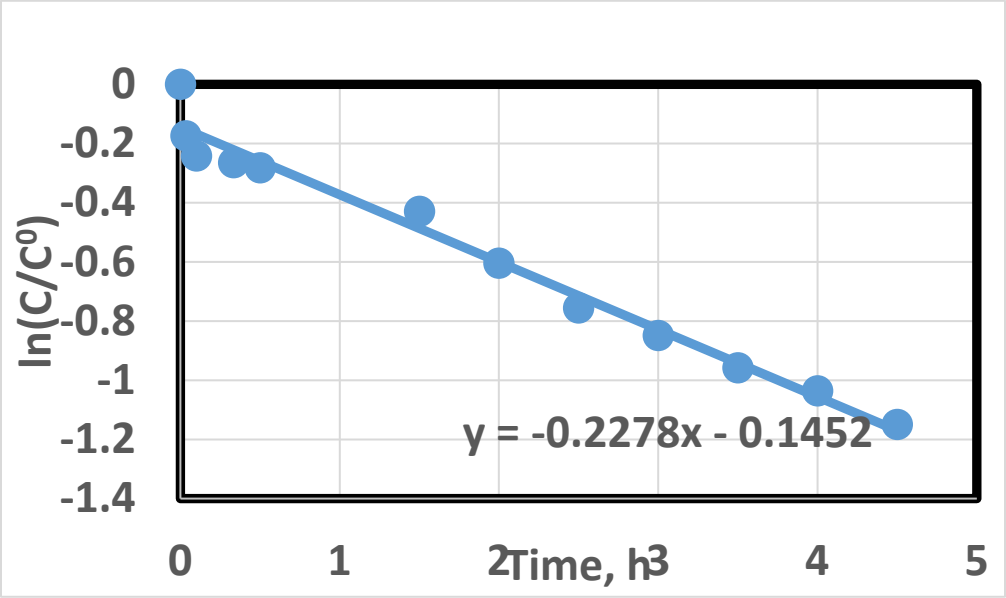
Procedure for computing half lives and decay constants

For each experiment naled and dichlorvos concentrations, C, were normalized by the initial naled concentration in molar units, $C_{n,0}$. Error bars on graphs correspond to the standard deviation of the normalized concentrations, $\frac{C}{C_{n,0}}$, for the replicated experiments. Rate constants and half-lives were computed by assuming a first order rate of degradation where:

$$\frac{C}{C_{n,0}} = -K\frac{d\left(\frac{C}{C_{n,0}}\right)}{dt}$$

Through integration, a linear relationship was established between the decay constant, K, and the natural logarithm of $\frac{C}{C_{n,0}}$ from which the rate constant was computed using the experimental data from a best fit line (K = slope of the line). The half life, t½, was then computed by reintegrating the first order rate equation from $\frac{C}{C_{n,0}}$ equal to zero to 0.5, and time from zero to t½ and then computing t½ algebraically.

Figure S2: Photoloysis of dichlorvos in DI water under simulated sunlight. This experiment was performed using a dichlorvos standard (no naled introduced). Rate constant was computed as 0.23 h^{-1} and half life is 1.6 h.



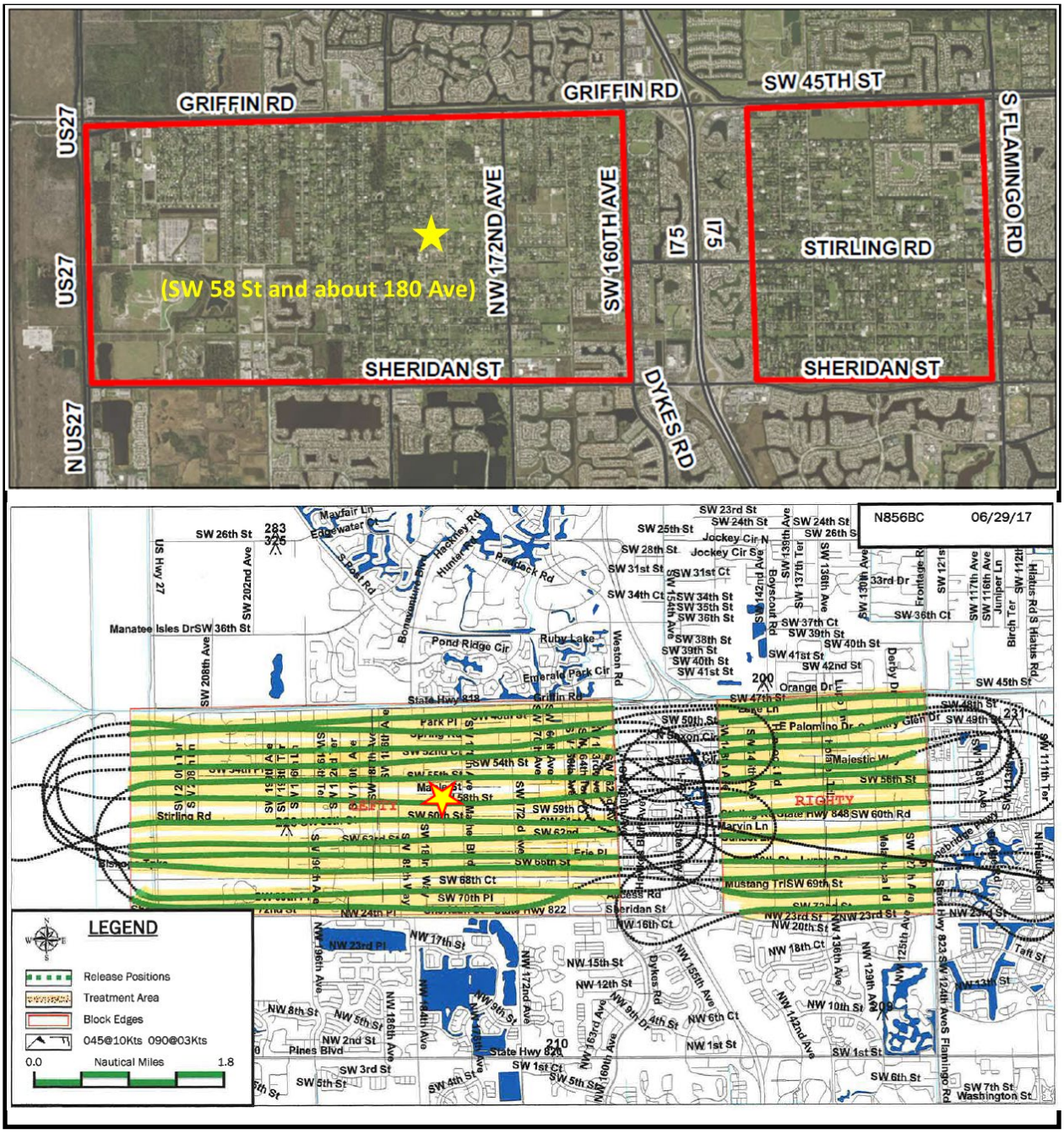


Figure S3: Flight path of airplane during June 29, 2017 aerial spray over the Southwest Ranches area of Davie, Florida, located in Broward County (courtesy of Broward County Mosquito Control) (top panel). The sampling site was set up in an open area at about 18000 SW 58 Street, Southwest Ranches, FL. Lower panel shows a satellite image of the area. The star corresponds to the sampling location.

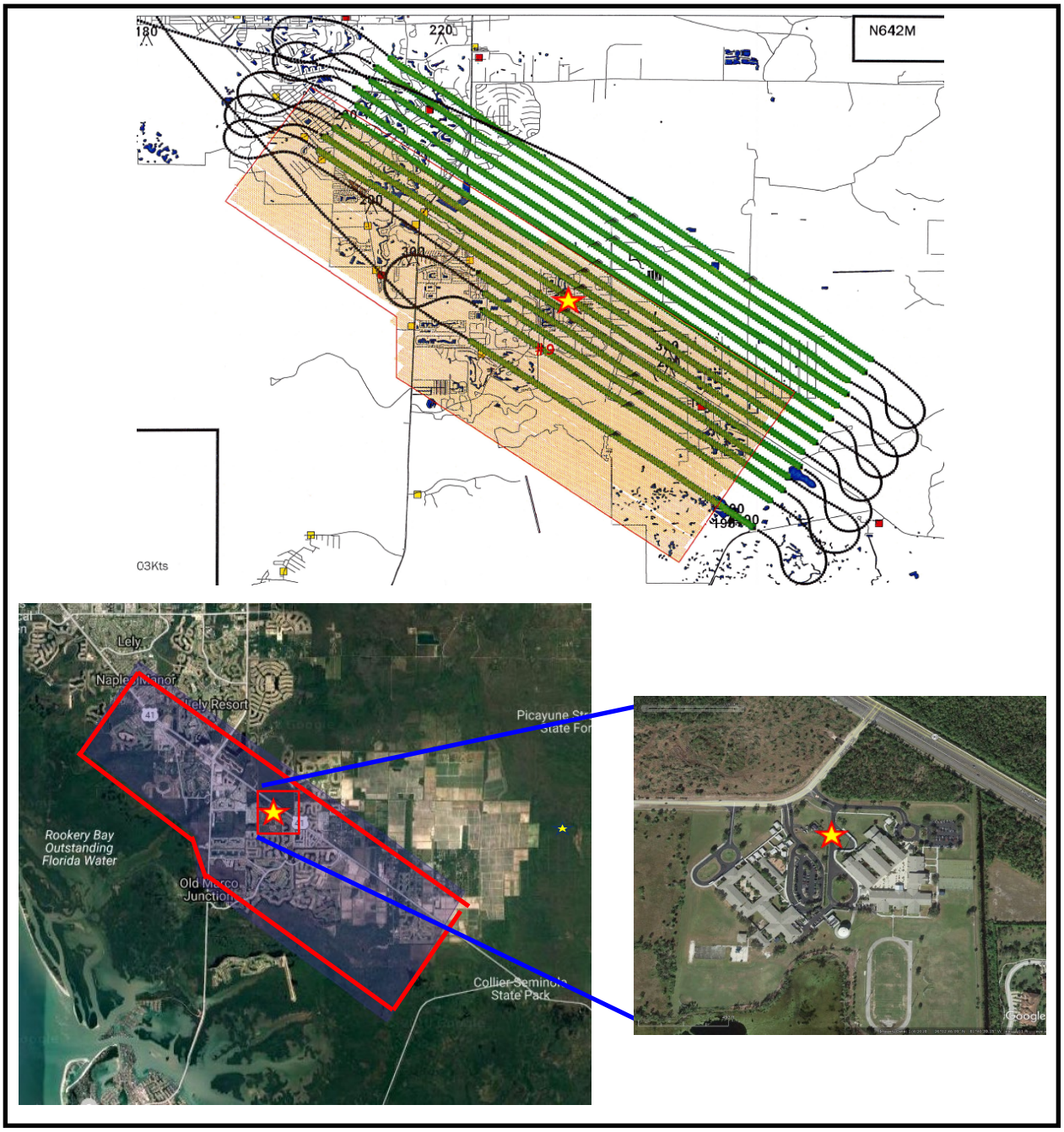


Figure S4: Flight path of airplane during August 28, 2018 aerial spray over the East Naples area of Collier County (courtesy of Collier Mosquito Control District) (top panel). The sampling site was set up in an open field area in the front of Manatee Middle School located at 1880 Manatee Road, Naples, FL. Lower panel shows a satellite image of the area within a zoom in of the specific sampling site. The star corresponds to the sampling location.

Details about the Aerial Flight Missions

The June 29 flight mission started at 5:03 am. The airplane used for aerial spray was a Britan Norman Islander fitted with a rotary atomizer (Micronair). The atomizer was calibrated to result in droplet sizes within label range - between 24 and 30 microns in diameter at ground level – at an application rate of approximately 0.5 oz/acre (Broward County Mosquito Control, personal communication). The target dose was 5.8 kg of active ingredient per square kilometer (equivalent to 0.5 fluid ounce concentration per air column acre with Dibrom® concentrate at a density of 15.1 pounds per gallon and 87.4 % purity by weight). The flight pattern – at an altitude of 91 m – consisted of parallel race-track patterns (275 to 300 meter swaths) over a 7.5 km by 3.7 km area with the last two swaths of the flight path occurring in the middle of this rectangular area immediately over the exposure location (Latitude and Longitude: 26˚02'48"N, 80˚23'20"W, see Figure S3 in supplemental text). The pass immediately over the exposure site occurred at 6:32 am local time and the last pass, immediately to the south, occurred at 6:37 am, which coincided with the time of the actual sunrise (civil twilight occurred at 6:05 am). The natural water samples were then transferred to the 1-L amber bottles in the same fashion as described in the main text. The 60-mL of ethyl acetate was added to these bottles within 30 minutes of exposure by 7:03 am. Samples were then placed in a cooler containing freezer packs and were immediately transported to the laboratory (Florida Spectrum Environmental Services, Ft. Lauderdale, FL) for analysis.

The August 28 flight mission started at 10:00 pm local time (sunset at 7:45 pm and sunrise at 7:00 am so it was completely dark at the time of the aerial spray). The airplane used

129 for aerial spray was a Short SC.7 Skyvan fitted with a wind-driven Micronair AU4000
130 rotary atomizer (Micron Group, Bembridge Fort, Sandown, Isle of Wight , U.K.). The
131 atomizer was calibrated to produce Dibrom® droplets within label range – achieving a
132 median droplet size of 28.9 microns – at an application rate of approximately 0.46 oz/acre
133 (Collier Mosquito Control District, personal communication). The target dose was 5.3
134 kg/km^2 (equivalent to 0.46 ounce per acre as described above). The flight pattern
135 consisted of parallel swaths at an altitude of 91 m, a swath width of 366 m and a speed
136 of 140 knots. A total of 13 swaths were made starting to the east of the sampling location
137 and finishing to the west. The flight path was offset from the target area by about 2
138 kilometers northwest to account for the wind speed and direction according to the
139 AGricultural DISPersal model (AGDISP) for Dibrom® Concentrate (See Figure S4 in the
140 supplemental text). Two swaths of the plane were observed to the east at 10:05 pm and
141 10:10 pm and three were observed to the right at 10:15 pm, 10:20 pm, and 10:25 pm.
142 The closest traverse to the site was observed at 10:15 pm. The four water samples were
143 collected for each water type. These samples were collected at 11:10 pm, 11:40 pm,
144 12:20 am, and 12:45 am. Upon collection, the samples were transferred to the 1-L amber
145 bottles in the same fashion as described above. Samples were then placed in a cooler
146 containing freezer packs and they were transported to the laboratory (Florida Spectrum
147 Environmental Services, Ft. Lauderdale, FL) at 12:00 pm for analysis as described in the
148 main text of the paper.

149 For each water type, a background sample was collected within 20 minutes prior to the
150 airplane flight. For the June 29 mission, the background sample was collected at 3:55 am

151 local time prior to the flight mission. The August 28 background water samples were
152 collected at 9:30 pm local time again prior to the flight mission.

153

154

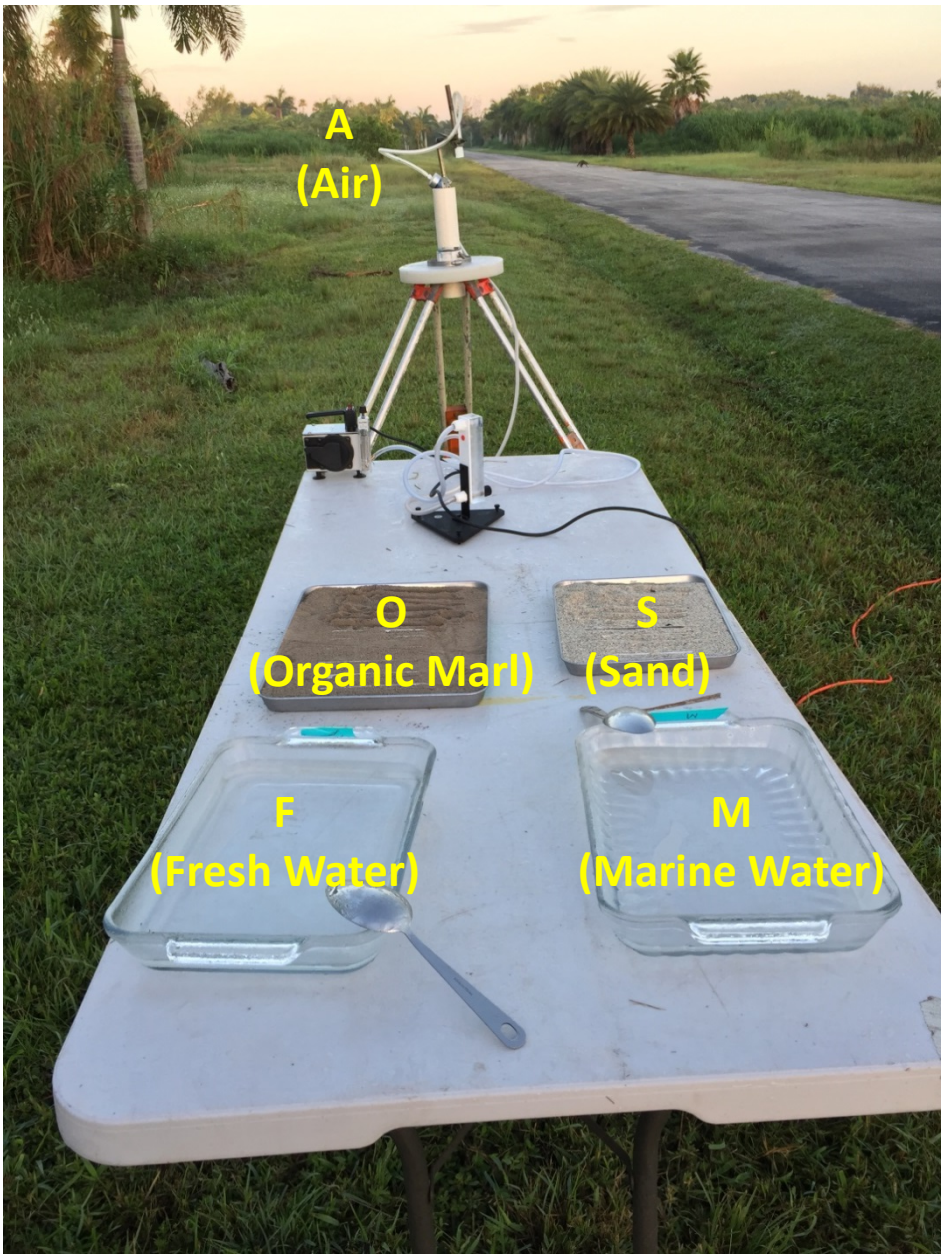


Figure S5: Field sample set up, illustrating how the water samples were laid out in the area impacted by full-scale aerial spray on June 29, 2017. Only results for water are presented in this paper as results for air and sand/marl were below detection limits on June 29.

Details about light conditions during each field sample collection effort

During the night hours, artificial light was needed in order to collect the samples. For the June 29 trip, the artificial light consisted of a handheld lantern and the headlights from a car aimed in a direction away from the samples to provide lighting for placing equipment in and out of coolers. The handheld lantern was taken to the sampling station only during sample collection. During the August 28 trip, a lit walkway was available about 600 feet from the sample collection station which allowed for set up of equipment and coolers under visible conditions. The samples were set up in complete darkness away from the lit walkway and a handheld lantern was used to collect samples. Again, this handheld lantern was used only during sample collection in order for researchers to be able to transfer the water samples from the trays into the amber bottles. The amber bottles were closed before walking them back to the lit walkway.