Monitoring the fate of methoprene larvicide for West Nile virus management

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Abstract Since the outbreak of vector-borne West Nile virus in New York City in 1999, the disease has spread across United States and Canada, resulting in the use of larvicides such as methoprene at catch basins for widespread urban mosquito control. Although the manufacturer has recommended a methoprene dosage for catch basin application, the effect of rainfall on this dosage is not known. A field study on the fate of methoprene pellets and ingots was conducted during the summer of 2004 at three catch basins in the City of Toronto, Canada. Water samples from each catch basin were collected daily and during rain storms and analyzed for methoprene concentration using gas chromatography mass spectrometry. It was found that: (1) the methoprene concentration at the catch basin sump fell below the minimum lethal concentration most of the time; (2) rainfall events greater than 25 mm flushed methoprene pellets out of the catch basin; (3) the higher the sump water depth, the higher the residual methoprene concentration at the catch basin sump; and (4) rainfall flushed methoprene from the catch basins into the storm sewer outfall at concentrations much lower than the detrimental level which might cause ecosystem damage.
Keywords Larvicides; methoprene; mosquito control; West Nile virus

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Introduction

West Nile Virus

Prior to the summer of 1999, West Nile virus (WNV) was never identified in the Western Hemisphere. Since the 1999 outbreak in the New York City metropolitan area, the disease has spread across North America. WNV is a mosquito-borne virus. It caused seven deaths when it first emerged in the United States in 1999. In Canada, the virus was first confirmed in birds in Ontario in 2001 and the first human case of WNV was confirmed in the Province of Ontario in September 2002. Infected people show various responses, from subclinical disease, to short duration flu-like illness, to frank encephalitis and death (Garmendia *et al.*, 2001). Ten species of mosquitoes have been shown to be carriers of the virus. Of these species, Culex mosquitoes are the most likely species to transmit the virus. At this time, there is no licensed vaccine to protect people against WNV (Health Canada, 2004). As of 31 October 2004, six human cases of WNV were reported in the City of Toronto as well as 31 mosquito pools and 18 birds tested positive for the virus. In 2003, there were 44 human cases of WNV reported to the City of Toronto as well as 17 birds and 55 mosquito pools tested positive for WNV (Toronto Public Health, 2004).

Life cycle of mosquitoes

Mosquitoes tend to breed in stagnant water in mid to late summer of extensive rainfall. They undergo four stages of their life cycle: egg, larva, pupa and adult, to complete their metamorphosis. The first three stages take place in water. Eggs are laid singly or in groups called rafts for some species on the water surface. Culex females lay their eggs on water that is high in organic material and stagnant. Most eggs hatch into larvae within 24 hours at 20–25 °C and up to several days if cooler temperatures are prevalent. Most eggs

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are laid in early May but egg-laying continues until fall. Larvae emerge and eventually change to pupa, which, in turn, change to adults. The whole process from hatched egg to flying adult takes between 4 and 14 days. In urban areas, catch basins with sumps provide a breeding ground for mosquitoes. Catch basins could experience flushing thoroughly during average rainfall and heavy rain of 102 mm could substantially reduce larval numbers (Geery and Holub, 1989).

Mosquito can be effectively reduced by source reduction, i.e. reducing the amount of standing water available for breeding; and controlled when they are larvae or adults. Products are called adulticides (mosquito repellants, e.g. DEET) and larvicides (e.g. oil, abate, Bti and methoprene). In general, the use of larvicides is preferred to the use of adulticides. Larval control is more effective at controlling mosquitoes and has the least effect on non-target species in the environment.

Larvicides

Methoprene, a juvenile hormone analogue, with IUPAC of Isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyldodeca-2, 4-dienoate, is an insect growth regulator for the control of mosquito larvae that was first registered for use in Canada in 1977. The synthetic methoprene compounds are structurally similar to insect's natural juvenile hormones and therefore referred to as juvenile hormone analogue. It prevents larvae from reaching maturity or reproducing by disrupting the action of the growth hormone, so its life cycle is disrupted. Methoprene works effectively during the late instar of larvae or pupal stage.

Methoprene is sold commercially as Zoecon Altosid and manufactured by Wellmark International. It can be a form of liquid, pellets, granules, as well as chalks. Pellets are shaped like grains of rice. Altosid chalks are available in an 'ingot' shape to allow the product to fit through the stormwater catch basin grate without lifting it. The pellets or ingots stay positioned in the water at the bottom of the catch basin sump, gradually breaking down and releasing methoprene into the water. Pellets and ingots are slow-release formulations, which can provide residual concentration up to 30 and 150 days, respectively. Application rates vary depending on the type of habitat, water depth and water quality (Wellmark International, 2003). Lethal concentration (LC50) of 0.3 to 2.3 μg/L has been reported for Culex mosquito species (Amin and White, 1984; Ali *et al.*, 1995).

Larvicides are most effective when used early in the mosquito season, from May to July when pupae begin to develop. In 2004, methoprene pellets (dosage of 0.7 g per catch basin) were placed at the 175,000 City of Toronto's owned catch basins during the summer months when the peak reproduction cycle of *Culex pipiens* was maximum. One of the reasons for using methoprene pellets is its longer persistence in water compared to others larvicides, which provide approximately 30 day mosquito control (McCarry, 1996).

Altosid pellets produced S-methoprene residues that peaked at 2.0 μg/L on the 7th day and declined to 0.2 μg/L at the 14th day after applicaton while the Altosid XR ingots produced the concentration peaked at the 2nd day with average concentration of 0.7 μg/L and declined steadily through the 4th day to 0.2 μg/L. The study was done in a static system (Ross *et al.*, 1994).

Most studies done on methoprene pellets or briquets are focused on static experiments. The efficacy of methoprene is assessed by larvae or adult mosquito reduction. Few studies have been done on the dissolution and decay rate of methoprene as well as investigation of any possible meteorological influences on the hydrodynamic properties of methoprene. Little work has been undertaken to determine the residual methoprene concentration and loadings within a drainage system during rain storms.

Methoprene is considered by the US Environmental Protection Agency (USEPA) as a 'least toxic' insecticide, and USEPA claims that "methoprene used in mosquito control programs according to label directions does not pose unreasonable risks to wildlife or the environment" (USEPA, 2001). It has very little non-target species toxicity and svirtually non-toxic to birds. However, there is some evidence of toxicity to fish, amphibians and freshwater invertebrates. Field studies of methoprene have demonstrated that there is little effect on non-target organisms including dragonflies, water boatmen and fairy shrimp (West Nile Virus Prevention and Control Program, 2003). However, methoprene levels in excess of 10 μg/L, could have detrimental effects on non-target invertebrates that are an important source of food for fish (Ross *et al.*, 1994). The draft Interim Provincial Water Quality Objective for methoprene for non-target and target species are 0.2 μg/L and 0.001 μg/L, respectively (MOE, 2004).

Study objective and scope

This paper describes a field monitoring study of methoprene pellets and ingots at three catch basins and the associated sewer outfall in the City of Toronto from July to September 2004. Two of these catch basins were dosed with one piece of Altosid XR ingot while the remaining catch basin was dosed with Altosid pellets of 0.7 g every month (July and August). The study objective is to determine the residual concentration of methoprene at the catch basins and the sewer outfall with and without rain storms.

Methods

Study location

The study site is located at a residential area served by a storm sewer system in the City of Toronto, Ontario, Canada. The storm sewer outfall conveys runoff from about 1200 catch basins and discharges directly to the Newtonbrook Creek of the Don River which, in turn flows through the middle part of Toronto into Lake Ontario. The Newtonbrook sewershed has a perimeter of 14,349 m and an area of 3,590,169 m^2. Three roadside catch basins (identified as W1, S1 and S2), closed to a storm sewer outfall, were selected for monitoring study. In addition, the sediment depth and water level of each catch basin are different. Using a vacuum truck, half of the sediment at W1 and all sediment at S2 were removed. S1 was almost filled up with sediment. Table 1 shows the sediment and sump characteristics of the three catch basins.

Monitoring program

Monitoring objective. The monitoring program focused on the measurement of rainfall, concentration of methoprene at the catch basins and the storm sewer outfall, and the flow at the sewer outfall.

In order to match the City of Toronto's larviciding schedule, 0.7 g of long methoprene pellets (average length 11.1 mm) were added to the catch basin W1 on 16 July and another 0.7 g of short methoprene pellets (average length 4.30 mm) were applied on 23 August. Altosid methoprene pellets are effective up to 30 days post-treatment and the

Table 1 Characteristics of catch basins

Catch basin	W1	S1	S2
Water depth (cm)	31.0	11.5	106.0
Sediment depth (cm)	52.0	66.5	3.0
pH	7.21	6.97	7.41
Temperature (°C)	20	22	22

second trial was needed to provide protection over the summer. The purpose of using long and short pellets in two trials was to examine the residual methoprene concentration of different sizes of pellets. For the catch basins S1 and S2, the larvicide used was methoprene ingots. Altosid methoprene ingots are designed to be effective up to 150 days, and no second trial was needed after the first application in July.

Monitoring equipment. Automatic wastewater samplers (American Sigma Model No. 1350) were installed at each of the catch basins to collect wet weather water samples. A long stainless steel tube was inserted into the top surface of the catch basin sump by drilling through the sidewalk and the wall of the catch basins. Dry weather water samples were collected through this steel tube using a small electric hand pump.

In order to monitor concentration of methoprene at the storm sewer outfall during wet weather, a concrete hut was installed right on top of the sewer outfall pipe and an opening was cored through the soil and the top of the sewer. An automatic sampler (American Sigma Model 900MAX) with a built-in area-velocity sensor was used to collect the wet weather water samples and flows. A tipping bucket rain gauge was installed on the roof of the concrete hut to measure the local precipitation. The rain gauge began data collection on 11 August 2003.

Sampling and analysis protocol. The dry weather residual methoprene concentration at each of the catch basins was monitored daily by the research team members. Water samples of 250 mL were drawn from the top 5–10 cm of the sump water surface through the steel sampling tube using an electric hand pump. Using automatic wastewater samplers, wet weather water samples were collected at the catch basins and the sewer outfall from July to September 2004 by the City of Toronto Works and Emergency Services. Water samples were stored in amber and teflon-lined jars to limit photolytic degradation of the methoprene, and all samples were preserved in ice during delivery to the laboratory for testing.

Methoprene concentration in water samples was analyzed by the City of Toronto's water and wastewater laboratory, using the United States Geological Survey (USGS) method of liquid–liquid extraction and gas chromatography/mass spectrometry (Zimmerman and Thurman, 1999). The USGS's method was developed for the determination of four mosquito insecticides (malathion, methoprene, phenothrin, and resmethrin) and one synergist (piperonyl butoxide) in water (Zimmerman and Thurman, 1999). The analytical method requires liquid–liquid extraction (LLE) and gas chromatography/mass spectrometry (GC/MS). Water samples were collected in glass-fibre bottles. At the laboratory, each bottle was assigned an identification number. A surrogate compound was added and a small volume of sample was removed from the bottle. The remaining sample in the bottle was then mixed with hexane. The hexane extract was then removed and evaporated under nitrogen within the internal standard. The sample components were separated, identified and measured by injecting an aliquot of the concentrated extract into a high-resolution, fused-silica capillary column of a GC/MS system under selected-ion mode (SIM). The compounds eluting from the GC column were then identified by comparing their measured ions and retention times to the reference ions and retention times obtained by the measurement of control standards under the same conditions used for the water samples. The concentration of each identified compound was measured by relating the MS response of the quantitation ion produced by that compound to the MS response of the quantitation produced by the surrogate standard (Zimmerman and Thurman, 1999). The USGS method detection limit ranged from 0.02 to 0.05 μg/L.

Results and discussion

Residual methoprene concentration at catch basins

Catch basin W1. Long methoprene pellets of 0.7 g were applied on 16 July 2004. As indicated in Figure 1, the methoprene concentration peaked at 4th day, and no second peak was observed. After the 1st peak, the concentration levelled off to non-detectable limit (< 0.03 μg/L) and never reached the lethal concentration (0.3–2.3 μg/L) in the 30 day effective period. The occurrence of the first peak and the failure to observe a second peak could be due to the rainfall event on days 4 and 5 where heavy rain (25–30 mm) might have flushed away the pellets.

On 23 August 2004, 0.7 g of short pellets was applied. No peaks were observed throughout the entire 30 day period, and the concentration stayed persistently at 0.05 μg/L. The rainfall event on 26th and 27th August might have flushed away the short pellets from the catch basin. The amount of rainfall could significantly affect the residual methoprene concentration through flushing of the pellets and resuspension of pellets in the catch basin sediments.

As indicated in Table 2, the methoprene concentration at W1 was always below the minimum lethal concentration (0.3 μg/L). Even though the methoprene concentration does not necessary imply ineffectiveness against the growth of mosquito larvae, the results may not show good potential to inhibit larvae growth. The effect of rainfall on the residual concentration of methoprene at W1 is clearly demonstrated.

Catch basin S1. One piece of methoprene ingot was used at catch basin S1. As shown in Figure 1, the residual concentration of methoprene peaked on the 2nd day and the second peak came 8 days later on the 10th day. The long wait to reach the second peak may be explained by the heavy rainfall event which occurred on the 4th and 5th day. The second peak went down again after another rainfall event on the 15th and 16th day. For each drop of residual concentration due to rainfall, the residual concentration went under the minimum lethal concentration (0.3 μg/L). However, the overall residual concentration at S1 was more favourable than that at W1. Even though the effectiveness of methoprene ingots is supposed to be 150 days, there are at least 12 days over the monitored period where the measured residual concentrations are above the minimal lethal concentration. The highest residual concentration went up to 2.6 μg/L. Moreover, the % of days for the residual concentration staying above the

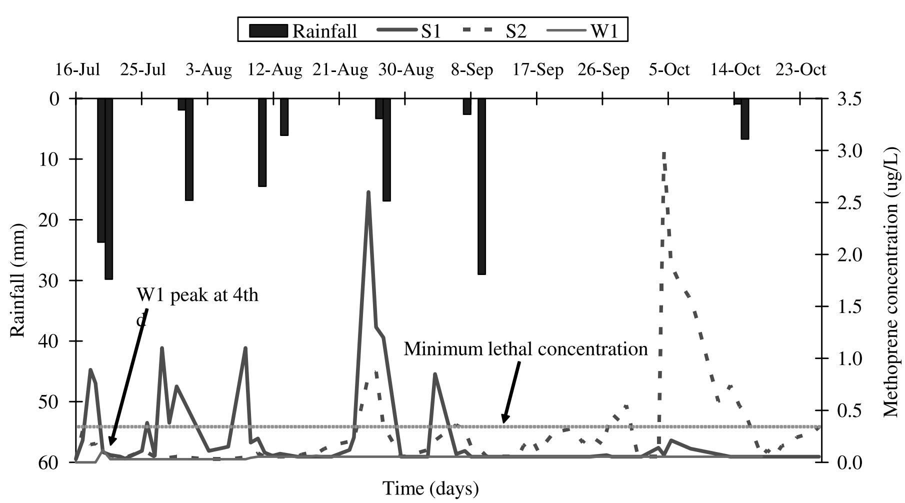

Figure 1 Residual methoprene concentration at catch basins

Table 2 Residual concentrations of methoprene at catch basins

Parameter	Catch basin		
	W1	S1	S2
Mean concentration (μg/L)	0.051	0.233	0.311
Standard deviation	0.013	0.427	0.486
Maximum concentration (μg/L)	0.12	2.60	3.00
Minimum concentration (μg/L)	0.03	0.03	0.03
Time to 1st peak (days)	4	2	1
% of days > limit (0.3 μg/L)	0.00	11.9	24.9

minimal lethal concentration is 11.9%. The ingot appeared not to be flushed away by rain storms and even the sump depth was only 0.1 m.

Catch basin S2. The residual methoprene concentration at catch basin S2 does not show a similar pattern to that of S1 (Figure 1). Low concentration of methoprene was observed in the first 30 days. It then climbed up gradually. After 4 October the concentration went up again and reached 3.0 μg/L. There were 24 days at which the methoprene concentration stayed above the minimal lethal concentration. As indicated in Table 2, the percentage of days in which the residual concentration at S2 was above the minimum lethal concentration is 25.9%.

S2 has a constant sump water depth of 0.85 m while S1 has only 0.1 m. Therefore, the large volume of sump water in S2 might prevent the ingot from being flushed or re-suspended during rainfall. Since it took a long time for the residual concentration of methoprene in S2 to go up above the lethal concentration, it is suspected that hydraulic turbulence at a catch basin sump may cause methoprene ingots to release methoprene at a faster rate. However, there were unusual cycles of methoprene concentration occurring in S2 in the late period of the field study when there was no rain over a long period of time in September and October. The methoprene ingot in S2 was not significantly washed or flushed away by the runoff; therefore, higher residual concentration remained in the water of S2.

The field study results in 2004 show that the residual methoprene concentration at a catch basin may be affected not only by the amount of rainfall and the associated turbulence flow, but also by the amount of sump water in the catch basin. This has been observed in previous field studies where shallow ponds had greater concentrations than deeper ponds (Hershey, 1994). However, it may not be entirely true when it comes to the investigation of catch basins S1 and S2. In the initial period, the residual methoprene concentrations in S1 exceeded those in S2. However, in the latter period, the residual methoprene concentration in S2 reached a higher level than that in S1. This observation suggests that the larger the amount of water at the catch basin, the greater the potential to retain a high residual concentration.

Wet weather methoprene concentration at the sewer outfall

Automatic wastewater samplers at the sewer outfall were triggered by the on-site rain gauge (located near the downstream end of the sewershed) to draw water samples at 10 minute intervals. As shown in Figure 2, the concentration of methoprene at the sewer outfall rises and falls like a hydrograph. In the initial period, the methoprene concentration stayed at a low value of 0.03 μg/L even when the rainfall was heavy. When the rainfall subsided, the concentration started to rise to 0.17 μg/L. Later on, the methoprene concentration declined due to flushing by another rain event. The highest concentration is much

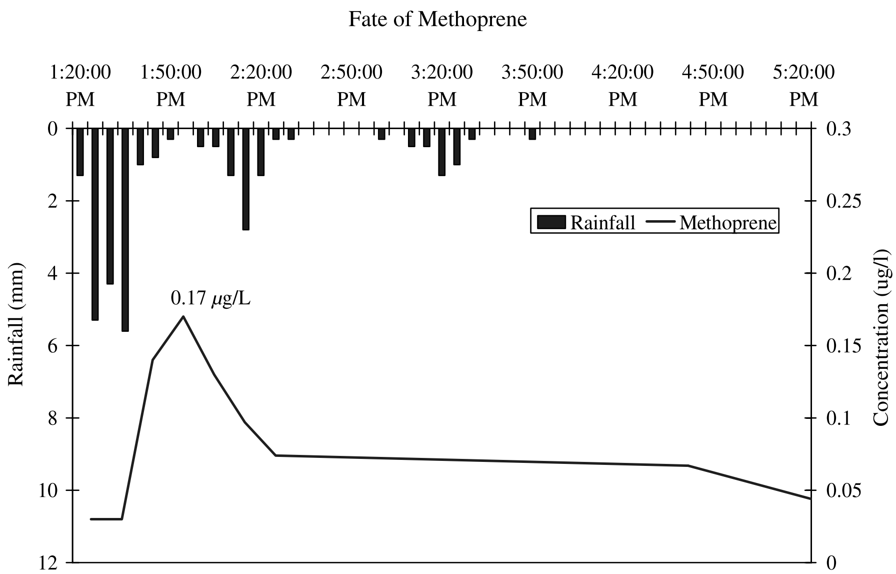

Figure 2 Methoprene concentration at the sewer outfall during a rain storm

lower than the USEPA recommended environmental concentration of 10 μg/L, and the ecotoxicity value for aquatic invertebrates of 360 μg/L.

Conclusions

From the field monitoring study, the following conclusions are drawn:

1. Rainfall of 25–30 mm may cause significant flushing of methoprene pellets at a catch basin. As a result, the residual concentration of methoprene at a catch basin sump may not be able to reach the required lethal concentration for larval control.
2. Methoprene ingots are more difficult to be flushed out from a catch basin during a rain storm than methoprene pellets.
3. Methoprene ingots can maintain the lethal methoprene concentration more often than methoprene pellets.
4. The higher the sump water depth, the higher the residual methoprene concentration.
5. The concentration of methoprene at the sewer outfall during rain events in 2004 did not exceed the USEPA's environmental concentration of 10 μg/L.

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