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THE FATE AND TRANSPORT OF METHOPRENE IN AN URBAN WEST NILE VIRUS MOSQUITO CONTROL PROGRAM

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A Thesis presented to Ryerson University

In partial fulfillment of the requirement for the degree of

Master of Applied Science in the Program of

Environmental Applied Science and Management

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ABSTRACT

Des Lauriers, Angelune, 2004. *The Fate and Transport of Methoprene in an Urban West Nile Virus Mosquito Control Program.* A thesis presented to Ryerson University in partial fulfillment of the requirements for the degree of Master of Applied Science in the Program of Environmental Applied Science and Management.

The recent occurrence of vector-borne West Nile virus in Canada has resulted in the use of larvicides for widespread urban mosquito control. The City of Toronto has focused its larviciding program on storm water catch basins as they are concentrated breeding grounds of the mosquito (Culex pipiens) most likely to carry West Nile virus. The City of Toronto undertook a larviciding program to control mosquitoes during the summer months of 2003. The larvicide approved for mosquito control in Canada is methoprene, commercially known as Altosid, in pellet formulation.

In order to determine the fate of the larvicide methoprene, the researcher, in conjunction with current studies at Ryerson University, the City of Toronto and the Ontario Ministry of the Environment, have undertaken a water quality monitoring study within the Toronto area. Three study catch basins in the Newtonbrook sewershed in Toronto, Ontario were dosed with methoprene (Altosid) pellets three times over the summer of 2003, at the recommended mosquito control dose of 0.7g. Water from each catch basin was sampled daily and analyzed for methoprene concentration, and mosquito larvae presence was observed. Precipitation, as well as the chemical composition of each of the catch basins was also monitored. A model catch basin in laboratory was also dosed

with methoprene pellets and sampled daily to observe methoprene concentration over time.

The fate of methoprene in the urban environment is of interest, to ensure that the larviciding program is not compromising human and environmental safety. It was found that rainfall flushes methoprene from the catch basins into the storm sewer outfall. The storm sewer outfall did not release methoprene at detrimental concentrations during the sampling period. Many factors such as physical dissolution, chemical degradation and catch basin water volume, affect the concentration of methoprene in a catch basin. In order to monitor the impacts of larviciding programs, comprehensive water quality monitoring and mosquito control efficacy should continue.

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CHAPTER 1

1. Introduction

Until recently, the threat of vector-borne disease was thought to be a serious problem only in tropical regions. However, mosquito-borne encephalitis has become a concern even in northern temperate climates (Hershey 1998) and West Nile virus (WNv) has even been detected as far north and west as northern Alberta (Health Canada 2004a). This has shifted the focus of mosquito abatement programs from wetlands near recreational and residential areas to control nuisance insects, to mosquito control for public health in urban areas. The very nature of pesticides creates risk of harm to humans, animals or the environment, as they are designed to kill or adversely affect living organisms. On the other hand, pesticides are useful in controlling disease carrying insects.

The recent occurrence of vector-borne WNv in the eastern United States and Canada has resulted in the use of larvicides such as methoprene for widespread urban mosquito control. Human and environmental safety is considered during implementation of pesticide use in urban environments (Racke 1993). Urban pesticide use is often the subject of controversy due to the high profile of these use patterns and proximity to humans (Racke 1993).

The City of Toronto has focused its larviciding program on storm water catch basins as they are concentrated breeding grounds for mosquitoes. A single catch basin may be small, but the collective water storage of the whole network of catch basins represents a large habitat with no natural predators. Along with storm water detention ponds, ditches, and wetlands, these breeding habitats cannot be removed. By design, catch basins hold water for extended periods of time, and due to their location underground, larviciding has been found to be labour efficient and effective against mosquitoes (Shapiro 2003), with low

environmental impacts. The species most likely to carry WNv is *Culex pipiens*, whose presence was confirmed in virtually all Toronto catch basins sampled (CoT 2003a).

As part of a WNv control program, the City of Toronto and surrounding municipalities undertook larviciding to control mosquitoes during the summer months of 2003, with completed applications in late June, July, and August, during the peak reproduction cycle of *Culex pipiens* (CoT 2003a). The sale and use of pesticides in Canada are regulated by the Pest Control Products Act, administered by Health Canada and managed provincially by the Ministry of the Environment (MOE 2003). The larvicide approved for mosquito control in Canada is methoprene, commercially known as Altosid, in pellet formulation (CoT 2003a).

It is the mandate of the Ontario Ministry of the Environment to ensure protection of the environment, including providing technical expertise regarding the use of pesticides to control mosquitoes and monitor water to ensure there are no adverse impacts on the environment (MOE 2003a). The City of Toronto is also responsible for monitoring water quality and related public health issues subsequent to application of larvicides as part of its public health initiatives for West Nile virus (CoT 2003b). In order to determine the fate of the larvicide methoprene, the researcher, in conjunction with current studies at Ryerson University, and the City of Toronto and the Ontario Ministry of the Environment, have undertaken a water quality monitoring study within the Toronto area. Surface water quality is of concern because it is particularly vulnerable to contamination by pesticides, as urban areas drain into the surface water system (Larson 1997).

The fate of methoprene in the urban environment is of interest, to ensure that the larviciding program is not compromising human and environmental

safety. The high level of scrutiny that exists regarding urban pesticide use indicates that there is a critical need for information on the behaviour of pesticides following use, and that the information be communicated to government regulators, pesticide manufacturers and formulators, pest product retailers, pest control professionals, and perhaps most importantly, consumers and homeowners (Racke 1993).

One challenge in mosquito control programs is choosing a control agent that is sufficiently specific to the target insect that it does not impact nontarget species or disrupt food webs (Hershey 1998). Evaluation of the environmental fate and persistence of a pesticide such as methoprene will determine the efficacy of the product, but also whether significant quantities of the product are being transported away form the treated site or reaching nontarget organisms (Racke 1993).

Public health experts warn that we have insufficient evidence to know how to control WNv-type diseases and how our control efforts might affect them (Wilson 2003). Filling in the data gaps in our knowledge will be essential in order to properly assess the risk balance essential to public health decisions regarding WNv.

WNv is predicted to become endemic to North America, the way malaria is endemic to tropical regions. Although it is a complex disease, a WNv specialist of the American Centers for Disease Control states that one thing is certain, where West Nile has been; it stays (Sibbald 2003). Pesticide application in New York City during 1999 and 2000 occurred as a response to an emergency situation, and was exempt from fulfilling state environmental impact assessment requirements (Lopez 2002). The New York City Department of Health recognized that WNv would be present in subsequent years, and that pesticide application could no longer occur without environmental reviews (Lopez 2002).

Because urban mosquito control programs continue as a normal yearly occurrence, it is critical to monitor for short and long-term environmental effects.

The outline of this thesis includes the project objectives and scope of the study, concluding in Chapter 1. Chapter 2 includes a review of the current issues relating to West Nile virus, urban mosquito control with methoprene and its associated environmental transport and impacts. This chapter also includes an examination of the current social and political climate of mosquito control, and alternatives to chemical mosquito control practices. A description of the study site including the sewershed and receiving waters is included in Chapter 3, which describes the methodology undertaken including the development and implementation of a field monitoring program and a laboratory experiment. Chapter 4 includes an analysis of the results collected from the site and the laboratory experiment, as well as the results from related studies, and a discussion of these results. Finally, Chapter 5 concludes the thesis with recommendations.

1.1. Objectives

Widespread urban application of mosquito larvicides in Canada is a relatively new occurrence, brought about by the arrival of WNv. Larvicidal control of nuisance mosquitoes and midges, and vector-borne diseases such as St. Louis Encephalitis in the United States has prompted studies monitoring the efficacy and impact of chemicals like methoprene (Niemi 1999; Hershey 1998; McCarry 1996). Few studies have focused on the transport of methoprene from catch basins through the storm sewer system to receiving waters, and in general, the hydrodynamic behaviour of this chemical is not known. Thus, it is important to understand the consequences of pesticide application for vector-borne disease control (Shapiro 2003).

The field study was conducted as part of a larger WNv water quality monitoring study in collaboration with the City of Toronto and the Ontario Ministry of the Environment (MOE). The ultimate goal of this project is to gain an understanding of the environmental impacts of larviciding programs. Thus, the objectives of this research project include the following:

- Determine the environmental fate of methoprene, to examine its behaviour in water and the concentration of methoprene over time when applied to urban storm sewer catch basins for mosquito control under local conditions in Toronto.
- Compare the field data to the methoprene decay rate obtained from a model catch basin in the laboratory.
- Assess the transport of methoprene in an urban mosquito control program and estimate the mass of methoprene discharged into Toronto surface waters, using Geographical Information Systems and a spreadsheet model of the Newtonbrook sewershed.

The findings of the research are expected to be applicable to future planning of mosquito control programs in the Greater Toronto Area, and lead to an increased effectiveness of such programs for reducing the human infection rate of West Nile Virus.

1.2. Scope

The objectives include the collection of water quality data and the outputs of a mass balance model. Based on these objectives, the scope of the research is limited to and includes the following:

- To characterize the concentration of methoprene in the field in three study catch basins and the storm sewer outfall of the Newtonbrook sewershed over three 30-day periods,
- To characterize the dissolution and degradation of methoprene over time in a model catch basin in a laboratory,
- To estimate the total outputs of methoprene from an urban sewershed.

Although the findings of this research may be useful for the epidemiological management of West Nile virus, the main focus of this investigation is to determine the transport of mosquito control larvicides in the environment. It is the hope of the researcher that the study findings may help in achieving the best balance between effective public health programs and ecological health.

CHAPTER 2

2. Current Knowledge of West Nile Virus Vector Control

2.1. West Nile Virus and Public Health

According to the American Mosquito Control Association, mosquitoes cause more human suffering than any other organism, as more than one million people succumb to mosquito born disease each year (Floore 2003). West Nile virus is an arthropod-borne virus (arbovirus); has a complex life-cycle involving a primary nonhuman vertebrate host and a primary arthropod vector (Floore 2003). Arbovirus cycles usually remain undetected until humans encroach on natural reservoirs of the virus or a host escapes due to some ecological change (Floore 2003).

The West Nile virus belongs to the Flaviridae family of viruses, and is related to Dengue fever, Yellow fever and St. Louis encephalitis (HC 2004a). Infection with West Nile virus does not always cause illness, but when it does, it causes a variety of symptoms that include fever, headache, body ache, rashes and swollen lymph nodes (HC 2004a). In rare cases, an infection with West Nile virus can result in meningitis, encephalitis and acute flaccid paralysis (HC 2004a). West Nile virus was first identified in 1937 in Uganda, Africa and arrived in North America in 1999. It has spread quickly through the United States and Canada, and has resulted in human and animal infections, some leading to death (Nosal 2003). The virus is spread through contact between birds and certain mosquitoes, humans and domestic animals are 'dead-end' hosts as they do not contribute to the transmission cycle (Floore 2003).

Different types of mosquitoes are responsible for different links in the transmission of WNv: "amplification" mosquitoes (e.g., *Culex pipiens* and *Culex*

restuans), "bridging" species (e.g., *Coquilettidia perturbans*) and human biters (e.g., *Aedes vexans*) (Nosal 2003). Amplification mosquitoes feed on birds and transmit the virus to other birds, increasing the size of the virus reservoir; while bridging and human biters feed on both birds and humans and are responsible for the transmission of WNv to humans. In Canada, 10 species of mosquitoes have been shown to be carriers of WNv, however it is more common in species such as *Culex* that feed on birds, and some infected species rarely feed on humans (HC 2004b). *Culex pipiens* have been shown to transmit the virus to their progeny, and maintain the ability to transmit WNv even after periods of inactivity such as wintering (Dohm 2002).

The Ontario Ministry of Health surveillance statistics show that there were 135 WNv positive mosquito pools identified in 2003, 56 of which were in Toronto (MOHLTC 2003a). In order to prevent and control the spread of WNv, public health strategies requiring cooperation among public health, public works, conservation area officials and elected representatives, must be undertaken.

At this time, there is no treatment, vaccine or cure for West Nile virus aside from supportive treatment of the symptoms; the only way to stem the spread of the disease is by risk reduction (HC 2004b). Researchers at the University of Alberta have developed a model to predict the risk of WNv in North America. This provides a new method of analysis to determine mosquito control levels, in order to maximize efficiency and minimize economic cost and environmental damage (Wonham 2004).

2.1.1. Integrated Pest Management

According to the U.S. Centers for Disease Control and Prevention (CDC), the key to minimizing an outbreak of WNv is surveillance, source reduction, and chemical control (CDC 2003). In Canada, the risk of WNv is managed by the

National Steering Committee, organized in February 2000 by Health Canada, including representatives from Health Canada, Provincial Ministries of Health, Conservation, Environment and Natural Resources, the Department of National Defense, Environment Canada, the Canadian Food Inspection Agency and the Canadian Cooperative Wildlife Health Centre. Their approach to WNv is an integrated pest management plan, which includes surveillance, education and prevention, and response (HC 2004b).

The American Mosquito Control Association describes the integrated mosquito management concept as "based on ecological, economic and social criteria and integrates multidisciplinary methodologies into pest management strategies that are practical and effective to protect public health and the environment and improve the quality of life" (Floore 2003). Integrated pest management programs have helped mosquito control in North America evolve from simple reliance on insecticide application (Rose, 2001). The effectiveness of mosquito habitat reduction and public education to promote personal protection is limited; therefore mosquito control measures including the use of larvicides and adulticides have continued to be an important part of a sustainable, integrated, North American response to WNv (Nosal 2003; Floore 2003, Rose 2001).

Surveillance is conducted to detect the presence of the virus as early as possible, in order to alert communities and take the necessary preventative measures. The extent and location of the virus is monitored throughout the summer season, as well as presence of mosquitoes and larvae. Surveillance activities in Canada focus on birds, horses, mosquitoes and humans (HC 2004b). The information collected helps guide municipal decisions regarding public health alerts and mosquito control activities, as well as contributing to long-term surveillance data to help refine future control strategies (Shapiro 2003).

Public education campaigns are essential to help people understand what the risks of WN and how they can protect themselves. Preventative measures include reducing mosquito habitat and breeding sites by eliminating standing water on private property; and promoting personal protection to avoid mosquito bites (Shapiro 2003). According to Shapiro (2003), "education also plays a key role in helping people understand what mosquito control is, how it works, why it is important and its potential health and environmental impacts." The last component of an integrated pest management plan is response: mosquito control. Safe and environmentally friendly chemicals are an essential part of vector and pest control (Mulla 1995).

2.1.2. Mosquitoes and Mosquito Control

Mosquitoes are insects belonging to the order Diptera; they have two scaly wings, and females have mouthparts forming a long piercing-sucking proboscis (Floore 2003). Mosquitoes can be annoying pests as well as transmit diseases to humans and animals. Only female mosquitoes require a blood meal and bite animals; *Culex* spp. prefer to attack at dusk and after dark, and prefer birds over humans and domestic animals (Floore 2003). *Culex* mosquitoes do not fly far from home, and only live a few weeks during warm summer months (Floore 2003).

The mosquito life-cycle has four separate and distinct stages, which are recognized by their special appearance: egg, larva, pupa, and adult (Floore 2003). Different species lay their eggs in different habitats, where water is a necessary feature. *Culex* species lay their eggs attached together to form an egg raft that floats on the surface of the water. *Culex* lay their eggs on the surface of stagnant water, in puddles, ditches, and catch basins and other places where water collects. The egg hatches into a larva which lives in the water and breathes at the

surface. Mosquito larvae will live in water from 4 to 14 days, depending on water temperature (Floore 2003). Larvae feed on microorganisms and organic matter in the water (Clements 1992). Eliminating breeding sites where possible and using larvicides will prevent emergence into a biting adult. A mosquito larva will grow and shed its skin in four molts, or instars, and after the fourth instar, emerges as a pupa. The mosquito rests and does not feed during the pupal stage of development, but it is mobile. When development is complete, the adult mosquito emerges from the pupal casing. A few days later, blood feeding and mating occurs (Floore 2003). Flying adult mosquitoes acquire WNv by biting infected birds, and the incubation period is approximately 2 to 3 weeks (Shapiro 2003). The length of each stage and the entire life-cycle differs from one species to another, and will vary with temperature (Floore 2003).

Controlling mosquitoes carrying WNv is part of most municipalities' mandate to protect public health (MOE 2003b). Under the Health Protection and Promotion act in Ontario, the local Medical Officer of Health (MOH) in each of Toronto's 37 health units is required to conduct a local risk assessment according to the 'West Nile Virus Preparedness and Prevention Plan for Ontario,' which determines the need for mosquito control in that jurisdiction (MOE 2003a). The MOH will decide to apply larvicide as a preventative measure if mosquito larvae are identified through surveillance and monitoring (MOE 2003b). Site-specific interventions based on knowledge of mosquito biology and local conditions include habitat modification, water management, sanitation and pesticides (Shapiro 2003).

Mosquito control pesticides are designed to act either on larvae (larvicides) or on adults (adulticides). The advantages of larvicides over adulticides are that they can be applied selectively to specific targets and can be applied in solid form, which limits human exposure from aerosolized drifting,

and can be formulated to be effective for varying lengths of time, from a few days to 150 days, reducing labour costs (Shapiro 2003). Finding and eliminating breeding sites to prevent larvae from emerging as biting adults is the most effective way of controlling mosquitoes (Floore 2003). The use of adulticides is generally reserved for a response to human WNv cases, or when a high level of human risk has been identified. There have been no adulticiding programs carried out in Ontario; however, the MOHLTC has retained licensed pesticide application companies that are trained in using ultra-low volume application equipment in case surveillance detects the need for increased mosquito control (MOE 2003a).

Resistance to certain larvicides and adulticides has occurred periodically, and cross-resistance may occur within a class of insecticide with the same mode of action. Sustained integrated mosquito control requires alternating use of different classes of insecticides, and resistance monitoring (Rose 2001).

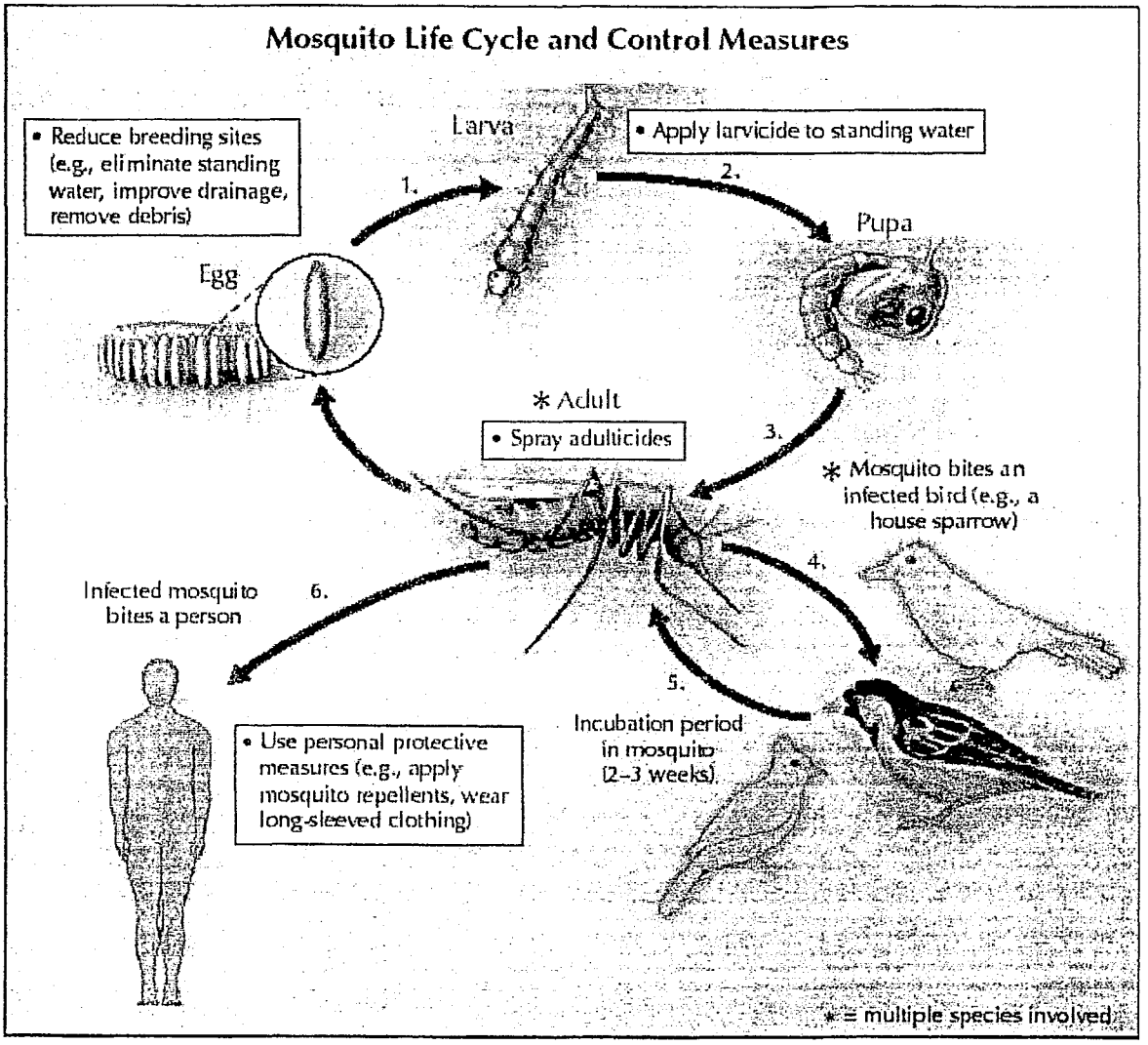

Figure 2.1 Schematic of the mosquito life cycle and control measures. (Shapiro 2003; drawing: Chesley Sheppard.)

2.1.2.1. Alternatives to Pesticides

Traditional mosquito control pesticides have contributed to an increase in vector resistance, and in turn to the spread of vector-borne disease, and were recognized in 1982 by the World Health Organization as unfeasible for long-term use (Collins 2000). Methoprene is less toxic to nontarget organisms than organophospate insecticides, but long-term effects of widespread use have yet to be established (Brown 2000; Chu 1997). Development of new public health insecticides is limited because it is expensive, regulatory approval is time consuming, and mosquito control is only a niche market compared with agricultural pest control (Rose 2001; Collins 2000). The need to control vector-borne disease may accelerate this process, especially to avoid resistance in vector populations. Efforts should be made to use the smallest amount of chemical pesticides needed as mosquito control becomes a yearly undertaking. Novel alternative approaches to chemical control, including biological control, should be developed and included in integrated pest management programs (SMCMAD 2003; Collins 2000). There are also new designs of mechanical mosquito traps available for domestic use, however the technology is expensive and some may kill other helpful insects (Rose 2001).

Biological controls include the natural presence or the introduction of indigenous predators that eat larvae and pupae, such as predacious mosquitoes (*Toxorhynchites* spp.; Collins 2000), dragonflies, birds, bats, larvivorous fish as well as fungi, protozoa and nematodes (SMCMAD 2003; Rose 2001). However, some natural mosquito predators when introduced to new habitats may also feed on other beneficial wildlife and upset the balance of a healthy ecosystem, or may not be readily available due to difficulties in rearing and storage (SMCMAD 2003; Rose 2001). In some areas of the U.S., such as California, mosquito fish are

provided by municipalities to the public for mosquito control of ornamental ponds and artificial water sources, however, the introduction of mosquito fish to natural waterways has been banned for over 15 years (SMCMAD 2003). The presence of biological controls such as birds and bats may not provide satisfactory alternative control, and aquatic predators may not be present in newly flooded areas, when water levels change, or when water quality is too poor (SMCMAD 2003; Rose 2001). The objective of employing biological controls is to decrease use and frequency of chemical controls, to reduce environmental impact and avoid development of pesticide resistance in the target mosquito (SMCMAD 2003).

Microbial agents for mosquito control exist as commercial formulations of *Bacillus sphaericus* and *Bacillus thuringiensis israelensis* (*Bti*), which are highly selective for mosquitoes, and proven to have little impact on nontarget organisms (SMCMAD 2003; Westchester 2001; Brown 2000; USEPA 2000). They are both naturally occurring bacteria that kill mosquitoes by disrupting the gut when it is ingested by the larvae (USEPA 2000). *Bti* is rapidly degraded by sunlight, and has a tendency to attach to sediment and settle out of the water column (Westchester 2001). *Bacillus sphaericus* is less likely to attach to sediment and settle out, and may have a greater sustained efficacy than *Bti*. A study comparing Altosid briquets (methoprene) and VectoLex (*Bacillus sphaericus*) found that they were both equally effective in controlling mosquitoes in catch basins, but that VectoLex was more economical and less labour intensive when applied by hand (Siegel 1999). Although not all biological controls are successful, they are an important component of an Integrated Pest Management plan. A combination of biocontrol agents, such as the planarian *Dugesia tigrina* or mosquito fish, and a chemical larvicide, such as methoprene, may offer optimal control (Nelson 1994; Mulla 1978).

2.1.3. Social and Political Climate of Mosquito Control

In the fight against WNv, public health units are faced with the challenge of balancing their mandate to protect humans from the risk of infection against the risk of human and environmental exposure to mosquito control pesticides (Shapiro 2003). Intensive media coverage has accompanied the WNv outbreak in Ontario and most of the southeastern parts of Canada, but less attention has been given to the impacts of pesticides on nontarget organisms (Wilson 2003). Some citizens are terrified of WNv and demand relief, and only careful forethought and planning using entomological expertise can provide acceptable results (Webster 2003). Provincial governments in Canada prefer a better-safe-than-sorry approach in preparing for WNv control, because they do not want to be accused of doing nothing (Sibbald 2001).

There has been concern over the use of pesticides in the fight against WNv, and many groups opposing the use of these maintain that "West Nile virus is a complex disease in the environment, and human health is directly linked to ecological health" (CCHE 2004). There is little doubt that pesticides do control mosquitoes when used according to directions, but there is doubt that the reduction in mosquito populations is significant enough to reduce human WNv infections (Shapiro 2003).

Many people are still unconvinced that the risk of WNv outweighs the health risk of pesticides (Hiscox 2003), and that substantial comparative risk-benefit analyses of the significance of disease impacts versus the human and environmental impacts of pesticides should be conducted when making regulatory decisions (Rose 2001). Anti-pesticide activists criticize the USEPA for failing to include an evaluation of the chemical breakdown products of pesticides in its risk assessment (Wilson 2003). They also claim that the true nature and threat of a pesticide is difficult for the public to understand because many of its

'inert' ingredients are kept as 'trade secrets' (Wilson 2003). Inert ingredients make up a significant portion of the material that is actually applied.

There is public concern that larvicides may have effects on untargeted beneficial aquatic wildlife (Rose 2001). Anti-pesticide groups maintain that the best defence against a vector-borne illness is a healthy ecosystem, and that public health initiatives must not compromise ecosystem health that includes a balance of mosquito predators such as birds, fish and dragonflies (Wilson 2003; CCHE 2004). However, if insecticides are used in a mosquito control program, these groups insist that it would only be scientifically responsible for public health authorities to conduct comprehensive monitoring and evaluation for effectiveness and adverse effects, using appropriate methods and distribute the findings to the public (CCHE 2004; Shapiro 2003).

Many critics of pesticide use cite past examples of inadequate testing as reason for greater caution to be used when approving pesticides, as demonstrated by the fact that almost 100 pesticides have been banned or restricted by the USEPA since their introduction (Wilson 2003). Generations may pass before the full impacts of a pesticide on public and environmental health may be detected, and its use prohibited (Wilson 2003).

Concern has been raised specifically on the use of methoprene in storm sewers (Hiscox 2003; Sibbald 2003). The Canadian Medical Association Journal reports that the New York Environmental Protection Agency, as well as Micheal D'Andrea, Toronto's manager of infrastructure asset management, are worried that water flushed from treated catch basins through the storm sewer system is washed out directly into natural waterways without being treated, where it may have adverse ecological impacts (Sibbald 2003).

2.2. Properties of the Insect Growth Regulator Methoprene

Methoprene has been favoured in recent years over other insecticides such as organophosphates due to their unfavourable environmental effects, and the development of resistance among several mosquito species (Sacher 1971; Schaefer 1972; Amin 1984; Brown 2000). Methoprene (isopropyl-11-methoxy-3,7,11-trimethyl-2-4-dodecadienoate) is sold commercially as Zoecon Altosid and manufactured by Wellmark International, in Schaumburg, Illinois (Wellmark International 2003). The primary use of methoprene is as an insect growth regulator (IGR) and belongs in the chemical group of juvenile hormone analogues (WHO 2003). The Canadian Pest Management Regulatory Agency (PMRA) classifies all pesticides that are applied directly to water, including methoprene for mosquito control as 'Restricted' (HC 2001). The Ontario Ministry of the Environment regulates the sale, use, transportation, storage and disposal of registered pesticides in Ontario under the Pesticides Act and Ontario Regulation 914. Methoprene use requires a license and a permit, and the applicator must pass examinations in order to carry out larviciding (MOE 2003).

Methoprene is considered by the U.S. Environmental Protection Agency (USEPA) as a 'least toxic' insecticide, and it states that "methoprene used in mosquito control programs according to label directions does not pose unreasonable risks to wildlife or the environment" (USEPA 2000). It has been in use commercially in the United States for mosquito control since 1974 (Mulla 1978). Health Canada describes methoprene as an insect growth regulator with a non-toxic mode of action, as it is not directly toxic but rather interferes with insect growth (HC 2001; USEPA 2001). It is indicated for use against standing water mosquitoes, including *Anopheles, Culex, Culiseta, Coquillettidia,* and *Mansonia spp.*, as well as adults of the floodwater mosquitoes, such as *Aedes* and *Psorophora spp.* from treated sites (Wellmark International 1999).

The Altosid pellet label lists permanent water and floodwater target sites including swamps, marshes, tires and other artificial holding containers, waste treatment ponds, ditches, storm drains and catch basins (Wellmark International 1999). Label directions indicate an application rate of 5.0-10.0lbs per acre (Wellmark International 1999), or 0.56-1.12 g per $1m^2$ of land area drained, which is calculated to be 0.7 g per catch basin in Toronto. The pellets sink to the bottom of the water and slowly break down, releasing the methoprene over approximately 30 days (MOE 2003b). The specific gravity of methoprene liquid is near to that of water, and tends to stay near the surface when in suspension, and therefore, according to the San Mateo County Mosquito Abatement District, no adjustment to the application rate is necessary for varying depths of water when treating larvae which live and breathe at the surface (SMCMAD 2003).

The formulation approved for use in Ontario and which is currently in use in the GTA is the pellet. These pellets contain approximately 4.25% per weight active ingredient, and 95.75% inert binders made of plaster and charcoal (Wellmark International 2003; SMCMAD 2003). After application to water, methoprene will degrade to several metabolites, including: photoisomers, methoprene acid, methoprene epoxide, 7-methoxycitronellal, and 7-methoxycitronellic acid (Degitz 2003; La Clair 1998). Ideally, pesticide safety should be evaluated in relation to its inert ingredients, breakdown products, and synergistic effects (SMCMAD 2003).

2.2.1. Efficacy

The effective level of methoprene concentration in mosquito habitat is considered to be 0.5-3.0 μg/L (SMCMAD 2003) and the lethal concentration (LC_{50}) has been reported as 0.3 to 2.3 μg/L for *Culex* (Amin 1984; Ali 1995). However, Schaefer (1973) found no clear-cut relationship between percent mortality and

concentration, and many studies have found residual concentrations effective (Floore 1991; Knepper 1992). When exposed to the chemical, the larvae seem to develop normally through their larval instars, but natural maturation through metamorphosis is interrupted and they die as pre-pupa (Sacher 1971). Recent laboratory studies of the effects of methoprene on two members of the *Culex pipiens* complex observed a prolongation of the intermolt period in each larval instar and in the pupal stage, and morphological changes in the larval-pupal and pupal-adult transformations (Gelbic 2002). Late or fourth-instar mosquito larvae are most sensitive (Sacher 1971; Schaefer 1972).

Schaefer (1972) found that *Aedes nigromaculis* larvae were controlled effectively in the lab with methoprene concentrations of 0.01 μg/L, and showed promising results in the field with 0.125lb/acre (14mg/m^2). *Aedes albopictus* were controlled by 2.2 μg/L methoprene in the lab (Ali 1995). An LC_{50} for *Culex quinquefasciatus* was achieved with 0.05 mg/L (50 μg/L) methoprene in the lab, and 90% or better emergence inhibition was obtained in the field with Altosid pellets at rates of 5.4 mg/m^2 active ingredient (A.I.) for flood water sites, 28 mg/m^2 A.I. for clean water and greater than 28 mg/m^2 A.I. for water with high organic content (Mulla 1995). This study concluded that controlled-release formulations of methoprene, such as pellets, provide excellent control of *Culex* broods (Mulla 1995). The Metropolitan Mosquito Control District in Minnesota has had success with Altosid pellets, with emergence inhibition at 87% during the first 30 days after application, and found that pellets maintained efficiency beyond the 30-day expected field life (MMCD 2001).

In stagnant freshwater test plots, Floore (1991) found that Altosid pellets applied at label rates achieved 92% emergence inhibition for 29 days, and continued to control up to 74% even at 41 days after application. In a field study examining efficacy of Altosid against *Culex pipiens* and *Culex restuans* larvae in

highly organic water conditions, 90-day briquets produced a 99% emergence inhibition in buckets and 70% inhibition in 20 urban catch basins over 107 days (Knepper 1992). The difference in control efficacy was attributed to the dynamic nature of the catch basin system, which experiences flooding and flushing during rainfalls (Knepper 1992). The slow-release formulation proved to release methoprene over the entire 15 weeks of the study and therefore methoprene remained in the system (Knepper 1992). The study focused on the efficacy of mosquito control, but did not examine the environmental fate or the concentration of methoprene over time.

Altosid briquet efficiency was evaluated in Illinois catch basins, and was found to be effective against *Culex* spp. in catch basins for one month, despite varying physical capacities and water volume of the catch basins (Siegel 1999). This study validated the label application rate of one briquette per 9.29 m^2 or 285 litres of water for 30 days (Siegel 1999). A field study in Michigan catch basins attained 82% emergence inhibition with Altosid pellets applied at a rate of 7 g per catch basin for *Culex pipiens* and *Cx. restuans* during a 15 week trial (McCarry 1996). Methoprene liquid larvicide was proven to be effective at 0.17 μg/L (LC_{90}) in controlling an important disease vector in Australia, *Aedes vigilax*, however it was found to be almost ineffective for two nuisance species, *Cx. sitiens* and *Cx. annulirostris* (Ritchie 1997). These studies show a recent interest in evaluating methoprene in different environments for public health uses. They outline its effectiveness, but do not investigate the environmental fate of the larvicide.

The granular formulation of methoprene, Altosid XR-G, was tested in the field and in the lab against several mosquito species including *Cx. nigripalpus, Ae. albopictus, Ae. taeniorhynchus*, and only in the lab on *Cx. quinquefasciatus* in Florida (Nayar 2002). The granular formulation was applied at 0.2mg/L and 0.4 mg/L for *Cx. quinquefasciatus* and at 0.02 mg/L and 0.05 mg/L for all other species, and gave

varying levels of emergence inhibition, with *Ae. taeniorhynchus* being most sensitive, and *Cx. quinquefasciatus* and *Ae. albopictus* most tolerant (Nayar 2002). Emergence inhibition remained high for 1-3 weeks in the lab for the different species, and 3-4 weeks outdoors in plastic tubs (Nayar 2002). Higher doses of methoprene did not result in higher sustained levels of efficacy for *Cx. quinquefasciatus* (Nayar 2002). The researchers compared the efficacy of methoprene to a new insect growth regulator, pyriproxifen, which proved to be more effective at inducing complete emergence inhibition for several weeks at lower doses, and to be less active against aquatic nontarget organisms (Nayar 2002). The results of the study support the World Health Organization Pesticide Evaluation Scheme's recent recommendations of the use of pyriproxifen for mosquito control at specified rates in certain habitats (Nayar 2002).

Mosquitoes were tested for resistance to methoprene over several generations and it was found that there is a possibility that resistance may develop in the field if intensive selection pressure is applied, but there was no cross-resistance observed from organophosphate resistant species (Amin 1984). Delayed sub-lethal effects in *Culex pipiens*, including reproductive failure were observed (Amin 1984). Some mosquito control authorities have expressed concern over the development of resistance to methoprene among mosquito species and advocate resistance management, and avoidance of sub-lethal dosages below the lower end of label application rates which may encourage resistance and may allow weakly resistant insects to produce resistant offspring (SMCMAD 2003).

Additional beneficial effects from methoprene treatment were observed in other trials, such as morphogenetic aberrations, reduced survival and bloodfeeding success, and a decline in reproduction and fecundity of mosquitoes that survived treatment (Mulla 1995; Ritchie 1997). This effect could delay the

evolution of methoprene resistance in the field, explaining the lack of observations of IGR resistance in wild populations (Amin 1984). Studies in Florida, where methoprene has been used for mosquito control for over two decades, show that lab strains and field strains exhibited the same susceptibility to the larvicide, indicating no development of resistance by populations of *Ae. albopictus* and *Cx. nigripalpus* (Nayar 2002).

2.2.2. Environmental Persistence

Health Canada states that methoprene is not persistent in the environment, and degrades rapidly in water; being susceptible to transformation by sunlight and microorganisms (HC 2001). Methoprene is not considered an oncogenic, teratogenic, or mutagenic compound (Wellmark International 2003), and is not known to bioaccumulate in animals that feed on mosquito and midge larvae treated with methoprene (SMCMAD 2003; WHO 2003). The Altosid material safety data sheet (MSDS) reports environmental fate half-life values for methoprene of greater than four weeks in water, less than 10 hours by photolysis, and approximately 10 days in soil (Wellmark International 2003). It is metabolized rapidly in soil, does not leach and is thus not expected to contaminate groundwater (USEPA 2001). Its solubility is reported as less than 2 mg/L (Wellmark International 2003).

Several formulations have been developed to slowly release methoprene at effective mosquito control levels over certain different periods of time: Altosid Liquid Larvicide, Altosid Briquets, Altosid XR Briquets, Altosid Pellets, and Altosid XR-G. At maximum label application rates for pellets, the sustained release of methoprene means that the actual concentration in standing water never exceeds a few micrograms per litre (SMCMAD 2003).

Degradation of methoprene is increased with sunlight, temperature, salinity and microbial action (Schaefer 1973; Westchester 2001). Microbial degradation in aquatic systems accelerates the breakdown of methoprene, reaching 80% degradation within 13 days in non-sterile pond water (Westchester 2001). Early field trials on methoprene found that "the compound appears to have a relatively low order of environmental persistence and a potential for minimal environmental contamination" (Sacher 1971). Field tests indicate that methoprene applied in the pure technical liquid formulation to prairie pools was undetectable after 24-48 hours (Schaefer 1973). Boxmeyer (1997) reported that the degradation of Altosid 150-day briquets under field conditions was influenced by the number of days submerged and exposure to sunlight, and that leftover mass of Altosid briquets did not contain methoprene concentrations of environmental concern.

Methoprene has a short environmental persistence, even for sustained-release formulations (Ross 1994). Concentrations of methoprene in freshwater microcosms, a small representative system of natural mosquito habitats, treated with several different Altosid sustained-release formulations were monitored to determine if the effective environmental concentration of methoprene would exceed 10 μg/L (Ross 1994). Methoprene concentration in the pellet-treated microcosms peaked at 2.0 μg/L on day 7 after application and then declined below detection limits (0.2 μg/L) after day 14, during a 35-day study (Ross 1994). No sample collected during the study contained concentrations higher than 6 μg/L. Laboratory and field tests have shown that methoprene formulations have a maximal rate of release of less than 4 μg/L, which is much lower than the USEPA recommended safe concentrations for organisms, which include a margin of over 200-fold (USEPA 2001). Therefore, exposure to methoprene for aquatic organisms is not anticipated to reach toxic levels.

2.2.3. Effects on Nontarget Species

In Canada, the Pest Management Regulatory Agency registers pesticides and only approves products that have been scientifically reviewed and found to be effective and safe for use with minimal risk to human health and the environment (HC 2001). Rates of application of pesticides for mosquito control are generally low, and the training required for licensed applicators reduces the risk to non-targeted organisms (Rose 2001). The application rate is selected from a range of doses; it must be sufficiently high to be efficient for mosquito control, yet sufficiently low to avoid nontarget impacts (SMCMAD 2003; Hershey 1998). The mode of action of methoprene, the interruption of insect metamorphosis, has no parallel process in mammals and is considered non-toxic (WHO 2003). It has been shown through toxicological assays to have no toxic or reproductive, carcinogenic, mutagenic, treratogenic, neurotoxic, endocrine, skin irritant or sensitizing adverse effects (WHOO 2003).

A comprehensive review of available scientific literature conducted by the Westchester County Board of Health in New York State is summarized in table 2.1. It was found that the most sensitive organisms are invertebrates; freshwater, marine, and estuarine, which are highly sensitive to acute doses of methoprene. Invertebrates represent over 95% of all animals, are omnipresent, and are significantly important ecologically and economically (USEPA 1998). There are few effects demonstrated on birds and mammals, and methoprene was only shown to be moderately toxic to warmwater fish such as the bluegill sunfish, and slightly toxic to cold water fish such as the rainbow trout (Westchester 2001). The most sensitive invertebrate tested in laboratory was *Daphnia,* which lost reproductive ability at 5 to 10 μg/L, which is 5-10 times higher than the expected environmental concentration for mosquito control (Westchester 2001).

Table 2.1 Review of laboratory derived toxicity values for methoprene on several nontarget organisms. (Westchester 2001)

Organism Tested	*Type of Test*	*Toxicity*
Mammals		
Human	dermal (sensitivity)	no positive response
Mice	chronic oral	brown liver pigmentation with 18-month diet of 1,000, and 2,500 ppm; no effect with 250 ppm; 18-month NOEL = 250 ppm
	developmental	no effect, materanal toxicity, fetotoxicity or teratogenicity; NOEL = 600 mg/kg/day
Rats	acute oral	LD50= 34,600 mg/kg (maximum contained in rat gut)
	subchronic oral	no effect, 90-days with 250, 500, and 1,000 ppm; increased liver weight with 5,000 ppm; 90-day NOEL = 500 ppm; LOEL = 1,000 ppm
	subchronic inhalation	21-day inhalation NOEL = 20 mg/l (highest dose tested)
	chronic oral	no effect, 2-year diet of 250, 1,000, and 5,000 ppm (86.9% acitve ingredient)
	chronic oral	2-year NOEL = 5,000 ppm (highest dose tested)
	oncogenicity	18-month oncogenicity NOEL = 250 ppm
	inhalation	no effect, 2,000 ppm; LC50> 210 mg/l
	reproduction	no effect, three generation study with 2,500 ppm
	teratology study	no effect, 1,000 mg/kg
Rabbits	acute dermal	LD50> 3,000-10,000 mg/kg
	dermal	no effect, 24-hour 0.5 ml (technical grade) on shaved, abraded, and unabraded
	subchronic dermal	no effect, 21-day exposure of shaved skin to 100, 300, 900, and 2,700 mg/kg/day; 21-day NOEL = 100 mg/kg body weight
	eye	no effect, 0.1 ml (technical grade)
	developmental	materanal toxicity and embryolethality

		(in utero) LOEL = 2,000 mg/kg/day; NOEL = 200 mg/kg/day
	teratology study	no effect, 1,000 mg/kg
Guinea Pig	dermal (sensitivity)	positive test for intradermal injection of undiluted methoprene; no effect topically
	inhalation	LC50> 210 mg/l
Dogs	acute oral	LD50= 5,000 - 10,000 mg/kg
	subchronic oral	no effect, 90-days with 250, 500, and 1,000 ppm; increased liver weight with 5,000 ppm; 90-day NOEL = 500 ppm; LOEL = 5,000 ppm
Birds		
Mallard Ducks	acute oral	LD50> 2,000 mg/kg
	subacute oral	LD50>10,000 ppm reproductive impairment with 30 ppm; no effect, 3 ppm
Bobwhite Quail	chronic oral	8-day dietary LC50 > 10,000 ppm
	reproduction	no effect, 30 ppm
Chickens	chronic oral	no weight loss with 0.005 and 0.01% methoprene food
Freshwater Fish		
Bluegill Sunfish	chronic exposure	96-hour LC50 = 1.52 ppm
	bioaccumulation	edible portions concentrated 550 and 950x ambient concentrations, residue excreted within 14 days of non-exposure
	exposure	LD50 = 4.62 ppm
Rainbow Trout	chronic exposure	96-hour LC50 > 52 ppm
	exposure	LC50 = 3.3 mg/l
	chronic exposure	96-hour LC50 = 106 mg/l
Channel Catfish	exposure	LC50 > 100 mg/l
Mummichog	exposure	96-hour LC50 = 124.95, NOEL = 24.68 mg/l
Mosquito Fish	exposure	no effect, 240-hour exposure with 1.0 ppm (fry)
Goldfish	exposure	no effect on locomotor activity with exposure to 0.2 ppm
Estuarine/Marine		
Pacific Blue-eye	exposure	96-hour LC50 > 4 ppm

Fathead Minnow	exposure	37-day NOEC = 48 ug/l and LOEC = 84 ug/l
Coho Salmon	exposure	96-hour LC50 = 876 mg/l
	exposure	LD50 = 32 ppm
Silverside	exposure	48-hour LC50 = 2.78 ppm
Freshwater Invertebrates		
Crustaceans		
Cladocera	exposure	48-hour LC50 = 0.0015 ppm
	chronic exposure	48-hour LC50 (technical methoprene) = 89 ppb
	chronic exposure	42-day maximum acceptable tolerance limit = 27 - 51 ppb
	exposure	24-hour LC50 = 0.51 mg/l; 48-hour LC50 = 0.34 mg/l
Copepods	exposure	144-hour ~10% mortality with 0.1 ppm
	exposure	72-houtr LC50 = 1-2.0 ppm (egg); 48-hour LC50 = 0.8 ppm (early larval); 48-hour = 5 ppm (late larval); 48-hour = 10 ppm (adult)
	exposure	24-hour LD50 = 0.67 ppm
Amphipod	exposure	96-hour adult (female) LC50 = 2.15 ppm; LC90 = 4.10 ppm; 96-hour adult (male) LC50 = 1.95 ppm; LC90 = 7.8 ppm; 24-hour young LC50 = 0.32 ppm; LC90 = 1.05 ppm
	exposure	no effect, 100 mg/L
	bioaccumulation	accumulated 66x ambient water and equal soil conc. of aged, radiolabelled residue
Crayfish	exposure	LD50 = 100 ppm
Mud Crab	exposure	arrested development of larvae with exposure to 1 ppm; no effect, exposure to 0.1 ppm
	exposure	no sublethal effect noted
Shrimp		
Freshwater Shrimp	exposure	LD50 = 100 ppm
	exposure	no effect on presence, density, or size with 150-day 1.5 ppb exposure

	exposure	LC50 = 14.32 ppm
White Shrimp	exposure	LD50 = 100 ppm
Pink Shrimp	exposure	LD50 = 100 ppm
Clam Shrimp	exposure	48-hour LC50 = 0.00015 ppm
Tadpole Shrimp	exposure	24-hour 40% mortality with 0.00075 ppm
Seed Shrimp	exposure	72-hour ~2% mortality with 0.5 ppm, 168-hour ~90% mortality with 0.01 ppm (nymphs)
Insects		
Mayfly	exposure	no effect, 1,000 ppm exposure
Honey Bees	exposure	65.5% formulation eliminated brood production; 10% mortality, 24-hour exposure to oral and topical combination with 1000 ug/insect; 168-hour >50% mortality with 0.05 ppm (nymphs)
Dragonfly	exposure	168-hour >50% mortality with 0.05 ppm (nymphs)
Beetle	exposure	no effect, 168-hour with 0.25 ppm (adults)
	exposure	no effect, 216-hour with 0.25 ppm (adults)
	exposure	48-hour ~30% mortality with 0.1 ppm (larvae)
	exposure	no effect, 48-hour with 0.25 ppm (adults)
Aquatic Midges	exposure	168-hour ~90% mortality with 0.01 ppm
Backswimmer	exposure	72-hour ~30% mortality with 0.01 ppm
Other		
Polychaete	exposure	no effect, 100 mg/l
Gastropoda	exposure	no effect regardless of concentration
Rotifer	exposure	no effefct, 224 g active ingredient/ha within experimental ponds
Estuarine/Marine Invertebrates		
Estuarian Mysid	exposure	100% mortality with 4-day exposure at 125 ug/l; decreased weight with rearing at 62 ug/l;reduced number of offspring per group with rearing in >8 ug/l; reduced number of offspring per female with rearing in >2 ug/l
Grass Shrimp	exposure	failure to complete metamorphosis with

		continuous exposure to 1,000 ug/l; reduction in metamorphosis completion with 100 ug/l exposure (R,S,)-methoprene
	exposure	no effect, exposure to 10x recommended dose of 0.02 ppm a.i. (Altosid SR-10)
Atlantic Oysters	exposure	48-hour TL50 = 0.269 mg/l (89.6% active ingredient) for normal embryonic development
Molluscs	exposure	48-hour LC50 = 10.6 ppm
	exposure	48-hour LC50 = 0.3 ppm
Amphibians		
Fowler's toad	acute exposure	LC50 for adult greater than 1.0 ppm
Bullfrog	acute exposure	LC50 for larvae greater than 10 ppm
Northern Leopard Frog	acute exposure	LC50 for larvae greater than 10 ppm
Fowler's toad	chronic exposure	22-day LC50 greater than 1.0 ppm
Bullfrog	chronic exposure	22-day LC50 greater than 1.0 ppm
Northern Leopard Frog	chronic exposure	22-day LC50 greater than 1.0 ppm

The fate and transport of methoprene in sustained-release pellet formulation from the stormwater sewer system to watersheds in Canada is largely unknown. A 2000 study examined the toxic contributions of 7 mosquito control pesticides in urban stormwater runoff. While the investigators did not specifically examine methoprene, they did conclude that at current application rates, these chemicals could affect nontarget organisms and confound stormwater and nonpoint toxicity evaluations (Milam 2000). The study examined the toxicity of urban stormwater runoff combined with mosquito control pesticides, but they did not look at the fate and transport of the chemicals from catch basins to receiving waters. The researchers advised that industries and municipalities preparing pollution prevention plans related to stormwater runoff should be knowledgeable about local mosquito control application schedules because runoff after a storm event could contain insecticides

overlooked as potential contributors to cumulative toxicity (Milam 2000). Degradation rates for liquid larvicides are faster than for solid formulations, which are likely to have greater long-term impacts (Pinkney, 2000).

Methoprene has long been shown to be highly selective for mosquito larvae, while non-target organisms, including well-known mosquito predators, exhibited high tolerance to the larvicide (Mulla 1979; Miura 1973). In general, this compound is biologically active against mature mosquito larvae; therefore it is effective against some related species such as chironomids (Mulla 1979; Ali 1991). Methoprene pellets have been proven as an effective control against nuisance midges in man-made and polluted waters, at an application rate of 5.6 kg/ha, the same rate as indicated by the Altosid label for mosquito control (0.56 g/m^2 – 1.11 g/m^2; Ali 1991). The U.S. Fish and Wildlife service is concerned that nontarget insect population reductions due to mosquito abatement may affect waterfowl, which depend on aquatic insects for food (Pinkney 2000).

Acute toxicity tests for methoprene on 35 aquatic organisms including Protozoa, Platyhelminths, Rotatoria, Annelida, Arthropoda, Mollusca, Chordata and Thallophyta, at 250 to 1000 times the recommended application rates, found that few adverse effects were observed, except for some sensitivity in aquatic Diptera, Cladocerans and Copepods (Miura 1973). In a field study examining the effects of methoprene on an aquatic ecosystem, Norland (1974) found a reduced abundance of several arthropod prey and predator species, including elimination of a major predator, the larval dytiscid beetle, which had also been identified as an organism sensitive to methoprene (WHO 2003; Steelman 1972). However, a 1999 Maryland study showed no significant reductions in insect populations due to spraying of Altosid Liquid Larvicide (Pinkney 2000). Populations of mosquito predators Odonates seem unaffected by methoprene treatments (Norland 1974; Pinkney 2000).

Miura (1973) reported ecosystem toxicity values from 900 µg/L to 5000 µg/L for methoprene, while a study by Ross (1994) reported that concentrations in microcosms treated with methoprene in several formulations never exceeded 6 µg/L. A short-term field study, in the spring and summer of 1989, found that there were no effects on nontarget benthic invertebrate biomass, density, or richness due to larvicide treatment with methoprene slow-release briquets in Minnesota wetlands (Hershey 1994). However, in a 6-year field study on the use of methoprene in granular formulation for mosquito control in wetlands in Minnesota including a 3-year pre-treatment monitoring period, Niemi (1999) found no effect on birds or zooplankton, but did observe a reduction in total insect densities and biomass, across various taxa. The researchers asserted that applications of methoprene can alter the structure and function of wetlands and that "it is unclear what the long-term consequences of insect reductions mean to wetland health" (Niemi 1999). Short-term studies have shown that nontarget insect populations affected by methoprene recover quickly once treatment is ceased (WHO 2003; Mulla 1979). Mulla (1979) states that the magnitude and extent of nontarget effects related to methoprene application depend on rates of application, the number of applications, and the type of habitat treated.

A USEPA review of research conducted in the early 1990s including the Estuarine Invertebrate Life cycle Study and the Octanol/Water Partition Coefficient Study, confirmed that methoprene is of low toxicity and poses little risk to humans and nontarget species (USEPA 2001). Until 1996, the USEPA had required that labels for solid Altosid products warn 'do not apply to known fish habitats,' and 'this product is toxic to aquatic dipteran (mosquitoes) and chironomid (midge) larvae' (USEPA 2001). The Altosid MSDS reports ecotoxicity values of methoprene for fish (bluegill trout) at 760 µg/L (LC_{50}) and at 360 µg/L (LC_{50}) for aquatic invertebrates (*Daphnia*) (Wellmark International 2003).

Although methoprene toxicity to fish is low, levels in excess of 10 μg/l could have detrimental effects on nontarget invertebrates such as *Daphnia*, which are an important source of food for fish and invertebrate predators, and contribute to healthy aquatic food webs and water quality as consumers of algae (Peterson 2001; Ross 1994; Hershey 1994).

A similar freshwater crustacean, *Moina macrocopa*, was found to be adversely affected by concentrations of methoprene greater than 0.05 mg/L (50 μg/L), but interestingly, at concentrations lower than 0.005 mg/L (5 μg/L) longevity and fecundity increased (Chu 1997). A recent study suggests that *Daphnia magna* may even exhibit sub-lethal adverse effects to methoprene exposure at concentrations lower than 0.2 nM (0.062 μg/L; Olmstead 2001). This means that *Daphnia* and other crustaceans might be affected by methoprene concentrations much lower than expected environmental concentrations from standard application rates, but exactly what the impacts at the community and ecosystem levels would be have not been determined (Olmstead 2001; Chu 1997).

The Australian atyid shrimp (*Caradina indistincta*), was used to compare ecosystem toxicity of 2 organophosphate mosquito insecticides, methoprene and *Bacillus thuringensis israelensis*, in freshwater marshes where presence of an arbovirus vector, *Culex annulirostris*, was being controlled (Brown 2000). The organophosphates, temephos and pirimiphos-methyl were found to be environmentally unsuitable, but methoprene and *B.t.i.* were found to effectively control mosquitoes and were safest for the nontarget shrimp, which are an important food source for native fish (Brown 2000). The lethal dose for the shrimp was found to be 550 times the expected environmental concentration of methoprene, and was second to *B.t.i.* in selectivity to the mosquito larvae (Brown 2000).

Sustained-release Altosid pellets applied according to label rates were found to control mosquitoes in a salt marsh, while having no adverse effects on other aquatic insects (Lawler 2000). However, the researchers agreed with earlier studies that warned that the use of sustained-release formulations must be monitored for the development of resistance in mosquitoes and initially undetected nontarget effects that could accumulate over time during from mosquito control (Lawler 2000; Brown 2000; Pinkney 2000; Niemi 1999; Hershey 1998). A 3-year study in Minnesota wetlands found that larvicide treatment significantly reduced the insect population, the richness of genera and increased the tendency to have one or a few genera dominate (Hershey 1998). The reduction in populations of insects, including predatory insects, may be due to the methoprene toxicity, or food web effects, or both (Hershey 1998). These effects were not observed until the 2nd and 3rd year of treatment, which demonstrates the need for long-term studies of ecosystem effects related to methoprene application (Hershey 1998).

In the interests of integrated pest management programs, adverse effects to the environment must be minimized, especially to natural predators of mosquitoes. Larvivorous fish such as the Australian Crimson-Spotted Rainbowfish (*Melanotaenia duboulayi*), and the mosquito fish (*Gambusia affinis*) in North America, can be used to complement conventional insecticide applications, however juveniles may be sensitive to these toxicants (Brown 2002; Milam 2000; Miura 1973). In a study on pulse-exposure effects of methoprene on juvenile and adult *Melanotaenia duboulayi*, it was found to have no acute toxic effects at 12.5 times the estimated environmental concentration (100 μg/l) (Brown 2002). The susceptibility of the planarian *Dugesia tigrina*, another potential biocontrol agent, to methoprene was evaluated in a 7-week field study in Mississipi (Nelson 1994). This mosquito larva predator was found to be

unaffected by methoprene, and the larvicide may have even had a stimulating effect on its asexual reproductive potential (Nelson 1994). Even though they do not emerge as adults, mosquito and midge larvae are not removed from the food chain by methoprene, and bioaccumulation of this larvicide has not been demonstrated in larvivorous animals (SMCMAD 2003).

Further concern has been raised regarding the degradation products of methoprene, and their effect on the environment. Many scientists have attributed fish and frog deformities to the methoprene metabolite methoprene acid, which binds to retinoic acid receptors (Degitz 2003; Sea Technology 1999; La Clair 1998; Kleiner 1997; Harmon 1995) although these results have been contested (Kaiser 1997; Lindahl 1998) and similar deformations were observed in *Rana pipiens* from exposure to ultraviolet light only (Ankley 1998). Recent studies confirm that methoprene itself is not toxic to *Xenopus laevis*, but that high concentrations of some degradation products such as methoprene acid (1.25 mg/l), methoprene epoxide, and 7-methoxycitronellal (>2.5 mg/l) can cause developmental toxicity to *X. laevis* (Degitz 2003). However, the researchers did not consider these chemicals as potent development toxicants to *X. laevis* because field applications of sustained-release formulations of methoprene would result in methoprene concentrations that would not typically exceed 0.01 mg/l (Degitz 2003). Studies with *Rana pipiens*, found that developmental effects related to methoprene were observed at lower concentrations of 0.5 mg/l (Ankley 1998). These differences could be attributed to experimental design or species sensitivity. The study did not test for any degradation products; therefore, given the aqueous instability of methoprene, the observed toxicity could have been the result of exposure to a metabolite of methoprene, rather than the parent material (Degitz 2003).

Methoprene acid has been found to bind to retinoid X receptors in mammal as well as insect cells, which stimulates gene transcription in vertebrates (Harmon 1995). Thus a metabolite of a chemical designed to mimic a juvenile hormone in insects can also affect mammals, and has been shown to have teratogenic effects on mice (Harmon 1995). This finding suggests that the potential bioactivity of a pesticide in the environment may have to be reexamined (Harmon 1995).

Few environmental protection agencies require comprehensive assessments of degradation products of a material added to the environment, yet many studies have found that methoprene degrades quickly into metabolites that may be more environmentally harmful than the original compound (La Clair 1998). Environmental concentrations of methoprene at rates significantly higher than allowed by the label most probably have the potential for adverse impacts in aquatic ecosystems (SMCMAD 2003). Mulla (1995) claims that insect growth regulators such as methoprene "have been successfully and safely used to date without any noticeable impact on nontargets and there are indications that this pattern of use will continue into the future." The possibility remains that methoprene and its metabolites may have unknown effects downstream from where it is applied, however, this is outside the scope of this field study. Uncertainties exist about the toxicity or teratogenicity of methoprene, therefore the investigation of the environmental dispersal of the larvicide continues even with the possibility that findings of negative bioactivity effects may be confirmed. It is anticipated that this research will contribute to a greater general understanding of the fate and transport of methoprene itself when applied to urban catch basins.

2.3. Provincial Water Quality Objectives

The province of Ontario sets numerical values, provincial water quality objectives (PWQOs), for satisfactory levels of contaminants in surface waters, for long-term protection of aquatic life and all aspects of the aquatic life cycles during indefinite exposure to the water (MOE 1994). These values are based on the best available scientific information, and are used to guide water quality management decisions as well as assess water quality and monitor the effects of waste effluent (MOE 1994). Scientific literature is reviewed for information on aquatic toxicity, bioaccumulation, and mutagenicity for each chemical. The final value is based on the lowest effect concentration reported, with an added safety factor (MOE 1994). When insufficient data are available to set a PWQO, standard protocol is followed to set an interim PWQO (IPWQO). Biological indicators may be a more direct measure of ecosystem health, and the MOE may identify informal 'benchmarks' in addition to IPWQOs, which may impact specific important ecological receptors (Hall 2004).

As part of this monitoring study, the MOE is currently developing a PWQO for methoprene with which to assess the impacts of catch-basin effluent (CoT 2003). The effect of methoprene on target organisms, mosquito larvae, was excluded from the analysis of ecosystem impacts (Hall 2004). An interim IPWQO was developed using available data from acute and chronic toxicity test results for several organisms, including the water flea, *Daphnia magna*; frogs, *Xenopus laevis* and *Rana pipiens*; as well as fish including the fathead minnow, *Pimephales promelas* (Hall 2004). A safety factor of 10 times the lowest reported effect concentration resulted in a proposed IPWQO of 0.2 μg/L to insure long-term protection of all nontarget species (Hall 2004).

Table 2.2 Summary of benchmarks adapted from Hall, 2004. Summary of benchmarks for environmental quality.

Organism	*Benchmark*
Fish	Acute: 1.6 mg/L
	Chronic: 0.084 mg/L
Invertebrates	Acute: 0.3 mg/L
	Chronic: 0.01 mg/L
Amphibians	Acute: 2.55 mg/L
	Chronic (Teratogenesis): 0.0016 mg/L
IPWQO	0.0002 mg/L

The predicted concentrations of 2-4 μg/L in the storm sewer system by the MOE compared to the IPWQO and the environmental benchmarks seem unlikely to cause adverse impacts on receiving aquatic ecosystems (Hall 2004). Mosquito control applications of methoprene in Ontario will occur in the summer and early fall, therefore methoprene will likely only be released to receiving waters during rain events. Rainfall will dilute methoprene concentrations, and storm sewer output concentrations will again be diluted when added to natural waters. Storm water discharges during rainfall events will probably last no more than a few days, but may result in an elevated output mass of methoprene, however, increased discharge would increase dilution. These concentrations are not expected to reach levels above the IPWQO or environmental benchmarks, however if they do occur for periods of time greater than a few months, ecological impacts may occur (Hall 2004). Continued monitoring of the larviciding program is essential to ensure protection of ecological functions.

2.3.1. Related Studies – South Etobicoke, Humber River and Toxicity

The Newtonbrook creek catch basin field study was part of a larger WNv monitoring study invclving the City of Toronto, the MOE, the University of Western Ontario, Peel Region, Halton Region, Environment Canada and local

conservation authorities. Related water quality monitoring studies in Toronto examined methoprene concentrations in the Humber River watershed and at storm sewer outfall sites in South Etobicoke. The Humber River was sampled at the intersection of the river and Steeles Avenue, and at the mouth of the river at Old Mill. The storm sewer outfall in South Etobicoke was located on the Humber River along the Kingsway just north of Bloor Street West. Water samples were collected from these sites in a similar manner to those from the Newtonbrook site. Samples from these sites were also analyzed at the same laboratory.

Aquatic toxicity bioassays on Rainbow trout and *Daphnia magna*, of the outfall water pre-dosage and post-dosage were conducted by the MOE. Methoprene efficacy studies were also carried out by the MOE and the CoT for the Toronto region (TPH 2004). The efficacy of methoprene on the larvae collected as part of this study was assessed at the MOE laboratory (Baker 2004). 48h toxicity experiment data for malathion reported an LC_{50} value of 1 μg/L for *Aedes quadrimaculatus* larvae, 1.23 mg/L for *Gambusia affinis* (Milam 2000).

The larvae were separated into groups by instar (one through four), and pupae. If there were sufficient larvae in the sample, ten individuals from each group were reared and evaluated. The groups were reared in 500 ml polyethylene terephthalate (PET) bottles, with 10 ml of dechlorinated, aerated water and fed NUTRAMIN® food (Baker 2004). Pupae were not fed. Mesh screens were fixed to the bottles using elastic bands. The bottles were placed in an incubator at 24°C, with a 16/8 light to dark ratio. Survival status, growth, pupal development and adult emergence were recorded daily. Larvae were considered alive if they moved within five minutes of observation, or with gentle agitation of the container (Baker 2004).

Pupae were considered alive if they moved in response to gentle agitation of their container. Pupae that did not respond were observed for up to three days afterward to assess survival. Adult emergence was considered as complete separation from the pupal casing and the ability to fly (Baker 2004).

Emerged adult mosquitoes were placed in a freezer for 30 minutes, then removed from the bottle using forceps and placed in small glass vials before mounting (Baker 2004). Adult mosquitoes were pinned using No. 002 pins and labeled with their locality, date and collector (Baker 2004). Adults were later subject to species identification and sex determination using a key by Wood, Dang and Ellis (1979).

The City of Toronto monitored water quality at storm sewer catchment outfalls in South Etobicoke. Methoprene levels above the minimum detection limit of 0.030 μg/L were not detected in water sampled from the outfalls during dry weather. The maximum methoprene concentration found during a wet weather event was 0.24 μg/L, and 14% of samples had concentrations greater than 0.10 μg/L (Gris 2003). Most of the samples in which concentrations were above 0.10 μg/L were collected during light or short duration rain events, approximately within one week of larviciding (Gris 2003). In comparison with literature values for ecotoxicity, the maximum detected methoprene concentration at the outfall would have to be exceeded by two to three orders of magnitude to effect adverse impacts on downstream biota (Gris 2003). These concentrations reflect only toxicity of the primary product, methoprene, and none of the secondary metabolites.

Methoprene was not detected in any of the 107 wet-weather samples taken from the Humber River monitoring sites in levels above the detection limit of 0.030 μg/L (Gris 2003). Malathion was detected in the Humber River at Steeles Avenue at 0.11 μg/L (Gris 2003). In a 90h toxicity test *Aedes albopictus* larvae were

considered tolerant to 1.043 mg/L malathion (Ali 1995). The 24 and 48 h LC_{50} values for freshwater cladoceran *Moina macrocopa* were in the range of 5-10 μg/L malathion (Chu 1997).

Aquatic toxicity bioassays on Rainbow trout (96 hour acute lethality test, single concentration) and *Daphnia magna,* (48 hour acute lethality test, single concentration) of the outfall water pre-dosage and post-dosage were conducted by the MOE (Table 2.3).

Table 2.3 Results of aquatic toxicity bioassays for the methoprene water quality monitoring from storm sewer outfalls.

	Newtonbrook Creek		South Etobicoke	
Date	Rainbow Trout Mortality	Daphnia Magna Mortality	Rainbow Trout Mortality	Daphnia Magna Mortality
July 4/2003	0%	0%	30%	0%
July 10/2003	50%	0%	N/A	N/A
July 14/2003	100%	0%	50%	0%
August 29/2003	N/A	N/A	0%	0%
September 18/2003	0%	0%	0%	0%

2.4. Catch Basins and Stormwater Management

Receiving little sunlight, catch basins provide excellent breeding refuges for *Culex pipiens* and contribute to the residual control of methoprene (McCarry 1996). In a survey of catch basins in the Greater Toronto Area, almost all catch basins tested positive for the presence of mosquito larvae (CoT 2003a). The abundance of larvae in catch basins is not correlated with pH, and is weakly correlated with warmer water temperatures and organic debris content (Geery 1989).

Larvae are flushed from catch basins during rain events, with low rainfall (7-17 mm) resulting in 22-34% larval reduction, moderate rainfall (22 mm) resulting in 45% larval reduction, and a heavy rain (102-127 mm) resulting in an 85-91% larval reduction (Geery 1989). Significant rainfall is necessary to flush the majority of larvae, as study results indicate that there are many *Culex* larvae remaining in catch basins after a normal rain (Geery 1989). For example, significant rainfall runoff caused flushing of larvae, as well as flushing and replacement of the catch basin water in a Michigan efficacy study of methoprene pellets (McCarry 1996). Mortality in the catch basins remained high throughout the study despite rainfall (McCarry 1996). Catch basin water is flushed during low flow storm events, with negligible contributions from the settled sediments (Morrison 1995). Higher flow storm events cause disturbance of the catch basin bed, which releases pollutants from the contaminated sediment (Morrison 1995).

2.5. Model of the Fate and Transport of Methoprene

In order to analyze the effluent concentration of methoprene from catch basins, and estimate the required frequency of application, a simulation model was developed (Behera 2003). The factors considered to affect the concentration of methoprene include the chemical characteristics of methoprene, rainfall

characteristics (rainfall volume, intensity and duration), runoff characteristics (volume, peak flows, duration, and land use), catch basin characteristics (size, volume, accumulation of sediments) (Behera 2003). Analysis of the model with different scenarios assists in estimating the required dose and application frequency for the WNv larviciding program.

The model was developed using a long term daily rainfall record which was determined to most closely represent the average climatic conditions for the study catchment. It was found that the annual rainfall volume of 1980 matches the long-term annual average for the Toronto region. The summer rainfall data from Pearson Airport in Toronto were selected. The analysis of the rainfall volume was calculated using STORM-type hydrology and daily runoff volume for the selected period (Behera 2003). The simulation model estimated the effluent of methoprene discharged during rain a event and predicted the methoprene concentration in the catch basin from the day of application until the chemical has been completely removed (Table 2.4).

The model is based on the following assumptions:

- Methoprene is applied approximately monthly, once every 30 days from the first application.
- The time interval for the model is daily (24 hours) and the day begins at midnight, 0:00 hours.
- If a rain event extended over more than one day, the amount of rainfall volume and corresponding duration was calculated on a daily basis.
- Runoff is calculated using a STORM-type hydrology model.
- A relationship between daily runoff and corresponding methoprene washout is assumed (Table 2.4). From experimental evidence, every runoff volume has been shown to have a certain potential to remove some or all methoprene mass from the catch basin (Sze, 2004). A small storm can remove part of the

available mass, while a large storm may remove all available mass. Based on the runoff volume, various remaining methoprene mass fractions are assumed. Experimental hydrodynamic data from a physical model of a catch basin have been used to determine that a flow of 9 L/s can dislodge and flush the pellet from the catch basin (Sze 2004). For the average storm duration for Toronto of 4.88 hr (Adams 2000), the critical flow for complete washout of the methoprene pellets is 158 m^3. Interpolation of values for the relationship between the mass of methoprene remaining and flow produces the values in Table 2.5.

- Daily methoprene mass is conserved and no daily decay is assumed.
- If there is no rainfall on a day, the daily mass of methoprene released from the pellet is assumed to stay within the control volume, and be available for washout during the next rainfall event. For example, consecutive dry days will result in higher methoprene concentrations in the catch basin water.

Table 2.4 Simulation model for the evaluation of methoprene application in catch basins.

B	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*
5/1/80	1	0	0	0	1	28.8	28.8	1.0	1.0	0.0972	0.010	0.000
...	...	...	...	...	...	...	...	...	...	...	...	...

Description of columns:

B. Date/month/year.

C. Day number of the trial.

D. Rainfall volume in mm during the 24 hours of the day.

E. Daily runoff volume in mm, using model: $V_r = \phi\ (V - S_d)$, where V_r is runoff volume, V is rainfall volume, ϕ is runoff coefficient and S_d is the amount of depression storage.

F. Daily runoff column, in m^3, based on contributing area and depth.

G. Methoprene remaining factor, which is based on daily runoff volume. The value comes from a lookup table based on an assumed relationship between runoff volume and methoprene remaining after the event.

H. The amount of methoprene mass left in the pellet at the end of the day.

I. This column is the same as H, except the negative values are considered as zero for physical meaning.

J. The amount of methoprene exiting the pellet at the end of the time step (day). If there is rainfall, more mass will exit; otherwise methoprene is released at assumed rate.

K. The amount of methoprene available for concentration within the control volume at the end of the day. If there is no rain the previous day, the mass is the sum of the previous day and the current day.

L. Runoff volume added to control volume. Control volume is based on the dimensions of the catch basin and assumed volume factor (e.g., 0.3 means that

30% of the total sump volume is available for control volume and 70% is filled up with sediment).

M. Methoprene concentration in the control volume based on the mass and volume available at the end of the time step.

N. Methoprene concentration discharged from the catch basin. If there is rainfall, resulting output concentration is produced from the available methoprene mass.

The following characteristics are used for the model:

- Runoff Coefficient: Residential = 0.6, Commercial = 0.9, Industrial = 0.9, Institutional = 0.9, Park = 0.3.
- Area drained per catch basin = 0.2 ha
- Depression storage per catch basin drainage area = 1.5 mm
- Initial dose of methoprene 4.25% of 700 mg = 29.75 mg
- Methoprene fully degrades within 30 days, releasing 1 mg per day over a month.
- Average daily runoff for the summer months is 7.4 m^3

Table 2.5 Methoprene washout factor per runoff volume used in simulation model.

Runoff Vol (m^3)	Methoprene Remaining
0	1.0
31.6	0.8
94.8	0.4
126.4	0.2
142.2	0.1
158	0
236.8	0
473.6	0

2.6. Summary of Current Knowledge

Because West Nile virus is considered a threat to public health in Ontario, during the summer of 2003, 605,607 catch basins were treated with methoprene in Ontario, 123,117 by the Toronto Public Health unit. In Toronto, there were 2 applications of 0.7 g per catch basin, for a total of 145 kg per treatment (MOE 2003a). In order to control the WNv threat without compromising ecological health, it is important to analyze and determine mosquito control levels, in order to maximize efficiency and minimize economic cost and environmental damage.

An integrated pest management plan is based on ecological as well as economic and social criteria. Long-term surveillance helps refine future control strategies. Current research on mosquito biology and methoprene has focused on the efficacy of the larvicide and refining its specificity. Efforts to reduce the quantities of larvicides or chemicals applied include research on alternatives to chemical control including biological control such as fostering healthy populations of mosquito predators and use of *Bti* and *Bacillus sphaericus*.

Intensive scrutiny from the public has put pressure on government agencies to research and monitor the risks associated with larvicide use and balance them against the risks of vector-borne disease. There is currently a large

body of research on the chemical properties and larviciding efficacy of methoprene. Efficacy monitoring has occurred in various regions where vector control programs have been implemented: California, Florida, Minnesota, Australia, and more recently New York State, Illinois and southeastern Canada. Urban mosquito control including larviciding in catch basins has been examined, but this has concentrated on efficacy measurement, and has not considered the environmental transport of methoprene.

Research on the environmental fate of methoprene has shown that it degrades rapidly in the environment. Research on its effects on nontarget species has demonstrated that it is safe for use with minimal risk to human health and the environment. However, contradictory findings on the effects of methoprene breakdown products on aquatic organisms points to a need for research on the transport of methoprene and the environmental fate of its metabolites.

This research is part of a monitoring study conducted in concert with the MOE and the City of Toronto to determine if the use of methoprene leads to adverse impacts on aquatic biota (CoT 2003a). The MOE is currently developing a provincial water quality objective against which the storm sewer outfall concentrations will be compared to establish whether methoprene is flushed from catch-basins in deleterious quantities (CoT 2003b).

Urban mosquito control programs have shown that the catch basin environment must be studied to determine the impacts of mosquito abatement programs downstream of the storm sewer system. In order to do this, this study increases the understanding of methoprene fate and transport by conducting a field study, laboratory experiments and a simulation model.

CHAPTER 3

3. Field Monitoring Program and Laboratory Experiment

3.1. Methoprene Field Study Materials and Methods

3.1.1. Sewershed Area

The entire Newtonbrook creek sewershed was surveyed on foot, and all catch basins as well as the land uses were marked on a map of the area. The catch basins and the land uses were then digitized on a map of the area using geographical information systems software, ArcGIS 8.3. Base map layers of the street grid, storm sewer index map, and topographical contours were provided by the Technical Services of the Works and Emergency Services at the City of Toronto. Air photos of the area flown in 1999 by the City of Toronto were provided by the Ryerson University Library.

The Newtonbrook neighbourhood is in the north-eastern region of Toronto (fig 3.1). The Newtonbrook sewershed outfall flows directly into Newtonbrook Creek, which is a tributary of the Don River. The Don River watershed is 86% urbanized, with a population of more than 800,000 people (TRCA 2003). The Don River flows through the heart of Toronto and has been under intense development pressures for the last 200 years (TRCA 2003). According to the Toronto and Region Conservation Authority, the Don River is one of Canada's most degraded urban rivers (TRCA 2004). The Don watershed is now only 7% forested, and has lost most of its wetlands, which threatens the river's ecosystem health (TRCA 2004).

The Newtonbrook drainage area has a perimeter of 14349 m and a surface area of 3590169 m^2 (fig 3.2). In the Newtonbrook sewershed, there are three main land uses: residential, commercial, institutional, and park and recreational (fig

3.3). There are no industrial zones in the Newtonbrook sewershed. The residential land use is 77% of the total sewershed area, while land for commercial uses is 11%, followed by institutional at 7%, and park and recreational at 5% (fig 3.4a). All of the catch basins in the Newtonbrook sewershed were marked on a map by survey on foot (fig 3.5). Each catch basin was then classified as draining land from one of the four land use categories (3.4b).

The City of Toronto larviciding program targets storm sewer catch basins on residential, commercial and urban park and recreational streets, however, industrial areas have been excluded (TPH 2004). The City of Toronto focused their larviciding efforts on residential streets because dipping surveys showed that there were few larvae in catch basins in industrial zones and along major arterial roads (TPH 2004). Methoprene pellets were not applied to catch basins on private property, such as driveways, parking lots and commercial lots. The catch basins marked on the final digital map reflect these conditions.

Rainfall data came from three separate gauges, E. Bales station at Bathurst and Sheppard (approximately 3.75km away), Mitchell field station at Church Ave. (approximately 1.125km away), and the Newtonbrook Creek station at Willowdale and Silverview, where data collection began on August 11th, 2003.

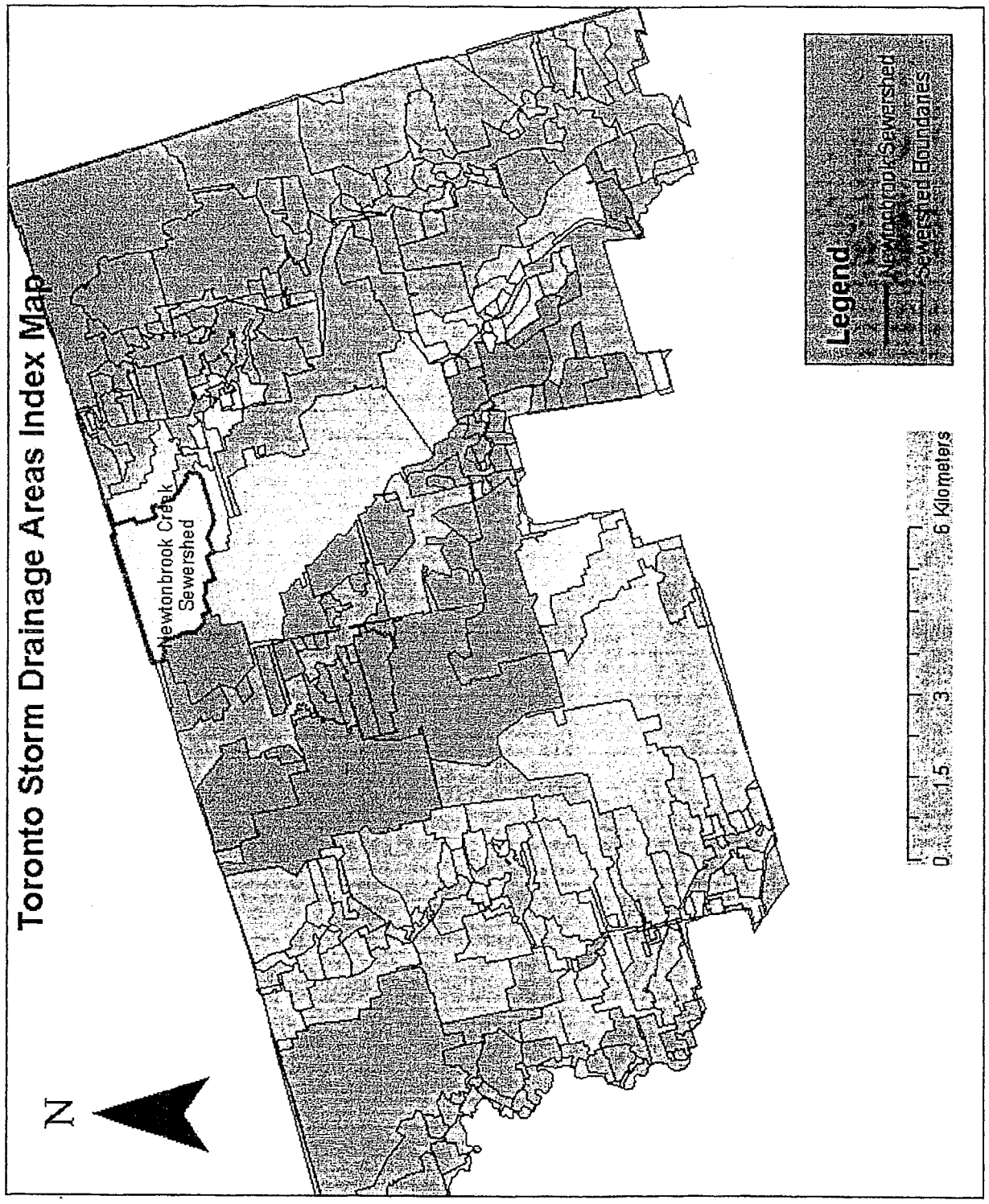

Figure 3.1 Toronto storm sewer drainage area map. Newtonbrook creek is in the northeastern region of Toronto. City of Toronto, 1999.

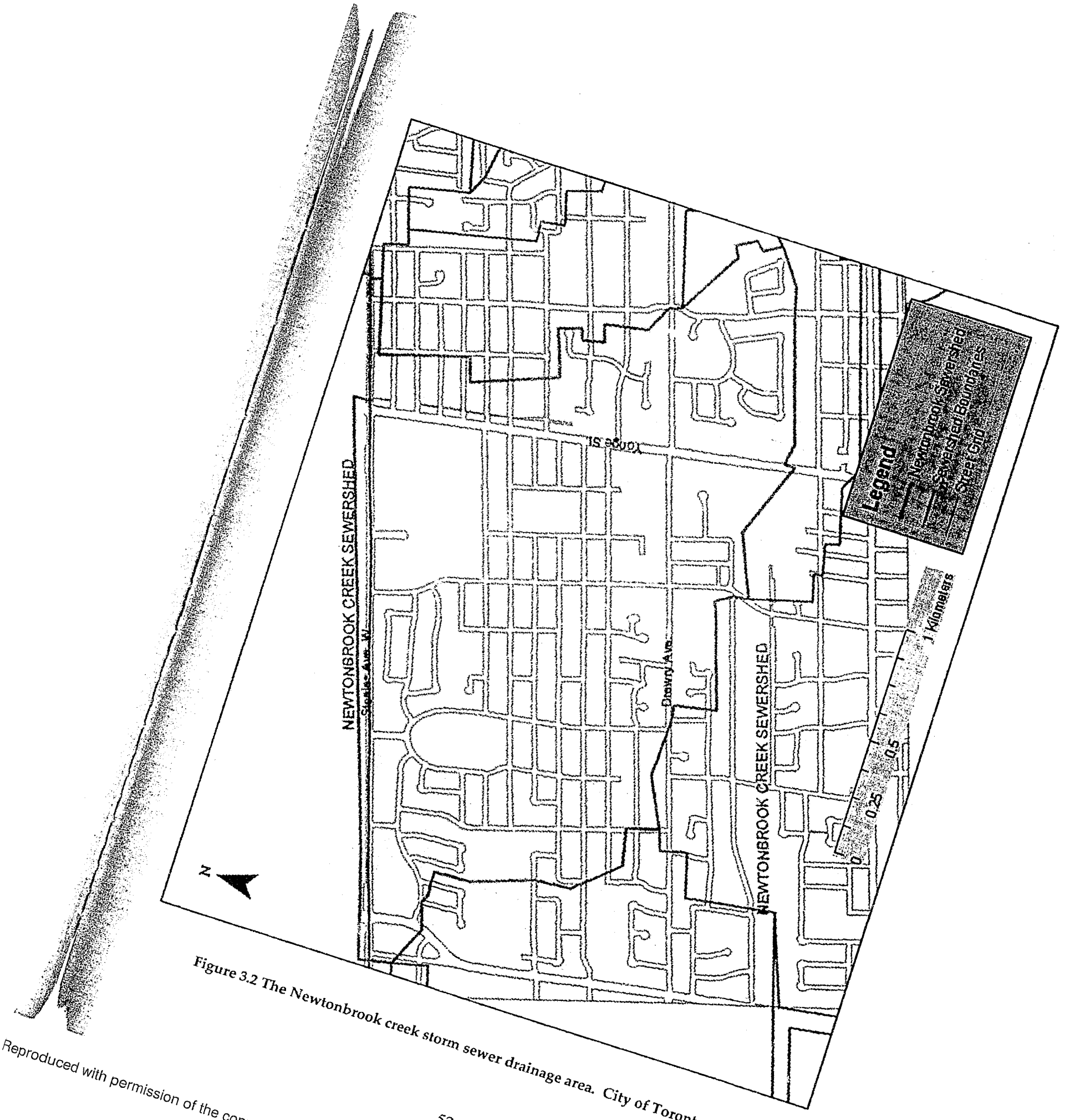

Figure 3.2 The Newtonbrook creek storm sewer drainage area. City of Toronto, 1999.

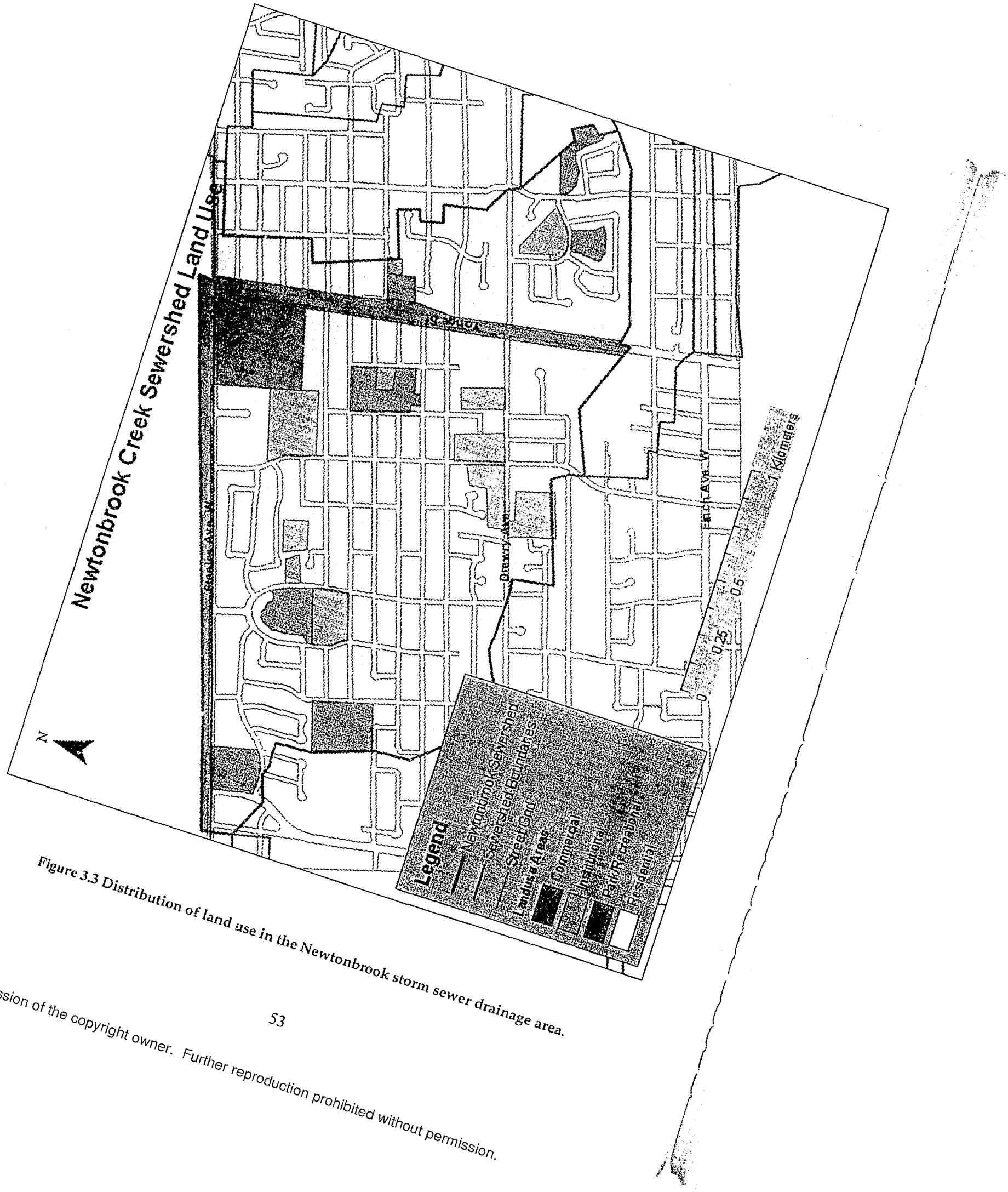

Figure 3.3 Distribution of land use in the Newtonbrook storm sewer drainage area.

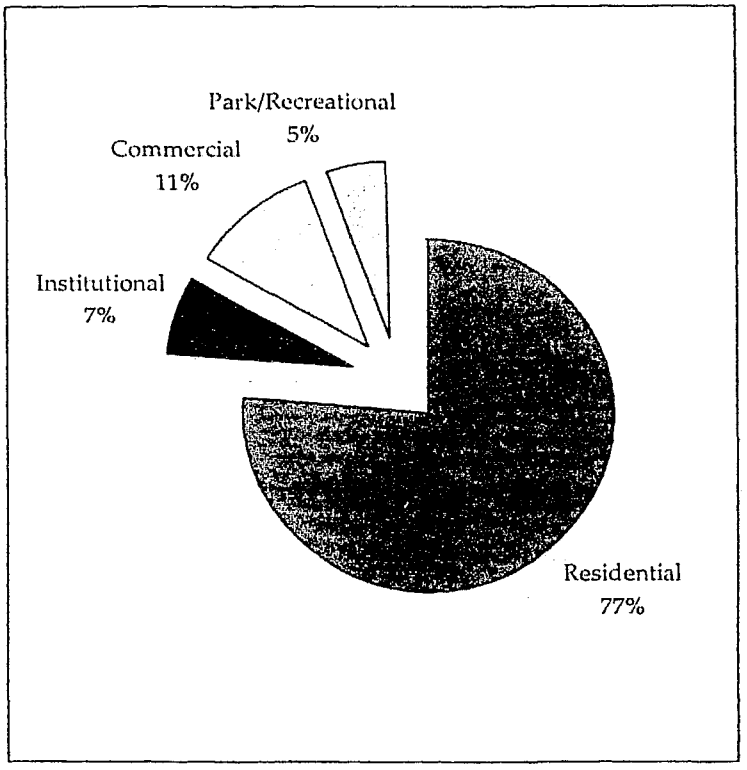

Figure 3.4-a Percentage of the Newtonbrook storm drainage area occupied by land uses.

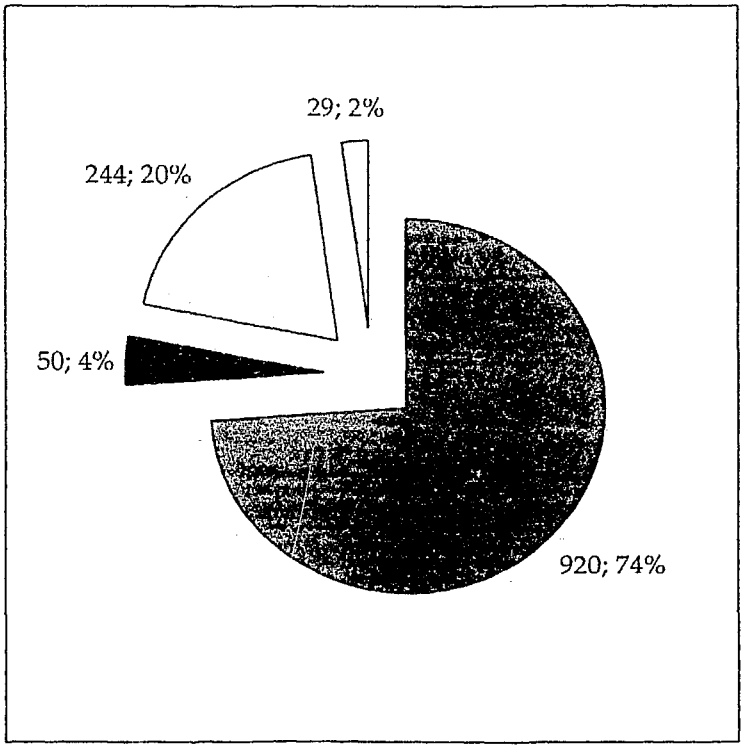

Figure 3.4-b Number of catch basins in each land use zone.

Figure 3.5 Overhead view of the monitoring site.

3.1.2. Monitoring Site Description

The study site at Silverview Road and Willowdale Avenue was chosen because of its proximity to the outfall of the storm sewer drainage area, and the City of Toronto's water quality monitoring hut in the grassy area on the northwest corner of the intersection. The location reflects the focus of the City of Toronto's larviciding efforts on residential areas. The study site consists of three catch basins at the intersection of Willowdale Avenue and Silverview Road in North York (Fig 3.6). Willowdale is a moderately busy main street lined with houses; Silverview is a quiet residential street with houses, a school and a small park. Both streets have many trees and lawns, which contribute to the perviousness of the area.

The catch basin on Willowdale is identified as W1; the two catch basins on Silverview are identified as S1 and S2 (fig 3.5-6). The outfall is located diagonally across the street from the three catch basins. Before the sampling program began, half the sediments in catch basin W1 and all the sediments in S2 were cleaned out by a vacuum truck in order to observe the effects of varying levels of water and sediment on the behaviour of methoprene (Table 3.1).

Table 3.1 Characteristics of the study catch basins.

	W1	S1	S2
Water surface (cm)	31	11.5	106
Sediment depth (cm)	52	66.5	3
pH	7.21	6.97	7.41
Basin temp. (°C)	20	22	22
Traffic volume	High	Low	Low

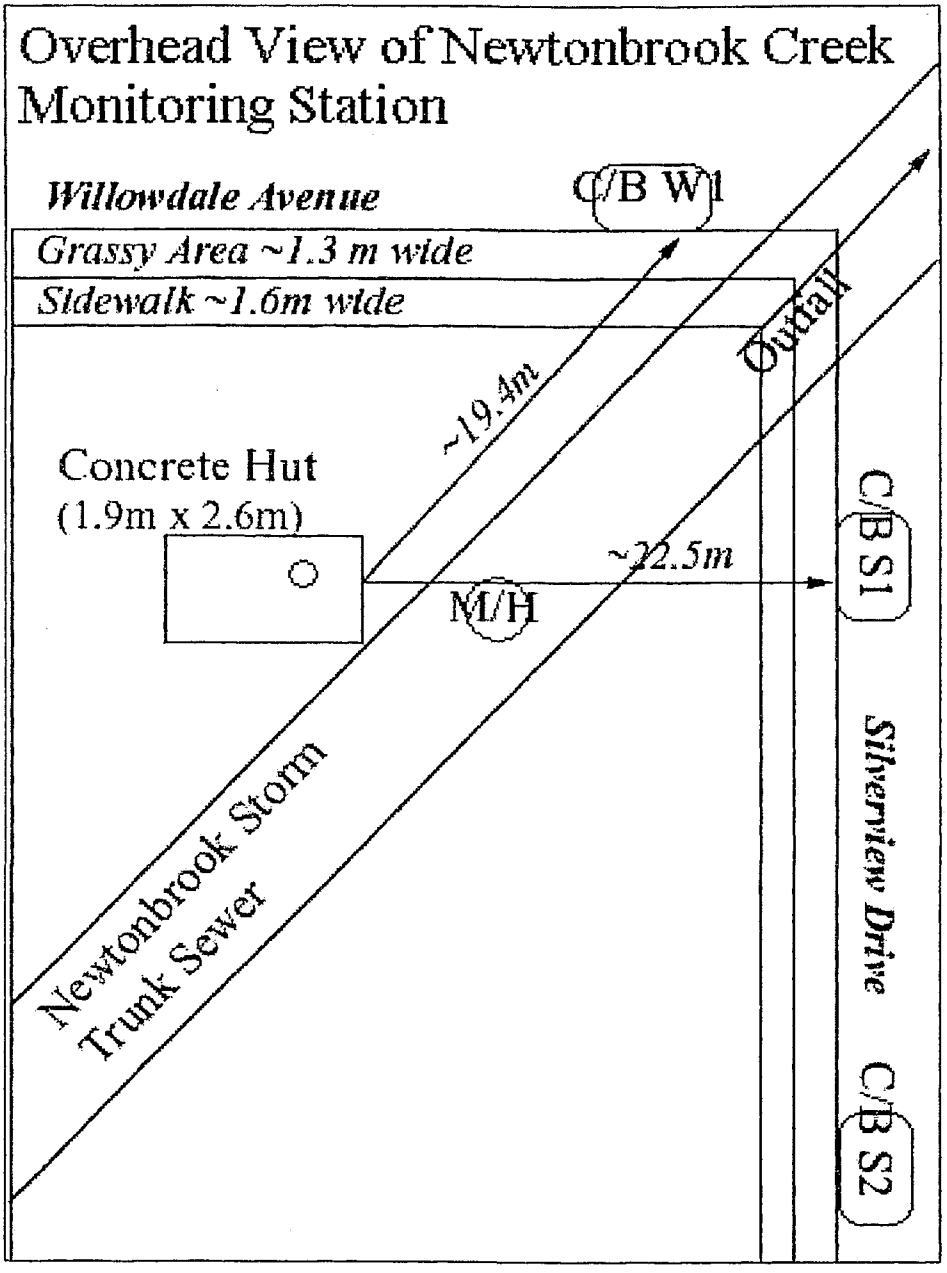

Figure 3.6 Schematic overhead view of the study site, showing the three catch basins, W1, S1 and S2, as well as the oufall and the monitoring hut.

3.1.3. Methoprene Application and Water Quality Monitoring

Larvicide dosages were 0.7g per c . basin, and applied July 4th, August 9th, and September 9th, 2003. These date coin ued with the City of Toronto's larviciding schedule of the area. The lar .. used was methoprene, in Altosid pellet formulation, which contains 4.25% active ingredient. Altosid solid sustained-release pellet formulations are designed to be effective for up to 30 days, according to label directions (Wellmark International 1999). Field trials in freshwater test plots in Florida (Floore 1991) and Illinois catch basins (Siegel 1999) also found that emergence inhibition was effective for approximately 30 days post-treatment.

The length and diameter of 3 batches of 50 pellets each were measured. The batches weighed 1.474 g, 1.565 g, and 1.490 g each. The pellet length distribution was more variable than the pellet diameter. The diameter of the pellets averaged 0.40 cm, with a standard deviation of 0.0080. The length of the pellets averaged 0.73 cm, with a standard deviation of 0.1717. The density of the pellets averaged 1.57g/cm^3, with a standard deviation of 0.0131.

Catch basin water was sampled daily using a small battery-operated mechanical pump fitted with a long stainless steel tube which was lowered into the catch basin. The sample was taken from the top 5-10 cm of the water surface (Hershey 1994; Schaefer 1973). Samples were stored in a cooler with ice until delivered to the lab within 48 h, where they were stored in a fridge at 4°C until they could be extracted as quickly as possible (Hershey 1994). The pump and tube were rinsed after being used at each catch basin. Water samples were analyzed daily for methoprene and malathion, twice weekly for dissolved organic carbon (DOC), and after the 3rd application; weekly for heavy metals (Table 3.2). After the installation of automatic wastewater samplers, (American

Sigma Model No. 1350) at each of the catch basins in August 2003, it was possible to use the built-in pump for daily sampling. Grab samples were taken from the outfall during rain events by City of Toronto Works and Emergency Services until the installation of the automatic sampler (American Sigma 900MAX) in the concrete hut (Fig 3.6).

The researchers did not use the traditional method of larvae collection known as 'dipping' (Geery 1989; MOHLTC 2003b); when water samples were taken from the catch basins with the electric pump, mosquito larvae were also taken up. The number of larvae seen in each catch basin was recorded as a qualitative observation and assigned a status and number: none = 0, few = 1, many = 2, which has no relation to the concentration of methoprene. Mosquito larvae were reared in the MOE laboratory. The presence of larvae simply indicates the need for a mosquito control program, but is not an indicator of the effectiveness of the methoprene, as it prevents emergence from the pupal stage and has no effect on the presence of larvae.

3.2. Laboratory Experiment Materials & Methods

A plastic tank was constructed with three spouts at different heights along its side (Fig.3.7), and filled with tap water with a temperature of 16°C and a pH of 7.26. The dimensions are approximately the same as a storm sewer catch basin, but the tank is round instead of square. The tank dimensions are: inside diameter: 600mm, thickness of tank wall is 10mm, and the faucet heights are: MT 900mm, MM 600mm, and ML 450mm from the bottom. The tank was filled with tap water to just above the top spout, which was 905 mm from the bottom. Methoprene pellets were added to the water, and the water was sampled daily from each spout, starting from the top down, in order to observe any concentration gradient that may have occurred. An amount of fresh tap water

equal to that withdrawn was added to the tank after each sampling. The first trial lasted 25 days, the second, 30 days; and the third, 25 days. The doses of methoprene used in each trial were as follows: 1st application 0.710g, 2nd application 3.534g and 3rd application 35.074g. These quantities were used in order to observe the concentration decay rates for the mosquito control field dosage, and for five and fifty times the field dosage; which would not be typically used in a field setting unless by accident.

The results of the first lab tank experiments prompted the researchers to conduct further testing to observe the effects of water depth and pellet size on the dissolution and decay of methoprene in quiescent conditions. These experiments were completed using the mosquito control dose of 0.7 g Altosid pellets, but varying the water depth and pellet size distribution. The first trial was run using two tanks, one filled with water to the lower tap (ML – 450 mm) and one filled to the middle tap (MM – 600 mm). The second trial was also conducted with two tanks, filled to the middle tap, but each dose of 0.7 g of pellets was composed of all short pellets, or all long pellets. The pellet length ranged from 4.293 mm to 11.582 mm. The dose of short pellets was composed of pellets near the short end of the length range, while the dose of long pellets was composed of pellets near the long end of the length range. The first trial lasted 25 days, and the second lasted 27 days.

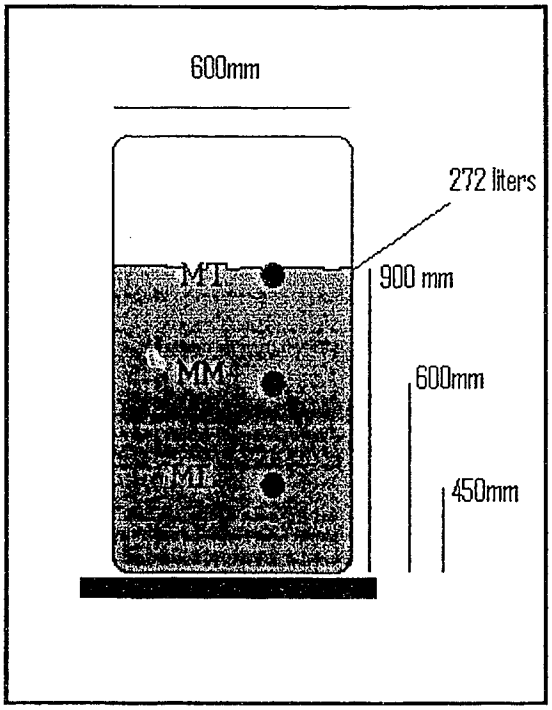

Figure 3.7 Lab tank schematic.

3.3. Sample Analysis & Handling

Methoprene concentrations in the collected samples were analyzed by the City of Toronto laboratory, using the United States Geological Survey (USGS) method of liquid-liquid extraction and gas chromatography/mass spectrometry for determination of mosquito insecticides in water (Zimmerman 2001). This method is used to acquire information about the fate and transport of mosquito insecticides through analysis of surface and ground-water samples. The surrogate standard terbuthylazine was used to assure quality control in sample analysis. Methoprene concentration results received from the City of Toronto Laboratory have surrogate standard recovery rates of between 22 and 66%. However, additional quality checks of the testing procedure were conducted by adding a known quantity of methoprene in a water sample and measuring the amount of methoprene; it was found that 95% of the added methoprene was detected by this analytical method. Water samples for methoprene analysis were collected in amber jars to limit photolytic degradation of the methoprene, and all samples were stored on ice during transit to the lab for testing (Table 3.2), which is consistent with the standard method (Zimmerman 2001).

Table 3.2 Sampling materials, handling, and analysis.

	Methoprene	DOC	Chloride	Heavy Metals
Size (ml)	250	500	500	500
Preservation	ice	ice + acid (H_2SO_4)	ice	ice + acid (HNO_3)
Jar type	Amber + Teflon-lined lid	Clear	Clear	Clear
Detection method	Liquid extraction and gas chromatography	Combustion total organic carbon analysis (Model 1020A)	Ion chromatography (Dionex IC25)	Inductively Coupled Plasma Optical Emission Spectrometry
Detection limit	< 0.03 µg/L	< 0.5 mg/L	< 0.1 mg/L	Varies per cation (0.001 – 0.012 mg/L)
Schedule	Daily	Bi-weekly	Bi-weekly	Weekly starting after 3rd application

3.4. Mass Balance of Methoprene in the Newtonbrook Sewershed

The model, with scenarios representing the individual characteristics of the three Newtonbrook study catch basins, was run by the researcher using rainfall data from 2003. The simulation model estimated the effluent of methoprene discharged during rain events and predicted the methoprene concentration in the catch basins from the day of application until the chemical was completely removed (Fig 3.8-10). The catch basins in the study site are all considered to drain residential areas, and therefore the runoff coefficient in the model remains 0.6. Each catch basin has a different water volume and sediment depth, therefore different effective volumes (Table 3.3). The output from the model representing the methoprene concentrations in the catch basins is compared to the actual methoprene concentrations (Figures 3.8-10).

Table 3.3 Catch basin characteristics of the Newtonbrook creek study site for the simulation model.

Catchbasin Size	W1	S1	S2
Base area (m^2) =	0.3721	0.3721	0.3721
Height up to sewer (m)	0.83	0.78	1.09
Effective vol. Factor	0.3735	0.1474	0.9724

An analysis of two rain events from August 16th and August 26th, 2003 required determination of outflow from the catch basins. Rainfall data used were collected at 5 minute intervals. Based on contributing area and depth, and assuming the flow into each catch basin is the same as the flow out, the following equation was used:

Daily runoff volume in mm: $V_r = \phi (V - S_d)$

- where V_r is runoff volume,
- V is rainfall volume,
- ϕ is runoff coefficient and
- S_d is the amount of depression storage.

The flow of the outfall was recorded during monitoring for the two rain events, as well as the outflow methoprene concentration. The concentration of methoprene in the catch basins was also determined from the daily monitoring data. The outflow of the catch basins was calculated based on the runoff volume and the area drained, 0.2 ha, by each catch basin.

Using the calculated flow out of the catch basins, and the concentration obtained from field values during rain events on August 16th and 26th, 2003, an output mass of methoprene was obtained (Tables 3.4-5). The results show that because the model does not take chemical decay into consideration, there is a great discrepancy between the model output, and the measured outgoing mass of methoprene. For the rain even on August 16th, the model values are approximately 25 µg, whereas the measured values are no greater than 0.0071 µg. If the model did account for the decay of methoprene, it is probable that the output would be closer to the measured quantities. For a dose of 0.7g of pellets, containing 4.25 % active ingredient, 29.75 mg of methoprene is applied to each catch basin. The model would have to include a release rate of approximately 1 mg per day, and a half-life of 28 days. The model values for August 26th are

closer to the measured values as it is nearly three weeks since application, and by that time, there is very little methoprene remaining to be dissolved in the control volume.

A second model iteration using runoff coefficients representing the four land use types in the Newtonbrook sewershed provided model values for the output volume and mass of methoprene from all catch basins in the drainage area. When these values were multiplied by the number of catch basins in each land use drainage area (Table 3.5-6), a total output volume and methoprene mass could be predicted from the model. Neither the model flow, nor the model output mass of methoprene were close to the measured values, and when the concentration is calculated, there are four to five orders of magnitude of difference between the model output and the actual measured field values.

These errors are due in part to the omission of chemical decay in the model, as well as a possible baseflow in the outfall which could contribute to the outfall volume. The propagation of these errors from the individual catch basin level to the entire drainage area is significant. This is evident in the wide gap between values obtained from the model, and those measured in the field.

Table 3.4 Model Rain Event 1 - Aug 16, 7:15 am to Aug 16, 9:25 am, 2003

	Field Values				*Model Values*		
Total	*Outfall*	*W1*	*S1*	*S2*	*W1*	*S1*	*S2*
Volume (m3)	3774.06	9.84	9.84	9.84	9.84	9.84	9.84
Mass out (μg)	43496.35	0.0044	0.0071	0.0000	25.17017	25.49847	25.49847

Table 3.5 Model Rain Event 2 - Aug 26, 3:05 am to 7:05 am, 2003

	Field Values				*Model Values*		
Total	*Outfall*	*W1*	*S1*	*S2*	*W1*	*S1*	*S2*
Volume (m3)	8941.80	1.2	1.2	1.2	1.2	1.2	1.2
Mass out (μg)	443130.21	0.0000	0.0004	0.0000	2.2595	0.0000	0.0000

Table 3.6 Model Rain Event 1 - Aug 16, 7:15 am to Aug 16, 9:25 am, 2003.

	Model Values			
Total	*Residential*	*Commercial*	*Institutional*	*Park/Rec*
Volume (m3)	9.84	14.76	14.76	4.92
Mass out (μg)	439110	658665	658665	219555

Table 3.7 Model Rain Event 2 - Aug 26, 3:05 am to 7:05 am, 2003.

	Model Values			
Total	*Residential*	*Commercial*	*Institutional*	*Park/Rec*
Volume (m3)	1.2	1.8	1.8	0.6
Mass out (μg)	53550	80325	80325	26775

Table 3.8 Model output of methoprene for Newtonbrook drainage area, for rain event August 16th, 2003.

	Vol (m3)	*Mass (ug)*	*# of c/b's*	*Vol (m3)*	*Mass (g)*
Residential	9.84	439110	920	9052.80	403.98
Commercial	14.76	658665	244	3601.44	160.71
Institutional	14.76	658665	50	738.00	32.93
Park/Rec	4.92	219555	29	142.68	6.37
Total			1243	13534.92	604.00

Table 3.9 Model output of methoprene for Newtonbrook drainage area, for rain event August 26th, 2003.

	Vol (m3)	*Mass (ug)*	*# of c/b's*	*Vol (m3)*	*Mass (g)*
Residential	9.84	439110	920	9052.80	403.98
Commercial	14.76	658665	244	3601.44	160.71
Institutional	14.76	658665	50	738.00	32.93
Park/Rec	4.92	219555	29	142.68	6.37
Total			1243	13534.92	604.00

Table 3.10 Comparison of model output volume and mass to measured outfall values.

	August 16th			*August 26th*		
	Volume (m3)	*Mass (g)*	*Conc. (mg/L)*	*Volume (m3)*	*Mass (g)*	*Conc. (mg/L)*
Model	13534.92	604.00	44.625	730.60	73.66	100.819
Outfall	3774.06	0.04350	0.012	8941.80	0.44313	0.050

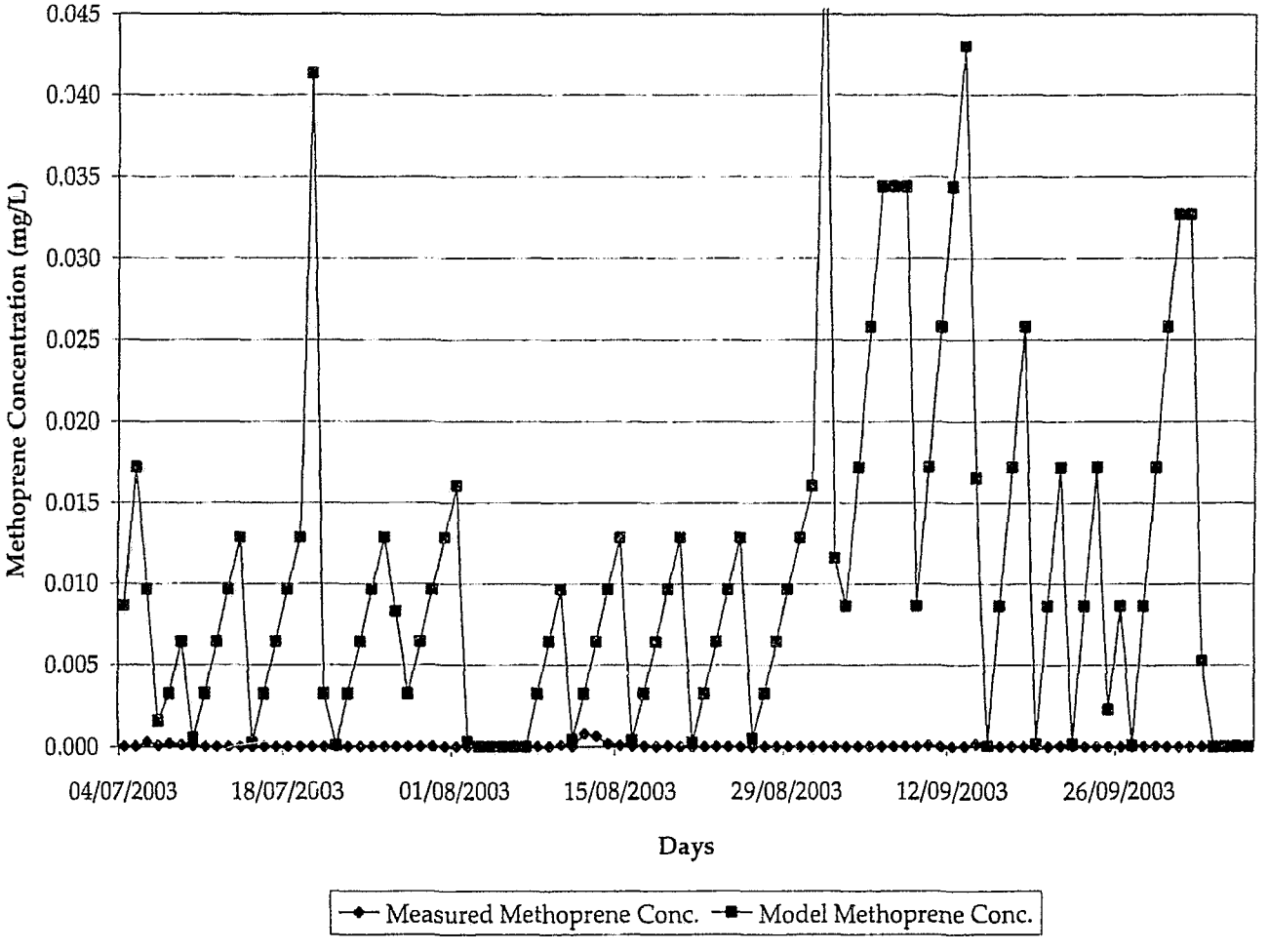

Figure 3.8 Comparison of model output and measured field concentrations for catch basin W1 using rainfall data for July to October 2003.

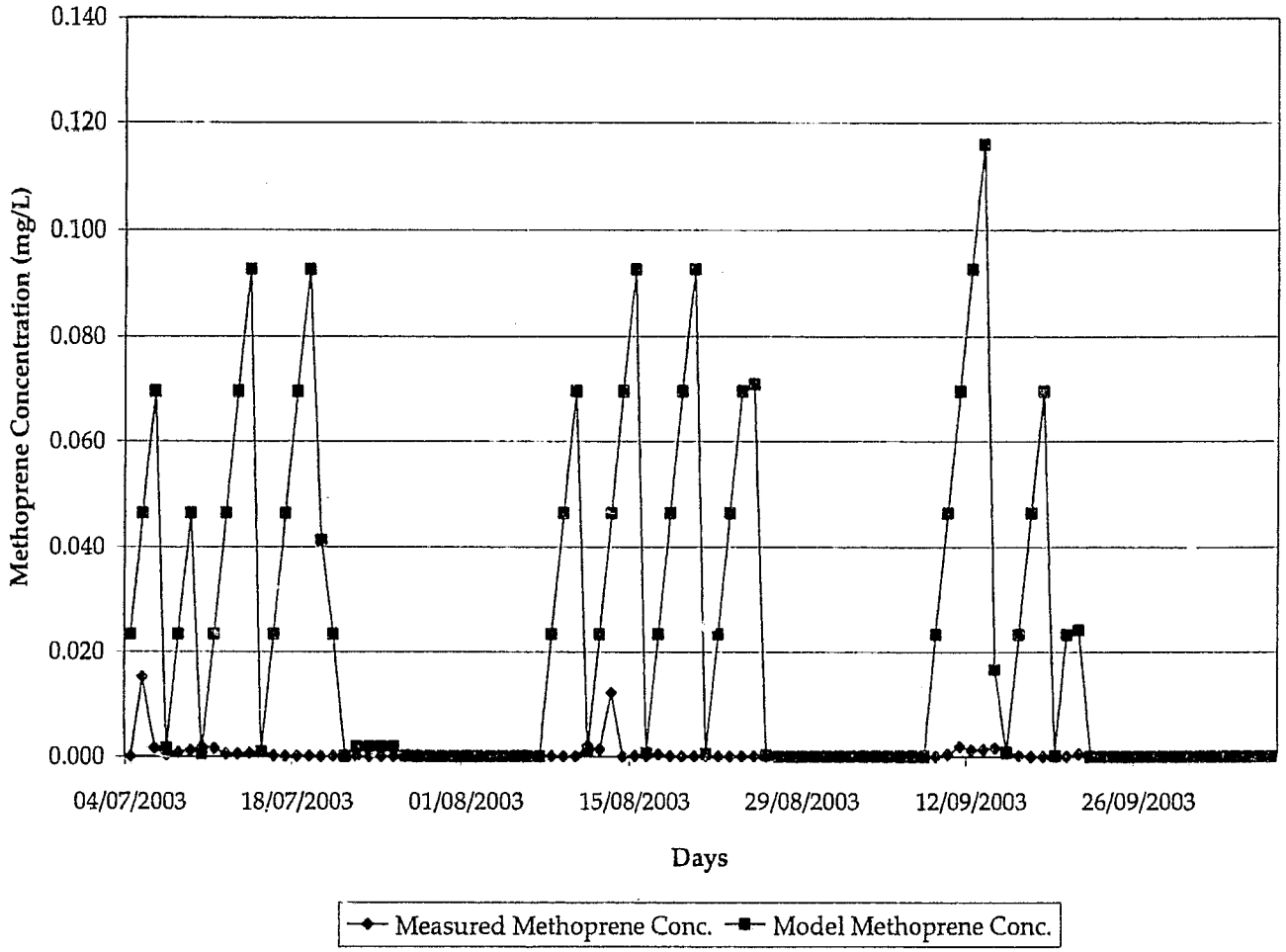

Figure 3.9 Comparison of model output and measured field concentrations for catch basin S1 using rainfall data for July to October 2003.

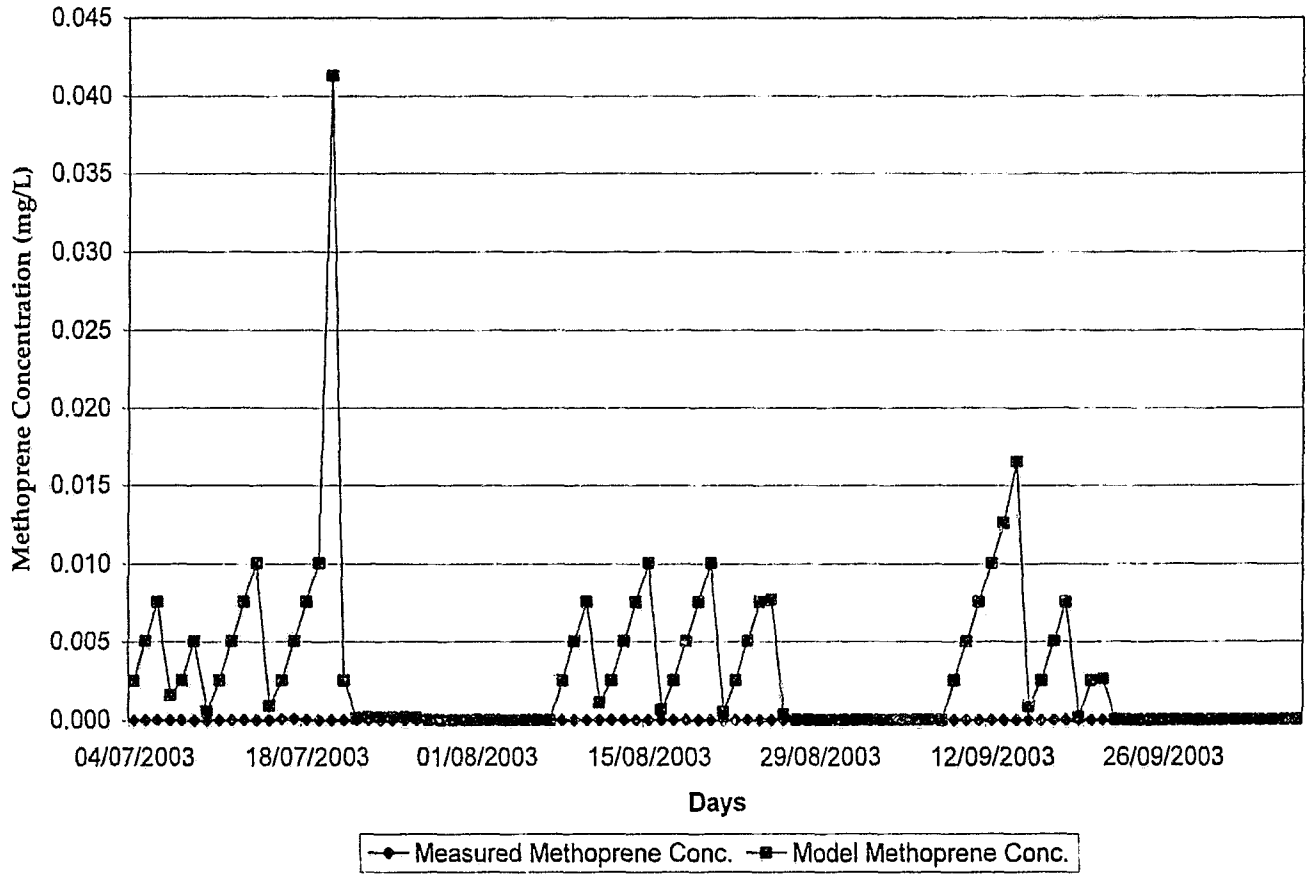

Figure 3.10 Comparison of model output and measured field concentrations for catch basin S2 using rainfall data for July to October 2003.

3.4.1. Simulation with Average Rainfall

In order to estimate the average daily expected methoprene concentration in catch basins in a typical Toronto neighbourhood, an analysis of the model with characteristics representing catch basins draining the area from each land use type in the Newtonbrook creek sewershed was run using rainfall data from 1980. The months of June, July and August were used, with applications assumed on the 1st of each month. The number of catch basins in the Newtonbrook storm sewer drainage area belonging to each land use type was calculated using the digital maps (Fig 3.4b and 3.5). There were 1243 total catch basins, 920 draining residential zones, 244 commercial, 50 institutional and 29 in park or recreational zones. To account for varying levels of sediments and water depth in catch basins in a typical neighbourhood, catch basins were assumed to have 50% effective volumes: the actual volume of water held in the basin.

A simulation for each land use type was completed using daily rainfall data for June, July and August 1980 from Pearson Airport (Fig 3.11). The industrial and commercial runoff coefficients are the same (0.9), so the output of methoprene is the same for these two land use types. The runoff coefficient for parks is much lower (0.3), therefore less methoprene is expected to be washed out during each rain event. This means that there is more methoprene remaining available for washout during subsequent rain events. Figure 3.11 shows that methoprene is expected to be quickly washed out in greater amounts from catch basins draining industrial and commercial areas, whereas the larvicide is washed out more slowly and over a longer period of time from catch basins draining residential and park areas.

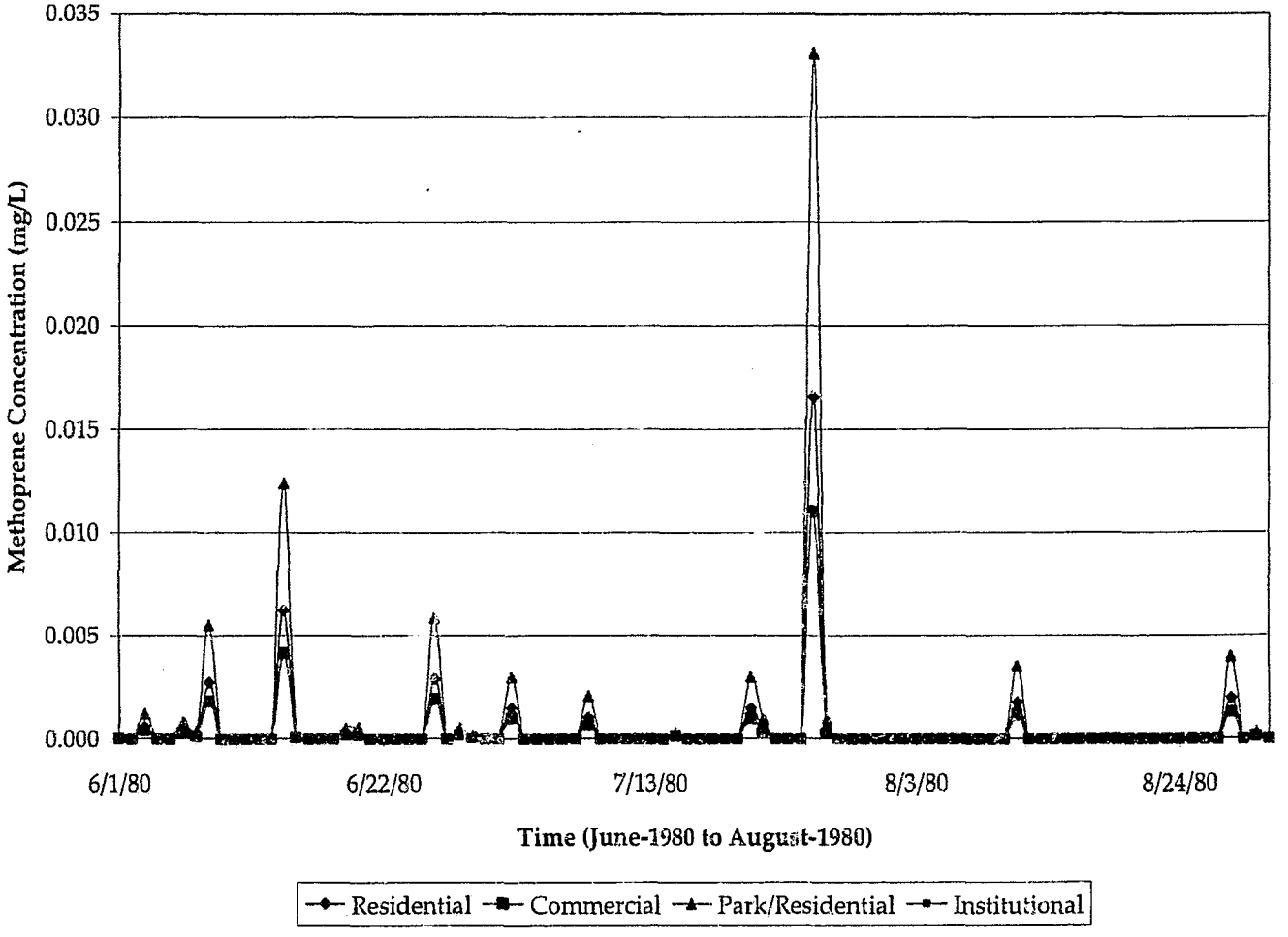

Figure 3.4 The output of the simulation model for each land use type using rainfall data from June to August 1980.

3.4.2. Recommendations and Application of Model to other Sewersheds

The simulation model for the application of methoprene to catch basins requires considerable further development. It is still useful to simplistically illustrate the process of flushing methoprene from the catch basins into the storm sewer system and out to receiving waters. If a decay function as well as ae hydrodynamic sediment resuspension function were incorporated into the model, it may become more useful in predicting outgoing concentrations of methoprene, and aid in the planning of applications of the larvicide.

Further improvement could include a more complex GIS application. The catch basins could be digitized in the map to include spatial coordinates, as well as connections to the storm sewers underground, and actual modelled runoff, not an assumed runoff coefficient. The construction of a network in GIS would allow the inclusion of travel time for concentrations arriving from the furthest catch basins. The catch basin response to rainfall over time could be predicted as a series of snapshots. Additionally, GIS can be used to estimate the discharge of methoprene to the environment on a watershed basis. The number of catch basins within a watershed can be estimated based upon the normal distance between catch basins and the length of roads. Together with the sewer network, the number of catch basins connecting to each sewer outfall can be determined. Finally, the cumulative mass of methoprene for each outfall can be computed using the improved simulation model.

3.5. Related Studies

The Newtonbrook creek catch basin field study was part of a larger WNv monitoring study involving the City of Toronto, the MOE, the University of Western Ontario, Peel Region, Halton Region, Environment Canada and local conservation authorities. Related water quality monitoring studies in Toronto examined methoprene concentrations in the Humber River watershed and at storm sewer outfall sites in South Etobicoke. The Humber River was sampled at the intersection of the river and Steeles Avenue, and at the mouth of the river at Old Mill. The storm sewer outfall in South Etobicoke was located on the Humber River along the Kingsway just north of Bloor Street West. Water samples were collected from these sites in a similar manner to those from the Newtonbrook site. Samples from these sites were also analyzed at the same laboratory.

Aquatic toxicity bioassays on Rainbow trout and *Daphnia magna*, of the outfall water pre-dosage and post-dosage were conducted by the MOE. Methoprene efficacy studies were also carried out by the MOE and the CoT for the Toronto region (TPH 2004). The efficacy of methoprene on the larvae collected as part of this study was assessed at the MOE laboratory (Baker 2004).

The larvae were separated into groups by instar (one through four), and pupae. If there were sufficient larvae in the sample, ten individuals from each group were reared and evaluated. The groups were reared in 500 ml polyethylene terephthalate (PET) bottles, with 10 ml of dechlorinated, aerated water and fed NUTRAMIN® food (Baker 2004). Pupae were not fed. Mesh screens were fixed to the bottles using elastic bands. The bottles were placed in an incubator at 24°C, with a 16/8 light to dark ratio. Survival status, growth,

pupal development and adult emergence were recorded daily. Larvae were considered alive if they moved within five minutes of observation, or with gentle agitation of the container (Baker 2004).

Pupae were considered alive if they moved in response to gentle agitation of their container. Pupae that did not respond were observed for up to three days afterward to assess survival. Adult emergence was considered as complete separation from the pupal casing and the abilty to fly (Baker 2004).

Emerged adult mosquitoes were placed in a freezer for 30 minutes, then removed from the bottle using forceps and placed in small glass vials before mounting (Baker 2004). Adult mosquitoes were pinned using No. 002 pins and labeled with their locality, date and collector (Baker 2004). Adults were later subject to species identification and sex determination using a key by Wood, Dang and Ellis (1979).

CHAPTER 4

4. Results and Discussion

4.1. Field Study Results

4.1.1. Methoprene concentration and rainfall

The S1 catch basin had the least amount of water in it (about 115 mm), hence the methoprene concentrations were very high compared to the other two catch basins which were highly diluted. The scales used for the methoprene concentration in the plots for W1 and S2 have a maximum value of 1 μg/L, however this scale is too restrictive for the elevated concentrations in S1 and a maximum value of 16 μg/L for methoprene concentration was used in plotting concentrations for this catch basin, to facilitate comparison of the methoprene concentrations over time. Mean monthly air temperatures for July, August, and September were 21, 20, and 15°C, and the sump temperatures were stable at around 20 to 22°C throughout the summer.

W1

1st Application (Fig 4.1): Methoprene concentrations were found to peak within two days, then peaked again at 4 days, and levelled off to non-detectable levels (<0.03 μg/L) 7 days after first application, the dip in the concentration could be due to flushing from a rain event on day 3. At 32 and 33 days after application, methoprene concentrations appeared at 0.04 μg/L, this may be related to turbulence in the catch basin sump due to a rain event that occurred on day 32.

2nd *Application* (Fig 4.2): Two peaks occurred at day 1 and day 3 after second application. The initial peak in concentration is much higher than it was after the first dosage, and reached a peak at 3 days, which may have been caused by flushing from a rain event on the 2nd day. The methoprene concentrations levelled off 11 days after the second application.

3rd *Application* (Fig 4.3): Again, two peaks in the methoprene concentration are observed, the first within 1 day, and the next is 6 days after application, but this one then drops off steeply. A large rain event on day 8 may have been responsible for flushing the methoprene. A small rise in concentration is observed 13 days after application which does not seem to be correlated to a rain event. Another increase in concentration appeared for only one day, 21 days after application, one day after a major rain event. It is clear from Figure 3 that the initial peak concentration is dependent upon the rainfall after application. The amount of rainfall affects the flushing of methoprene and resuspension of pellets in sediments.

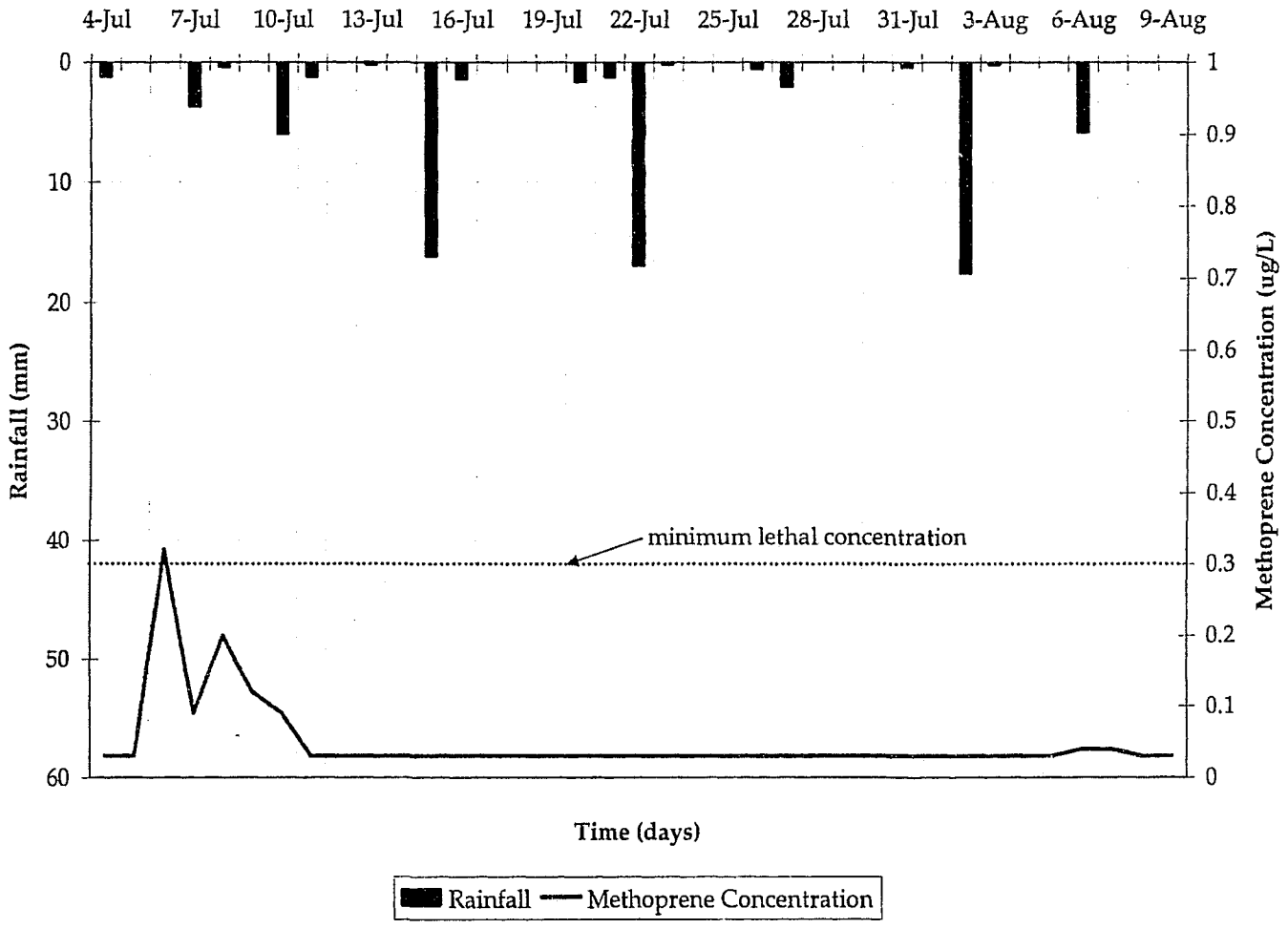

Figure 4.1 Rainfall in Newtonbrook region and methoprene concentration in catch basin W1 after July 4th application.

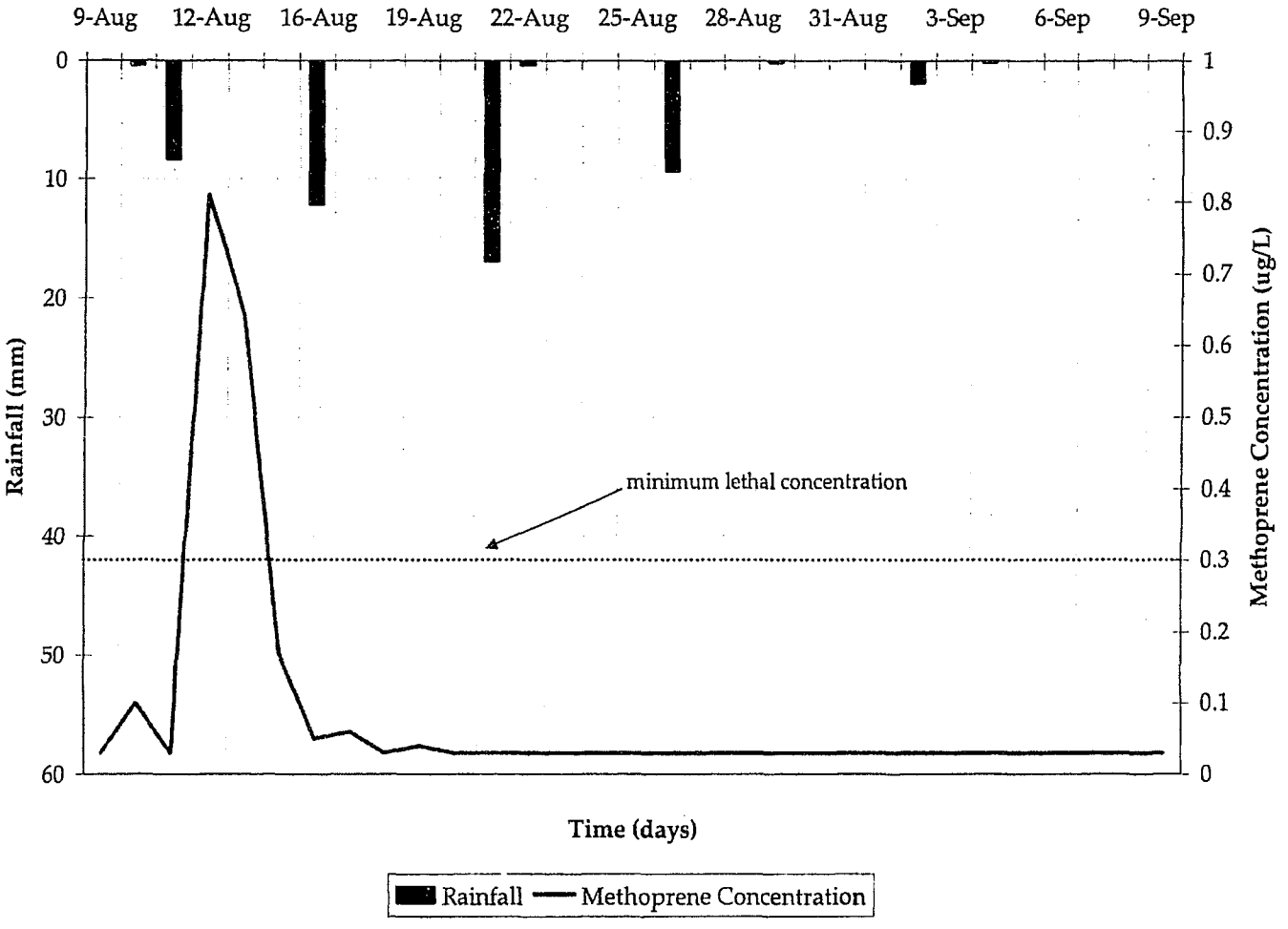

Figure 4.2 Rainfall in Newtonbrook region and methoprene concentration in catch basin W1 after August 9th application.

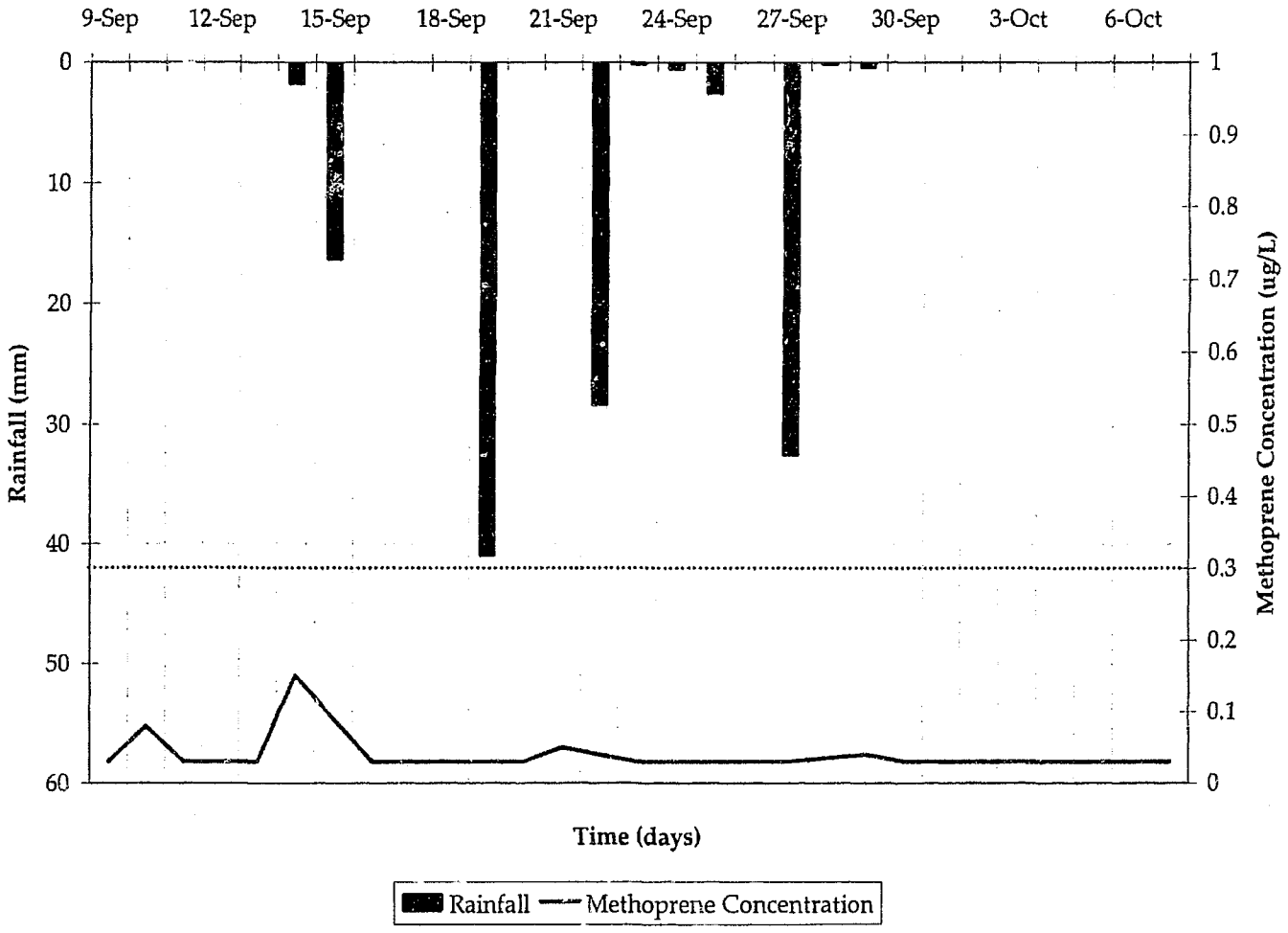

Figure 4.3 Rainfall in Newtonbrook region and methoprene concentration in catch basin W1 after September 9th application.

S1

1st Application (Fig 4.4): The peak in methoprene concentration occurred one day after application, with a second peak after 6 days. After 19 days, concentrations were under detection limits, with a few rises in concentration between 23 and 27 days, and at 34 days. With the higher concentrations present in this catch basin, it is easier to observe a correlation between rain events and rises in methoprene concentration, as on days 7, 12, and 19 after application. However, the rainfall data used are from a station located further northwest, this may explain why small peaks on days 25 and 27 did not occur in tandem with rain events, and that a rain event on day 30 has no corresponding increase in methoprene, if any chemical is left at all.

2nd Application (Fig 4.5): A low first peak in methoprene occurred after the second day, then at day 4 concentration rose to a high peak of 12 μg/L, and decreased sharply within 2 days. After 19 days, concentrations were under detection limits. The rain event on day 7 may have been responsible for flushing of the dissolved chemical and subsequent rise in concentration from resuspension. A rain event on day 12 corresponded with a small peak in the methoprene concentration.

3rd Application (Fig 4.6): A first peak occurred at day 2 after third application, two small peaks are observed, but are much lower than in previous months. Methoprene concentration levels dropped to non-detectable levels 10 days after application, and appear again for one day at 22 days after application, after a small rain event. A major rain event occurred 20 days after application, but there was no corresponding rise in concentration. This may be due to complete dilution, degradation and flushing of the chemical.

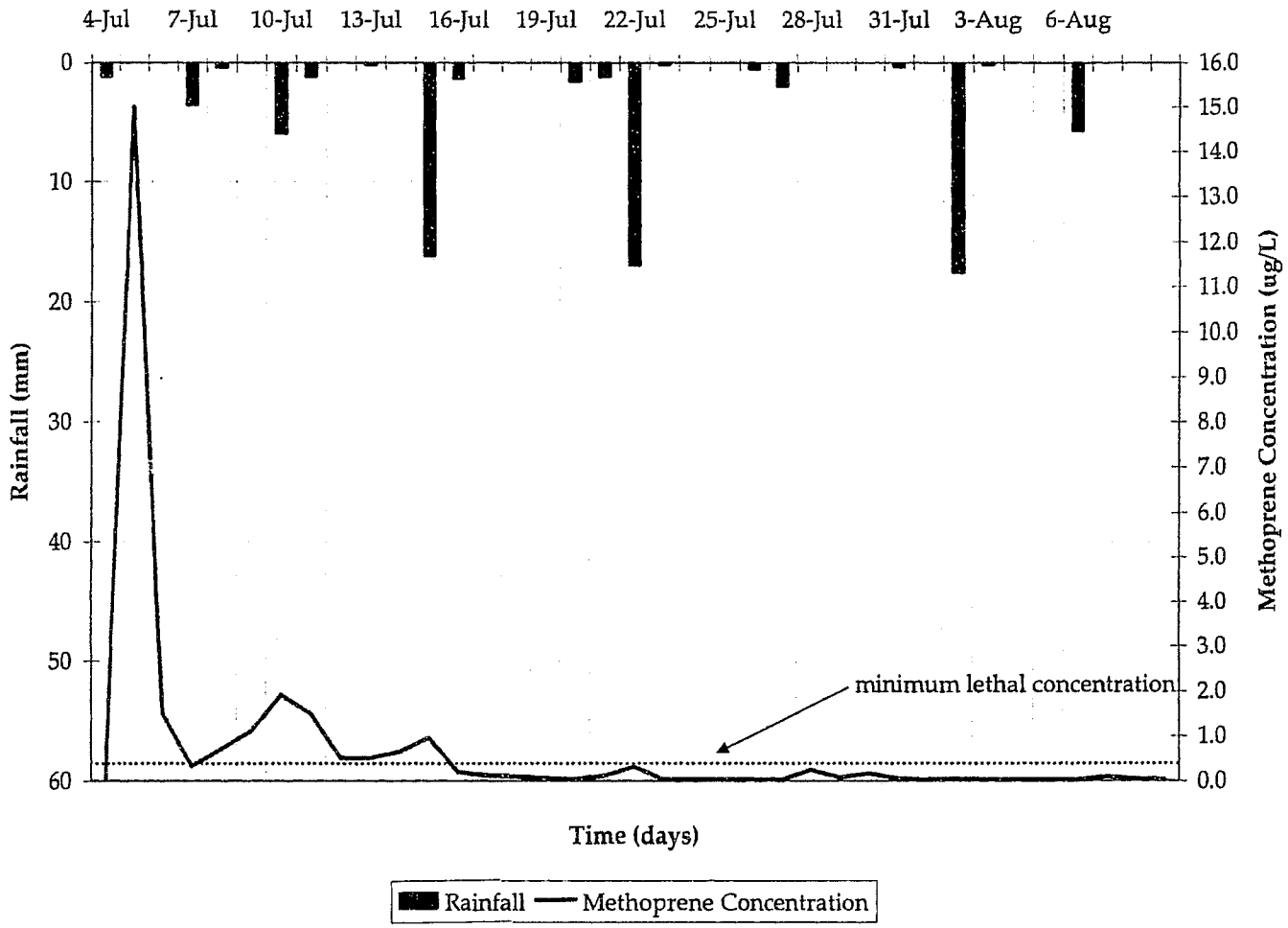

Figure 4.4 Rainfall in Newtonbrook region and methoprene concentration in catch basin S1 after the July 4th application.

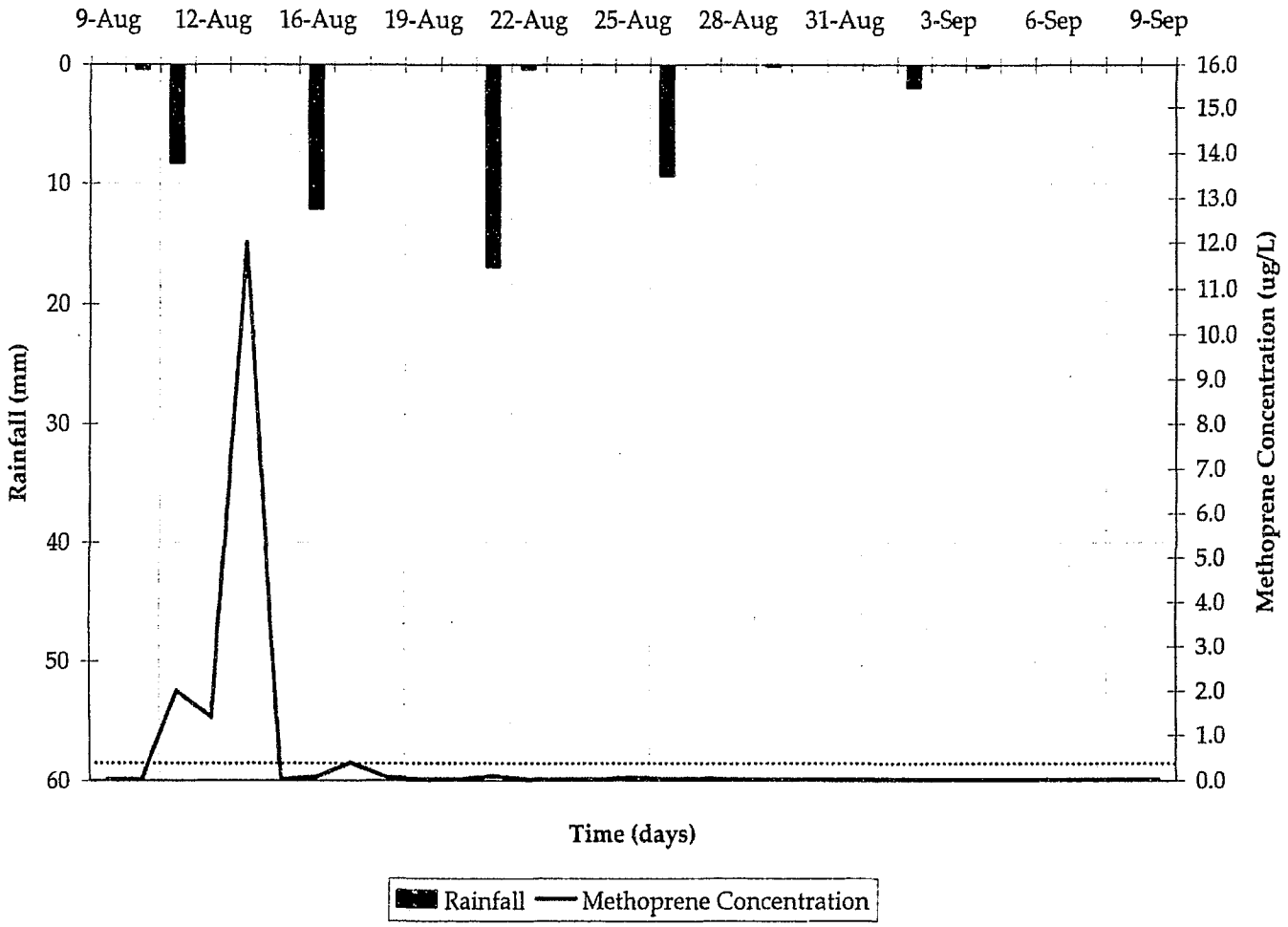

Figure 4.5 Rainfall in Newtonbrook region and methoprene concentration in catch basin S1 after the August 9th application.

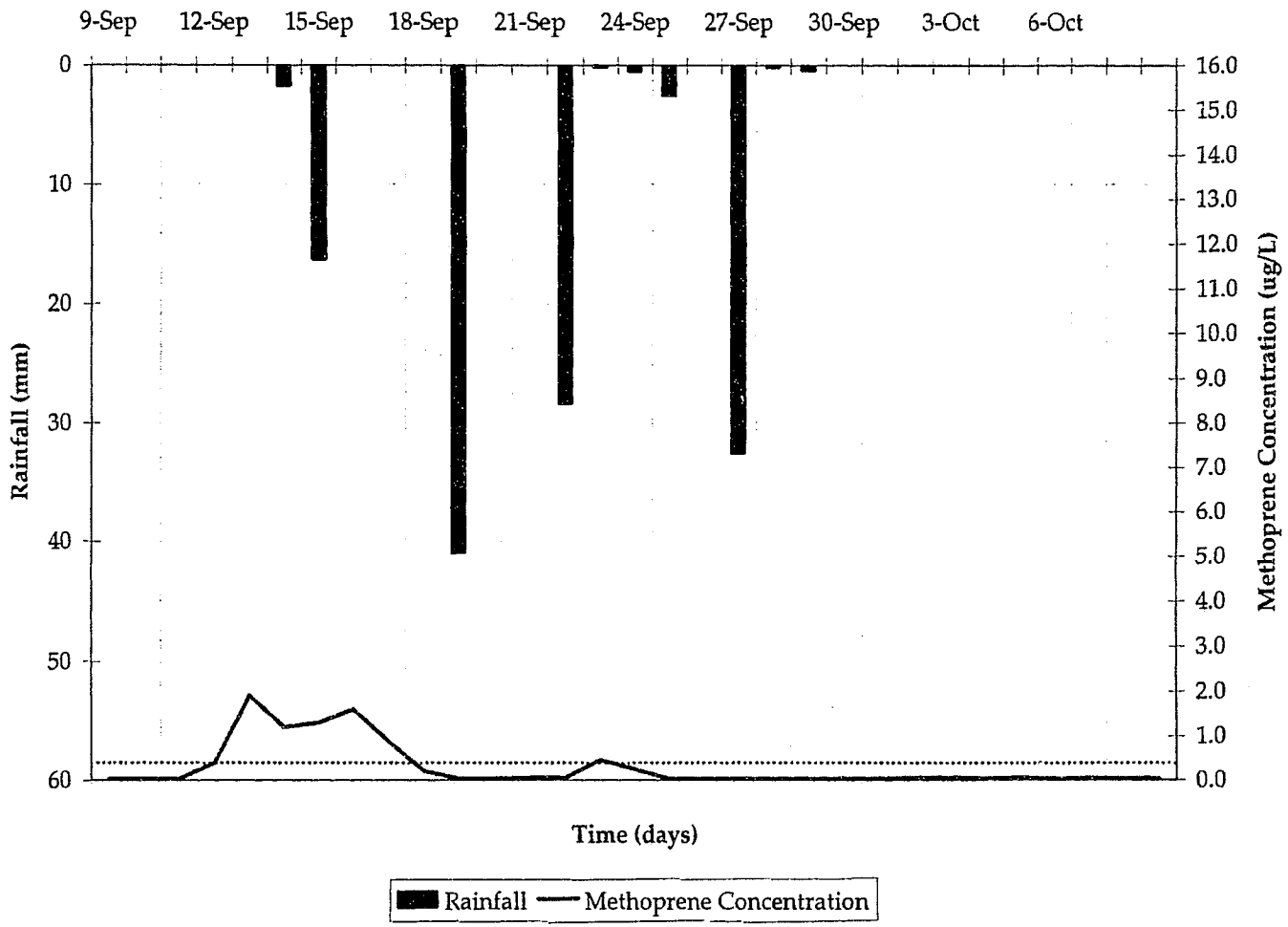

Figure 4.6 Rainfall in Newtonbrook region and methoprene concentration in catch basin S1 after September 9th application.

S2

1st Application (Fig 4.7): Methoprene concentrations were undetectable until 11 days after application and peak at day 12, showing small concentrations (0.05-0.06 μg/L) for 2 days only and then were undetectable for the remainder of the trial. The water volume in this catch basin caused the methoprene concentration to be so dilute that it was barely present at detectable levels. A rain event on day 11 may have been responsible for the slight rise in concentration on day 12, probably caused by turbulence in the sump water.

2nd Application (Fig 4.8): The same trend is observed as the one during the 1st application. However, methoprene concentrations were undetectable until 20 days after the second application, when methoprene was detectable (0.07 μg/L) for one day.

3rd Application (Fig 4.9): Methoprene concentrations remained undetectable immediately after application, with no detectable levels until 22 days after application. However, this time the familiar two-peak curve is observed over a period of 5 days. A rain event of 16.3 mm, 7 days after application appears to have no effect on methoprene concentrations, but an event of greater magnitude (32.6 mm) 20 days after application may be related to the subsequent appearance of methoprene concentrations in the sump water.

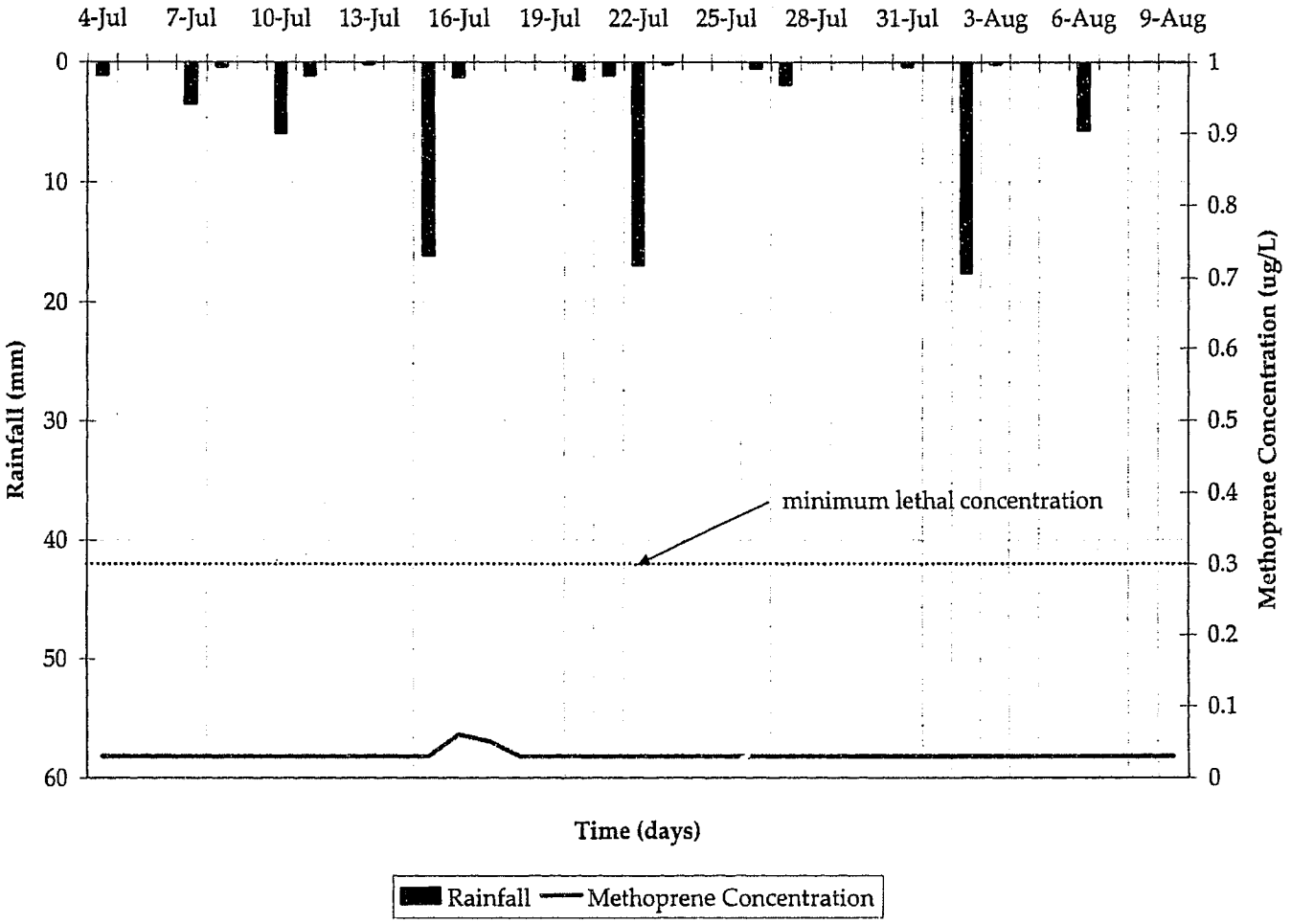

Figure 4.7 Rainfall in Newtonbrook region and methoprene concentration in catch basin S2 after July 4th application.

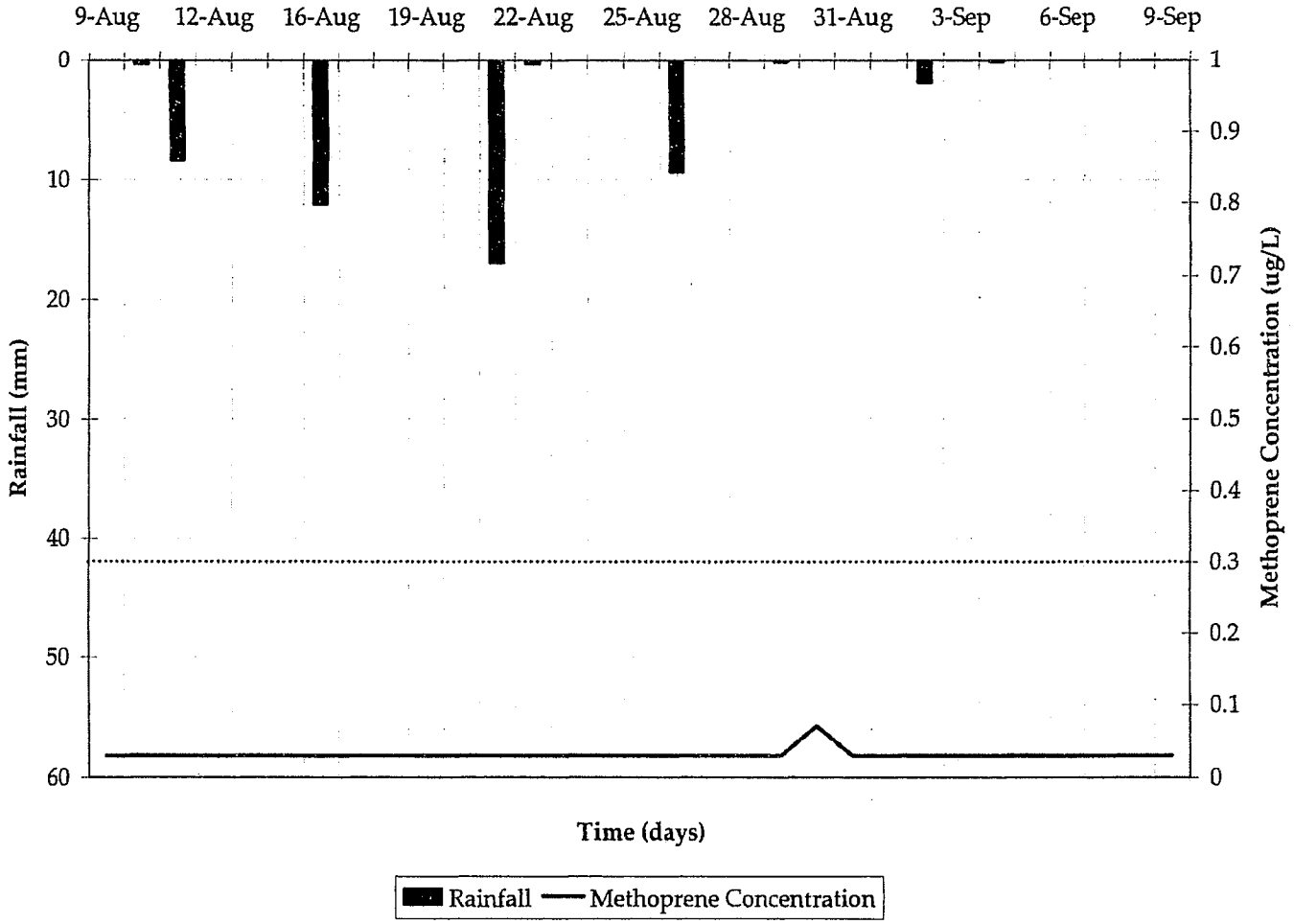

Figure 4.8 Rainfall in Newtonbrook region and methoprene concentration in catch basin S2 after August 9th application.

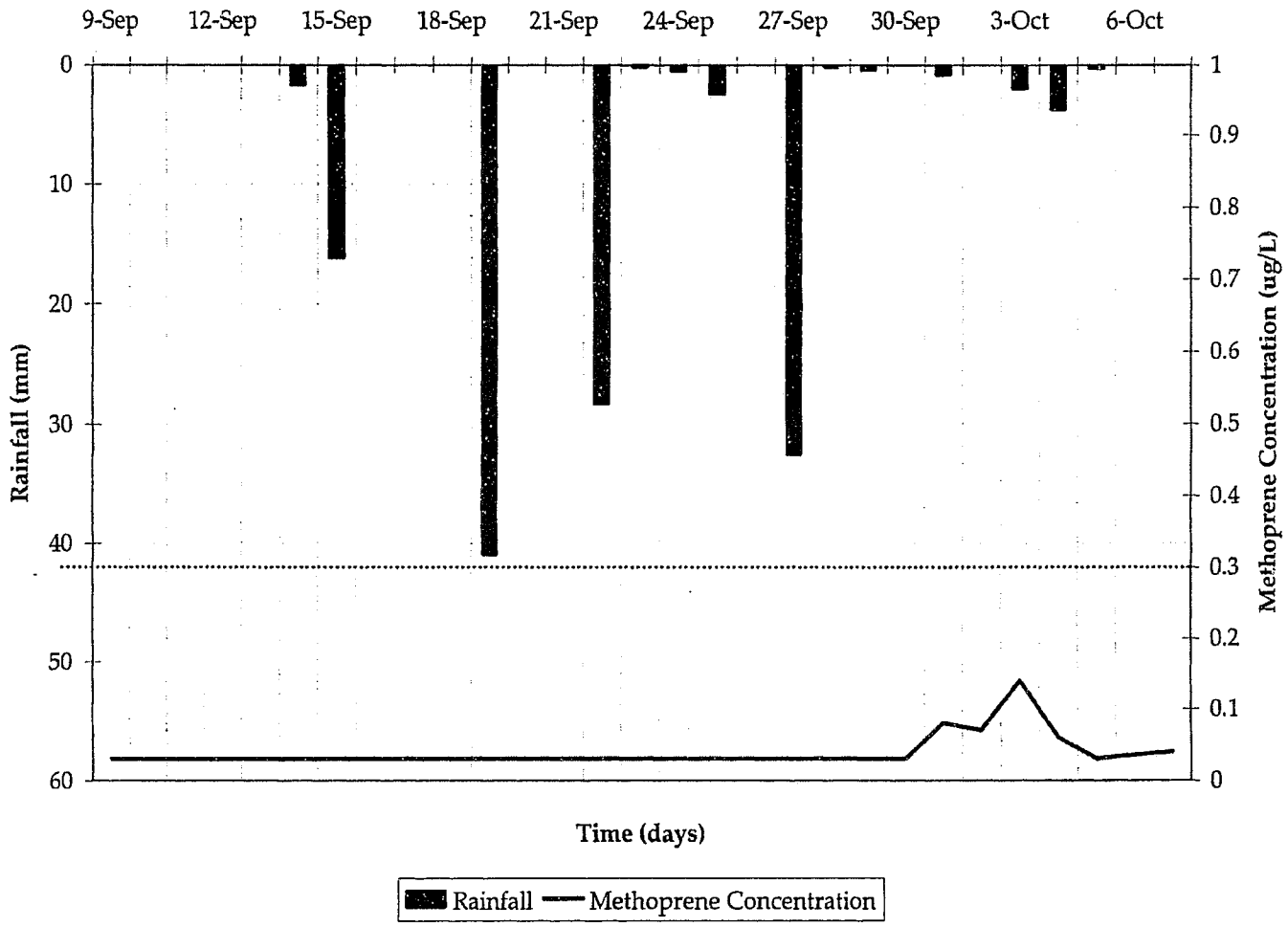

Figure 4.9 Rainfall in Newtonbrook region and methoprene concentration in catch basin S2 after the September 9th application.

The concentration of methoprene in the catch basins is affected by the amount of water stored in the catch basin. This has been observed in previous field studies where shallow ponds had greater concentrations than deeper ponds (Hershey 1994). For the experiment, S1 had not been vacuumed out and had the least amount of water; as a result the methoprene concentration was least affected by dilution, and concentrations were detectable even 35 days after application. S2 and W1 contained more water and had much lower methoprene concentrations. The less water in a catch basin, the longer the methoprene concentrations remained detectable (>0.03 μg/L).

The shape of the methoprene concentration curves for the catch basins may be due to the gradual breakdown of the pellets in the first few days, and subsequent peaks after the first 4 or 5 days could be due to resuspension caused by turbulence in the catch basin water due to rain events. Rain events may also be responsible for flushing of the chemical, although both of these results are difficult to correlate directly with rainfall. The magnitude of the rain event may also dictate whether the chemical is flushed or if it only causes resuspension or both. Methoprene concentration curves for W1 and S1 for the month of September, which had the heaviest rains during our study period, show that after the initial two-peak curve, small rises in concentration are detected one to two days after a heavy rain event.

Because rainfall data came from three separate gauges, the E. Bales station at Bathurst and Sheppard, the Mitchell field station at Church Ave., and the Newtonbrook Creek station at Willowdale and Silverview, some rain events that affected only the Willowdale and Silverview area are not considered in the rainfall data as the gauges were too far away to record events localized in that area. The presence of methoprene in the storm sewer outfall indicates that the chemical may have been flushed from the sewershed and into receiving waters.

This may have ecological implications for other invertebrates and amphibians downstream.

4.1.2. Larvae presence and rainfall

The presence of mosquito larvae was observed in all three catch basins over the course of the field study. The W1 (Fig 4.10) catch basin had the least frequent larvae observations, while S1 (Fig 4.11) and S2 (Fig 4.12) were usually filled with larvae. No larvae were detected in any catch basin after September 18th. All mosquitoes sampled from the Newtonbrook catch basins were identified as *Culex spp.*, 90% of which were identified as *Culex pipiens* (Baker 2003).

In order to assess the efficacy of methoprene at preventing larvae emergence, late instar larvae must be collected and reared. Due to the insufficient number of late 3rd and 4th instar larvae present in the collected samples, no efficacy statements can be made at this time. This indicates the need for further studies. Most adult emergences occurred from samples collected later in the larviciding program; however, there is not enough data to attribute this to the efficacy of methoprene. Rain may have been a factor in determining the presence of mosquito larvae in the catch basins; however, it is difficult to observe a direct correlation. Geery (1989) observed flushing of larvae from catch basins during rain events, the greater the rainfall, the more larvae were washed out.

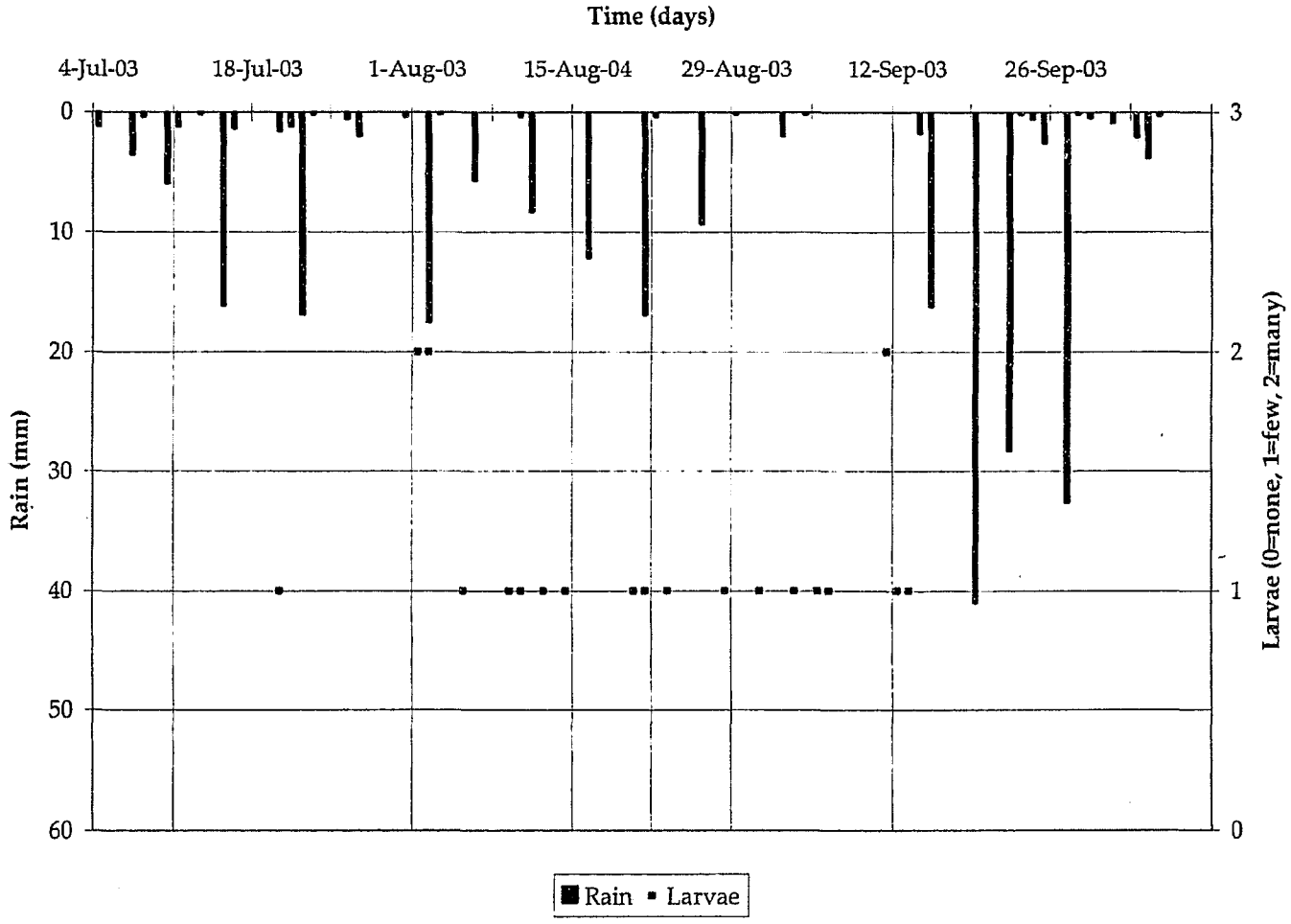

Figure 4.10 Rainfall in Newtonbrook region and number of larvae observed in catch basin W1 during summer 2003.

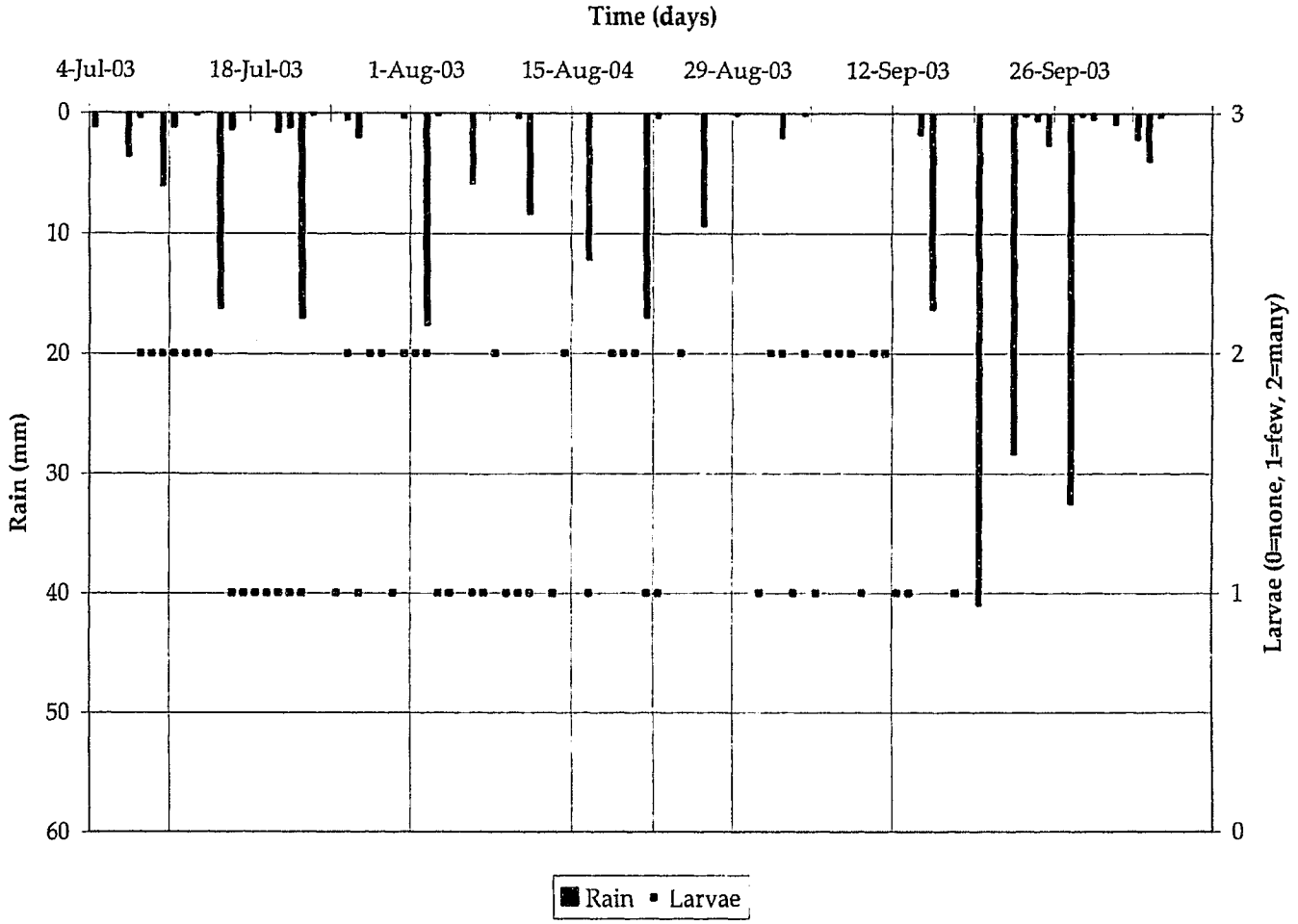

Figure 4.11 Rainfall in Newtonbrook region and number of larvae observed in catch basin S1 during summer 2003.

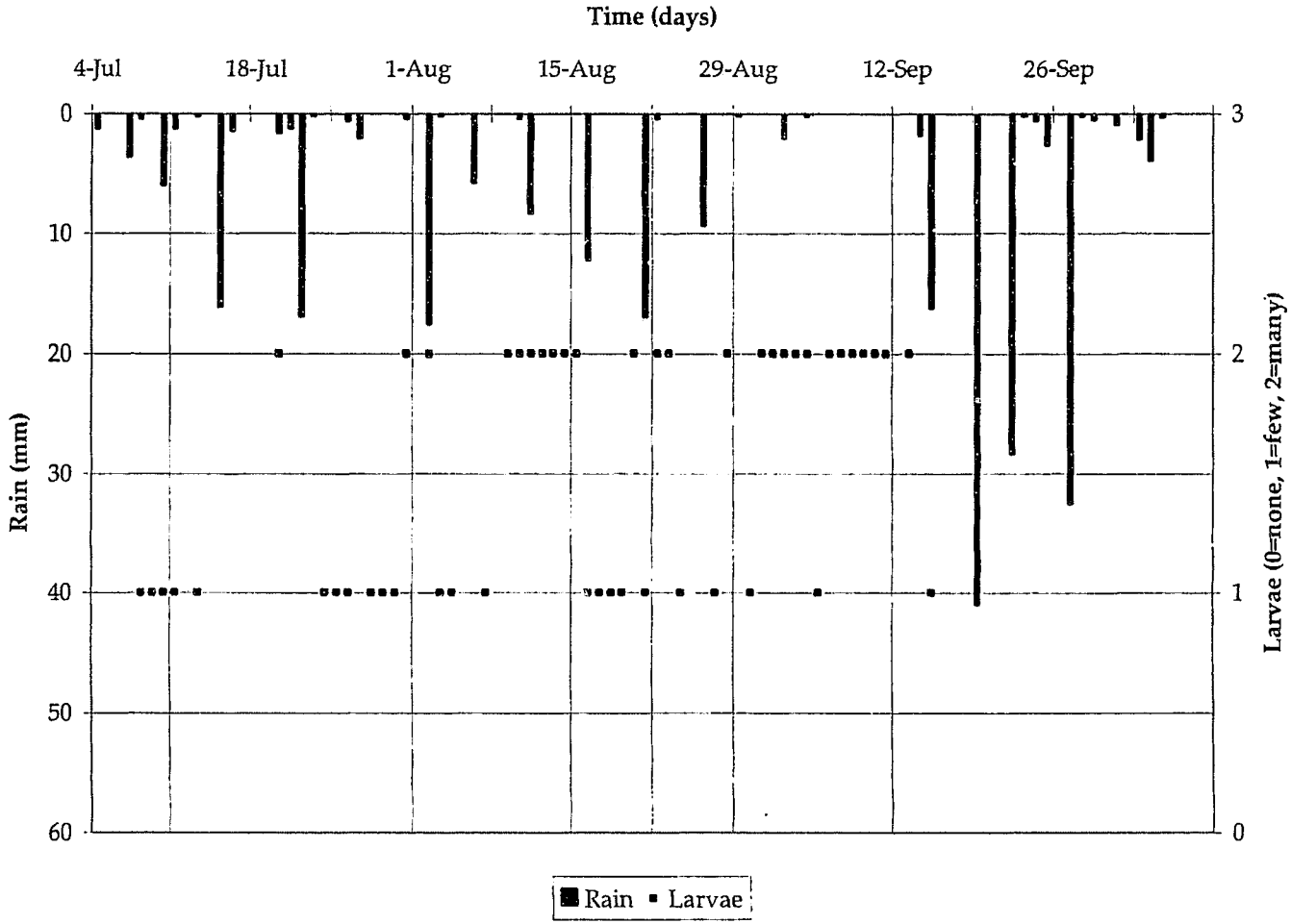

Figure 4.12 Rainfall in Newtonbrook region and number of larvae observed in catch basin S2 during summer 2003.

4.1.3. DOC and Chloride vs. Larvae

Chloride and DOC concentrations were monitored weekly to assess if these could be factors in the number of larvae observed. All three catch basins had similar concentrations of DOC (4.13-15), which is an important source of nutrition for *Cx. Pipiens* larvae (Clements 1992). W1 had the highest chloride concentrations (Fig 4.13), and also the lowest number of larvae observed. While chloride was present in both S1 (4.14) and S2 (4.15), the concentration was lower than in W1, and many larvae were observed frequently in both catch basins. The presence of chloride ions may indicate high salt concentrations which can be detrimental to larval growth (Clements 1992).

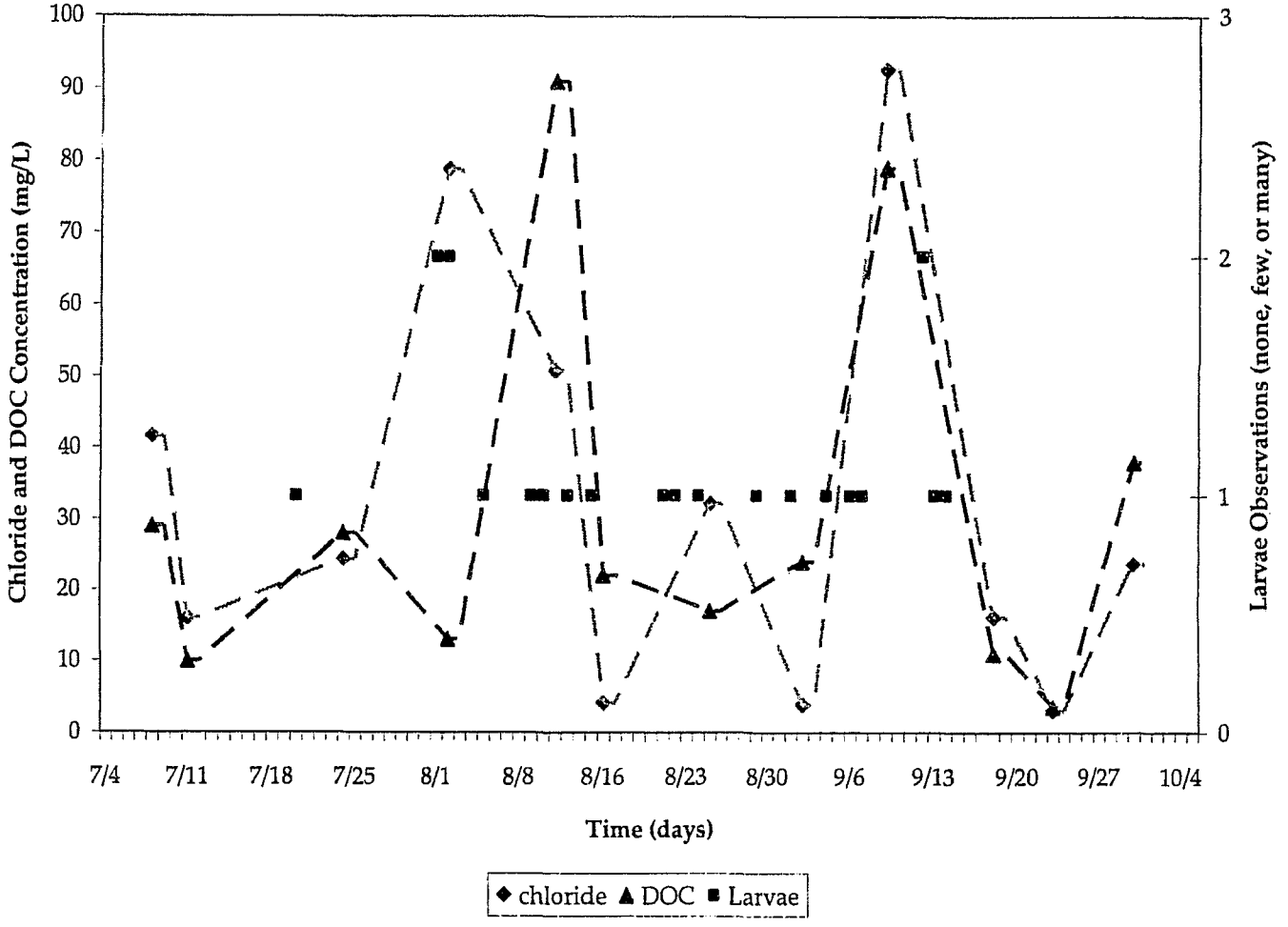

Figure 4.13 Chloride ion and dissolved organic carbon concentration and number of mosquito larvae observed in catch basin W1 during July, August and September 2003.

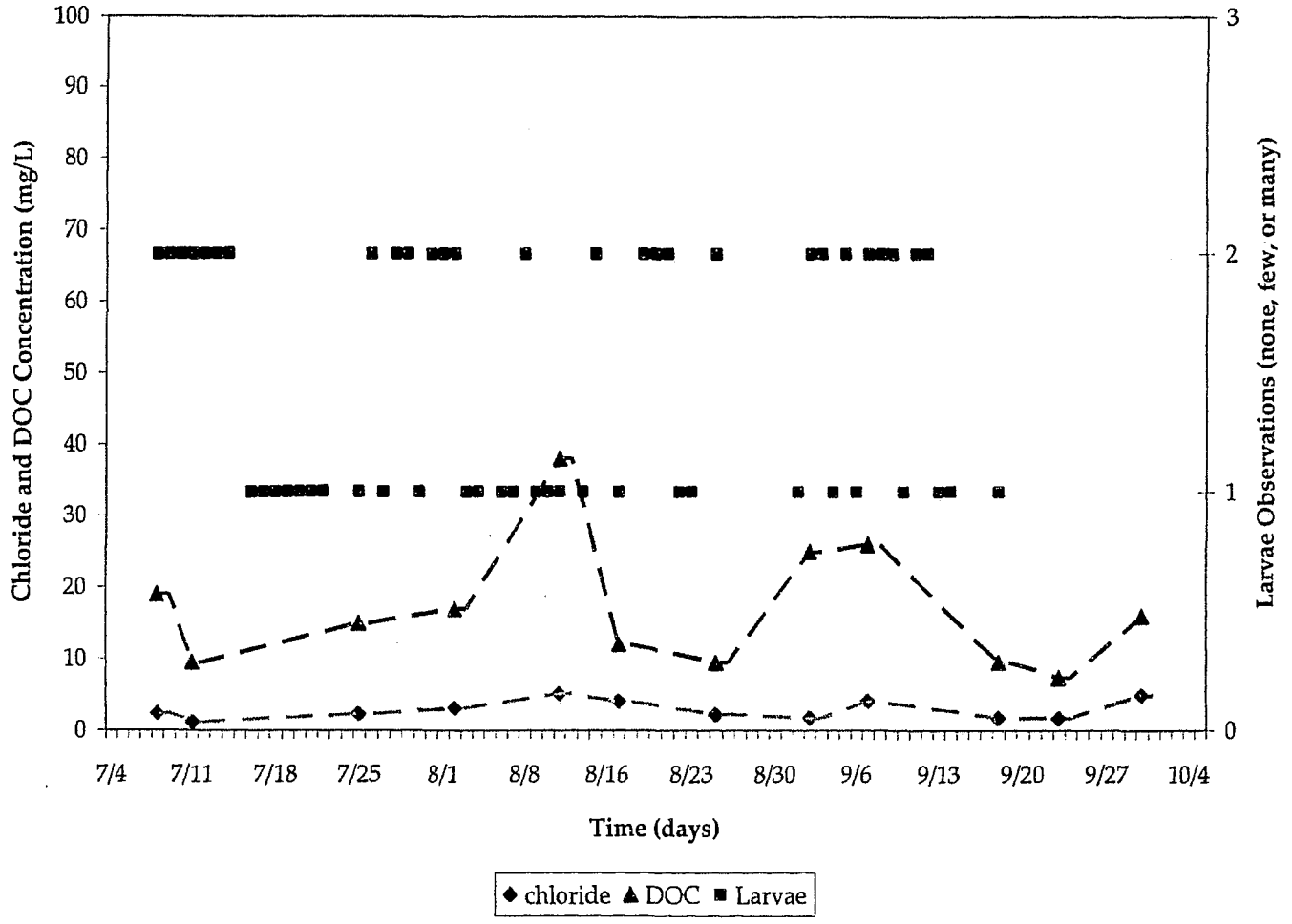

Figure 4.14 Chloride ion and dissolved organic carbon concentration and number of mosquito larvae observed in catch basin S1 during July, August and September 2003.

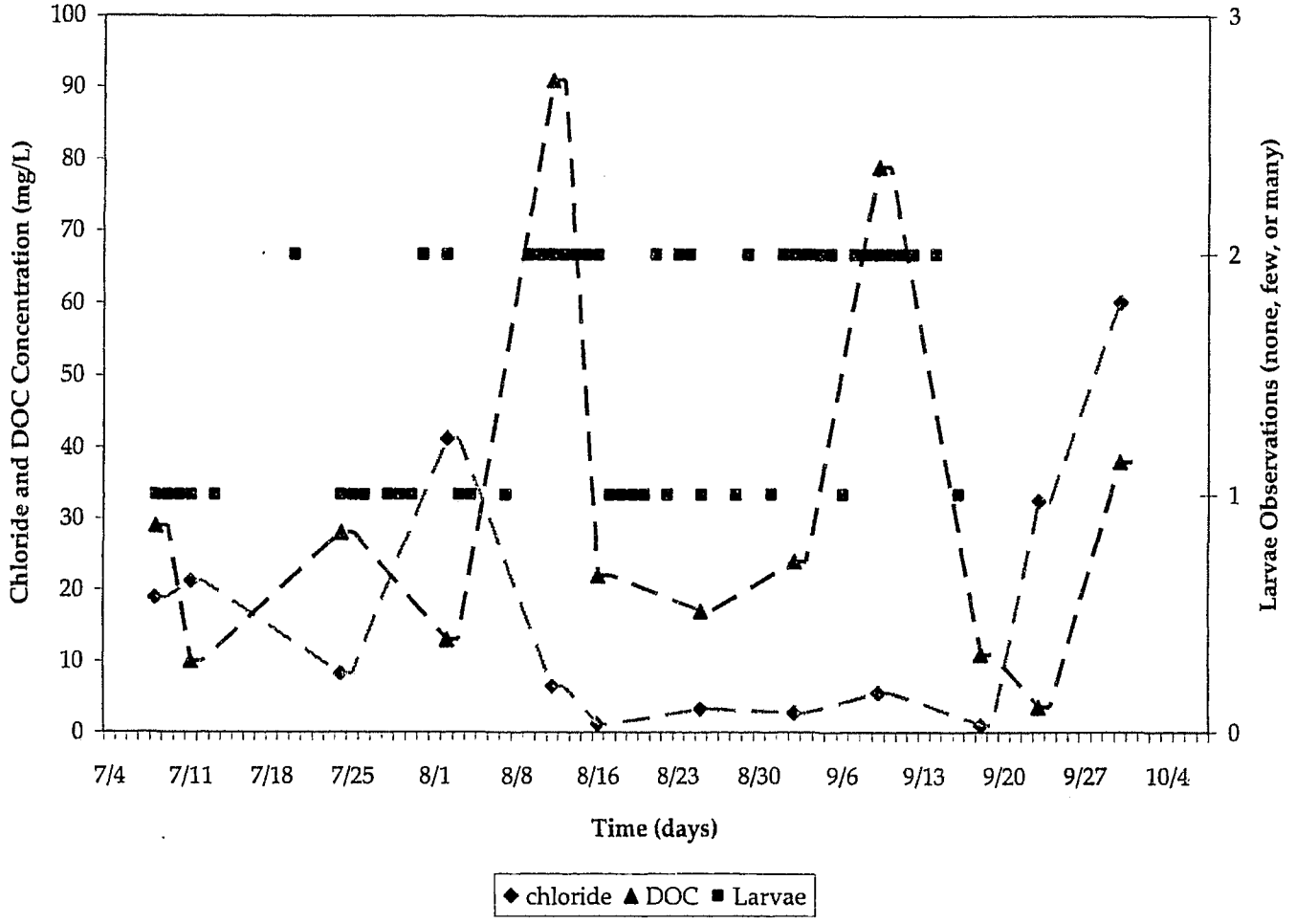

Figure 4.15 Chloride ion and dissolved organic carbon concentration and number of mosquito larvae observed in catch basin S2 during July, August and September 2003.

4.1.4. Malathion

The City of Toronto analytical laboratory that handled the water samples from the catch basins simultaneously tested for methoprene and malathion, another pesticide used as an adulticide for domestic, municipal and agricultural use (USEPA 2002). Malathion was detected in catch basin W1 (Fig 4.16) only, beginning July 20th, and was present regularly until September 20th. This and other unidentified pesticides may have contributed as additional inhibition to larval growth in this catch basin.

Malathion concentrations would have an opposite relationship with rain than methoprene, as rain entrains malathion from surrounding areas into the catch basins, increasing the mass present in catch basins, however, large volumes of rainfall will also dilute catch basin concentrations of malathion. The presence of malathion in catch basin W1 may have affected the larvae population, although no causal connection can be made between changes in malathion concentration and larvae observations. As it was not applied by the City of Toronto, the occurrence of malathion in catch basins is disturbing. It originates from domestic uses, and will be carried to natural waters through the storm sewer, having impacts on downstream organisms.

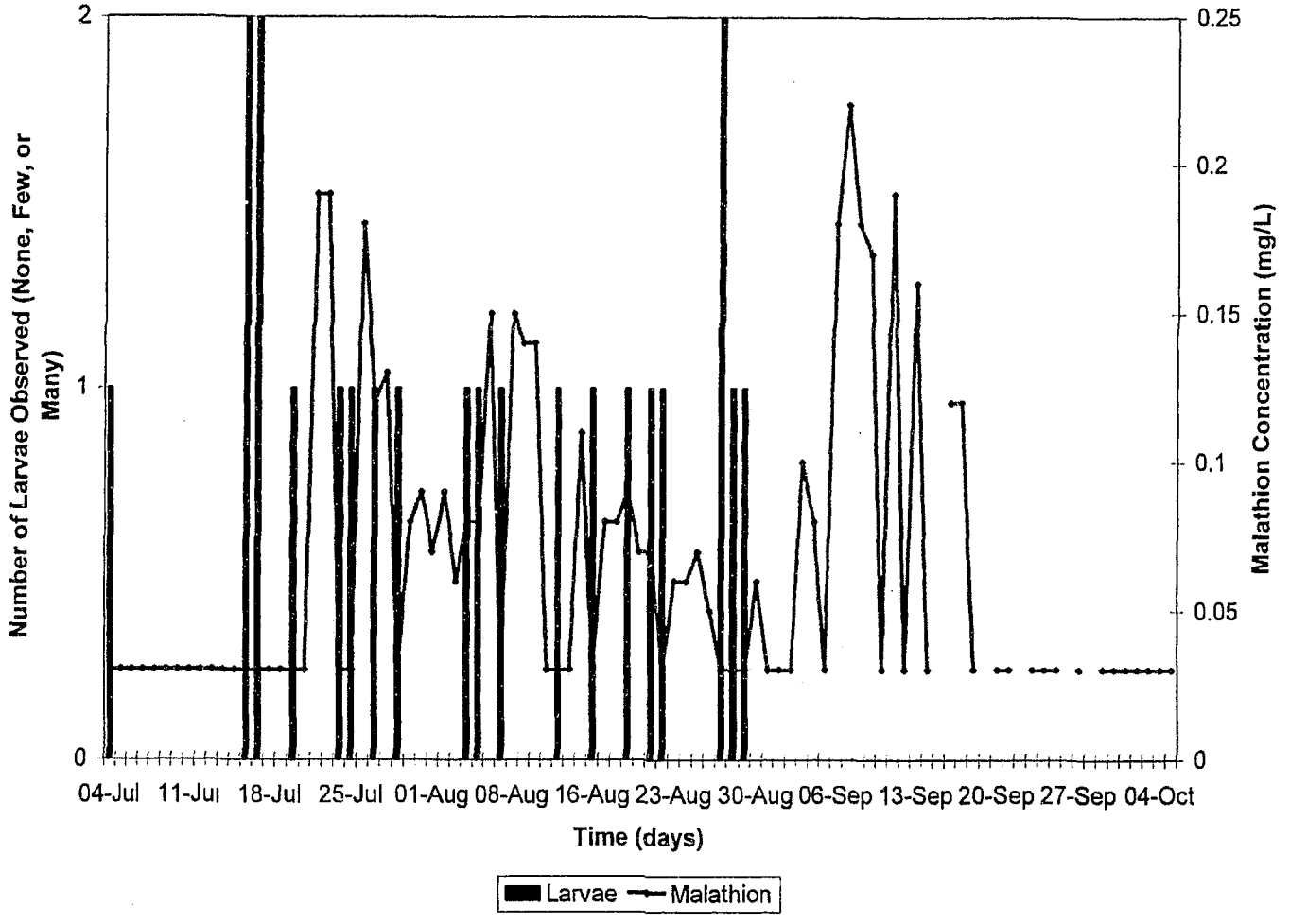

Figure 4.16 Malathion appeared in catch basin W1 on July 21st. This may have been a contributing factor in larvae control.

4.1.5. Heavy Metals and rainfall

In order to identify other possible inhibitory factors to larval growth, water samples from the catch basins were analyzed for heavy metals. Heavy metals were monitored weekly, but only after the 3rd application of methoprene as this factor had not been considered previously in the study. The most common metals detected were iron, manganese and aluminum, with a maximum concentration of 7.98 mg/L of iron in W1 (Fig 4.17). There were no occurrences of cadmium, molybdenum or tin, and a concentration of 0.008 mg/L of lead was detected only once in catch basin W1. Heavy metal concentrations were highest in W1, probably due to the higher amount of traffic on Willowdale Avenue, where car exhaust and debris enters the storm sewer. The second highest heavy metal concentrations were found in catch basin S1, followed by S2.

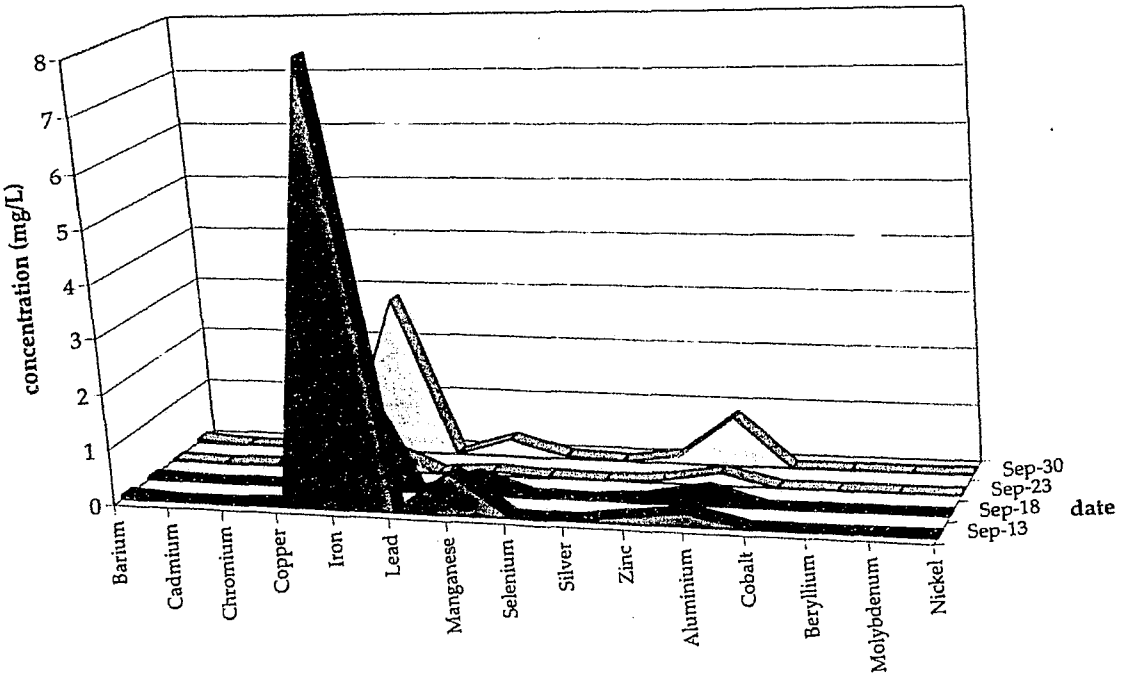

Figure 4.17 Highest heavy metal concentrations were found in catch basin W1 during the month of September, 2003.

4.1.6. Outfall methoprene concentration and rainfall

The storm sewer outfall was sampled during 10 separate rain events over the course of the field study. The events on July 7th, August 26th and August 6th are shown in Figures 4.18, 4.19-a and 4.20-a. Rain gauge data from the Newtonbrook station were only available after August 11th, and therefore data from E. Bales station and Mitchell fields were used for events prior to that day. The storm events that occurred in September were not sampled due to equipment failure.

In general, an increase in flow and in the concentration of methoprene (Fig 4.19-b, 4.20-b) in the outfall can be observed during rain events. The shape of the curves suggest a balance between the processes of dissolution and decay. of The highest methoprene concentration observed in the outfall was 0.24 μg/L (July 7th), which is well below the USEPA recommended environmental concentration of 10 μg/L, and the ecotoxicity value for aquatic invertebrates of 360 μg/L, under the conditions monitored.

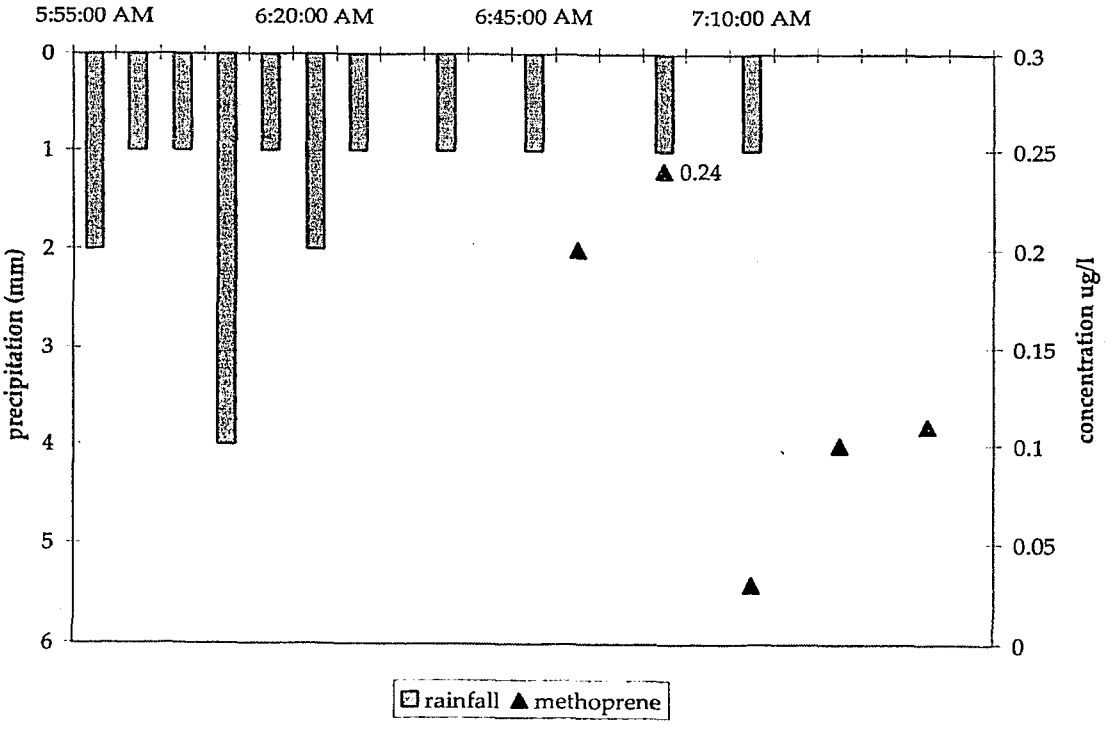

Figure 4.18 Concentration of methoprene in the Newtonbrook outfall during a rain event July 7th.

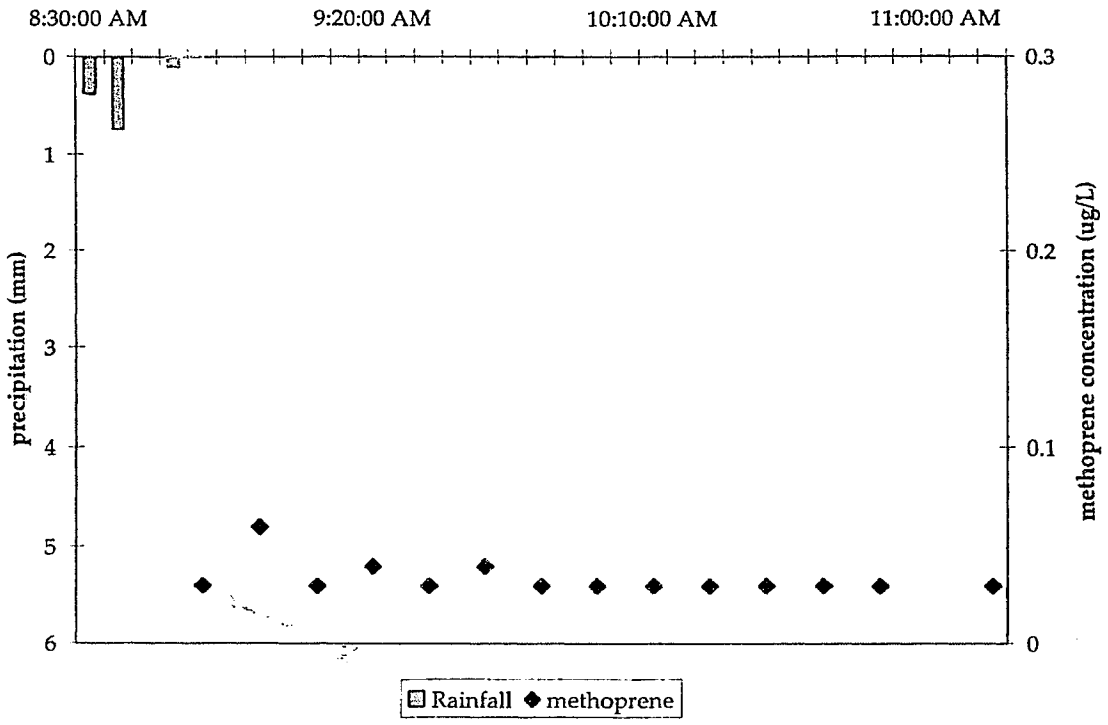

Figure 4.19-a Concentration of methoprene in Newtonbrook outfall during a rain event August 16th.

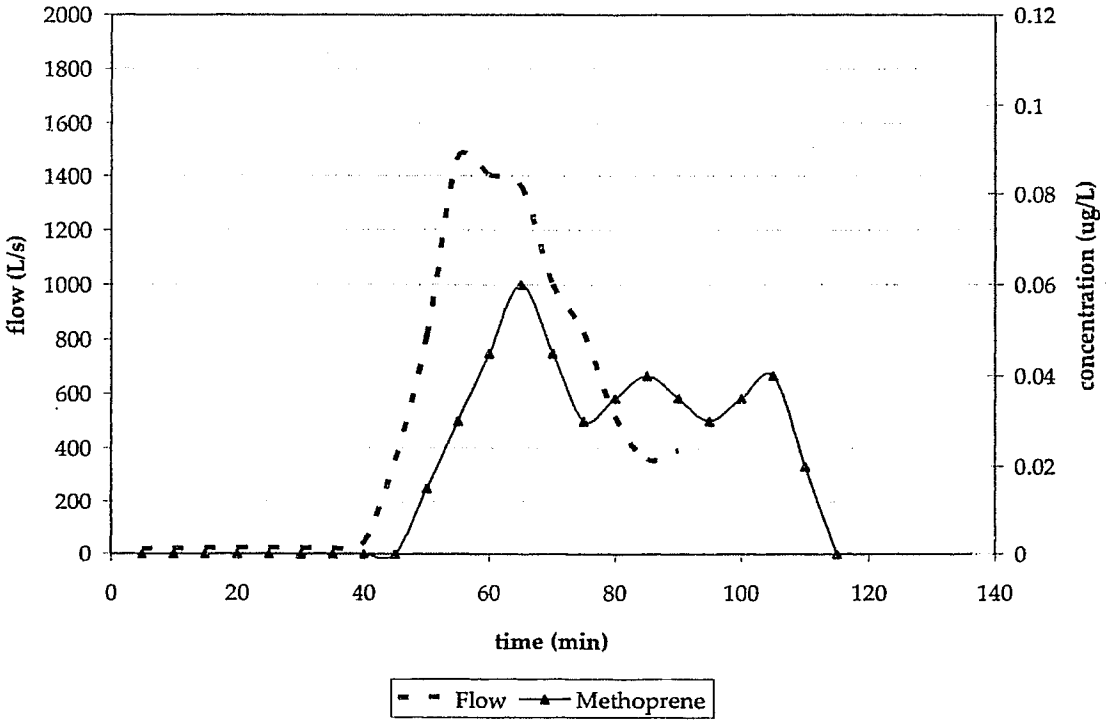

Figure 4.19-b Concentration of methoprene and outfall flow during a rain event August 16th.

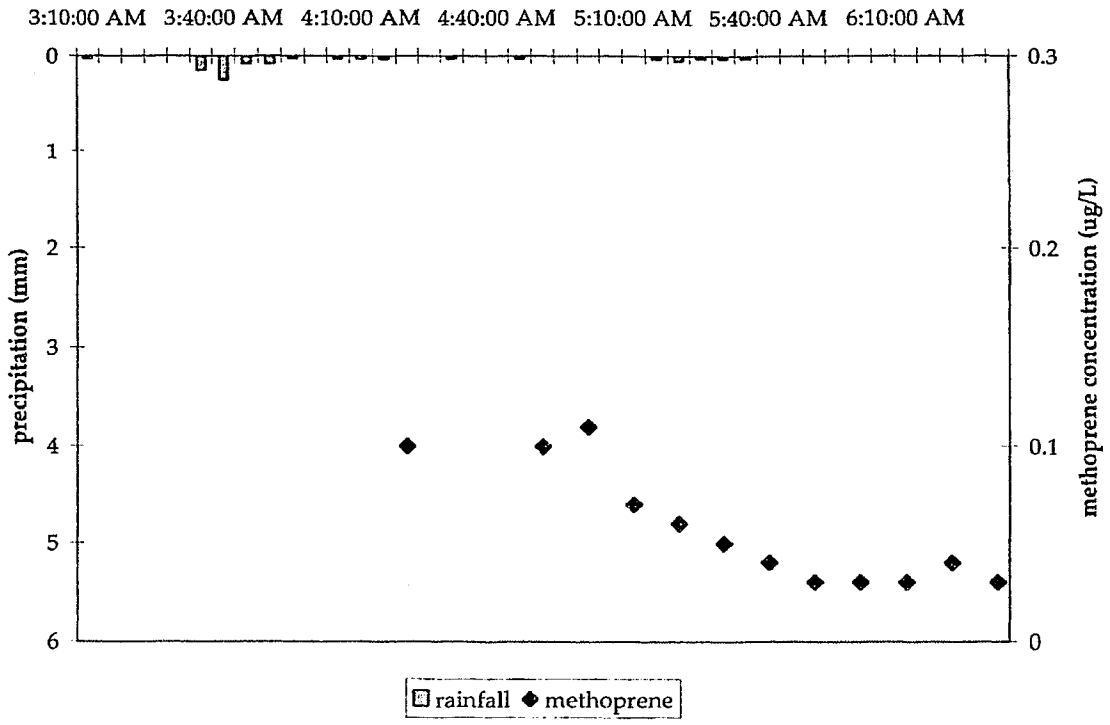

Figure 4.20-a Concentration of methoprene in Newtonbrook outfall during a rain event August 26th.

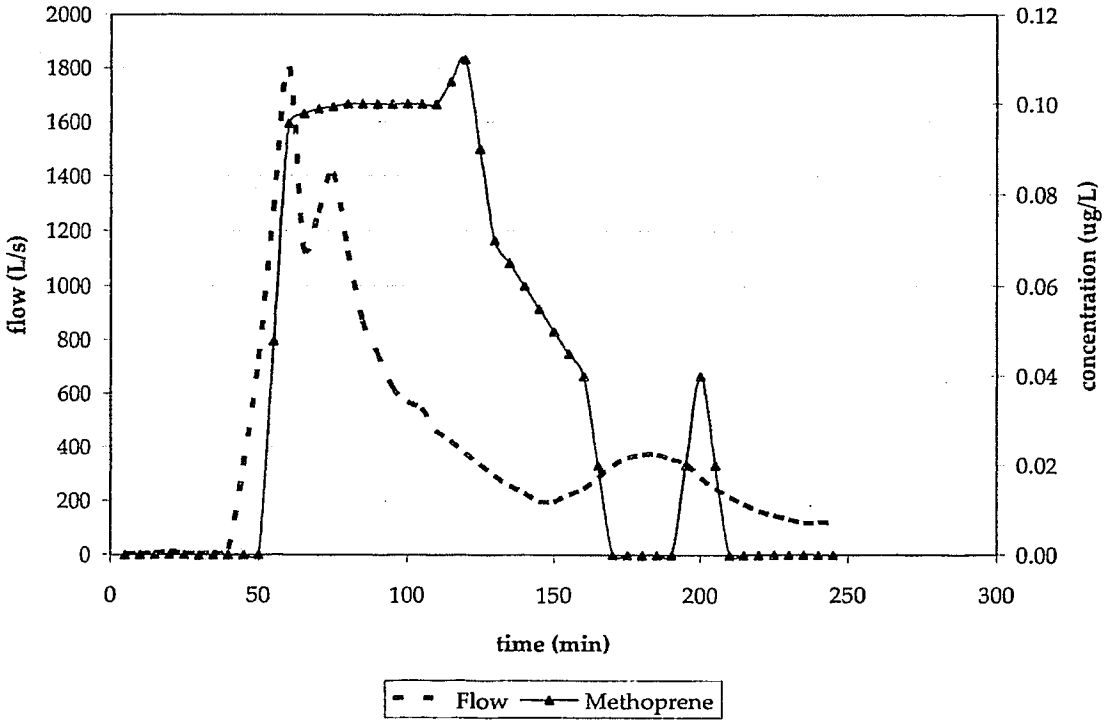

Figure 4.20-b Methoprene concentration and outfall flow during a rain event August 26th.

4.2. Laboratory Experiment Results

In order to observe the behaviour of methoprene under quiescent conditions, a model catch basin tank was dosed with methoprene under the controlled conditions in a laboratory. The results of the first batch of trials prompted questions about the effect of water depth and pellet size on the concentration of methoprene over time in static water (Table 4.1).

Table 4.1 Experimental setup of lab tank.

	1st batch			*2nd batch*			
Water depth (mm)	900	900	900	600	450	600	600
Pellet mass	0.710	3.534	35.074	0.708	0.728	0.724	0.713
Pellet size	Random	Random	Random	Random	Random	Long	Short

4.2.1. Full tank & random size, 0.7g, 3.5g, and 35g

The doses of methoprene for the first batch of trials in the laboratory tank were 0.7 g, 3.5 g and 35 g in 272 litres of tap water for the 1st, 2nd and 3rd trial, respectively. The shape of the curves for methoprene concentration over time for each of the spouts; MT, MM, and ML, seem to be parallel to each other (Fig 4.21), and exhibit the double-peak shape observed in the catch basin concentrations, for the majority of the experiment. Samples from the top spout showed that in general, concentrations at the surface of the water were slightly higher than at the middle or the bottom, which is desired as *Cx. Pipiens* larvae live at the interface between air and water.

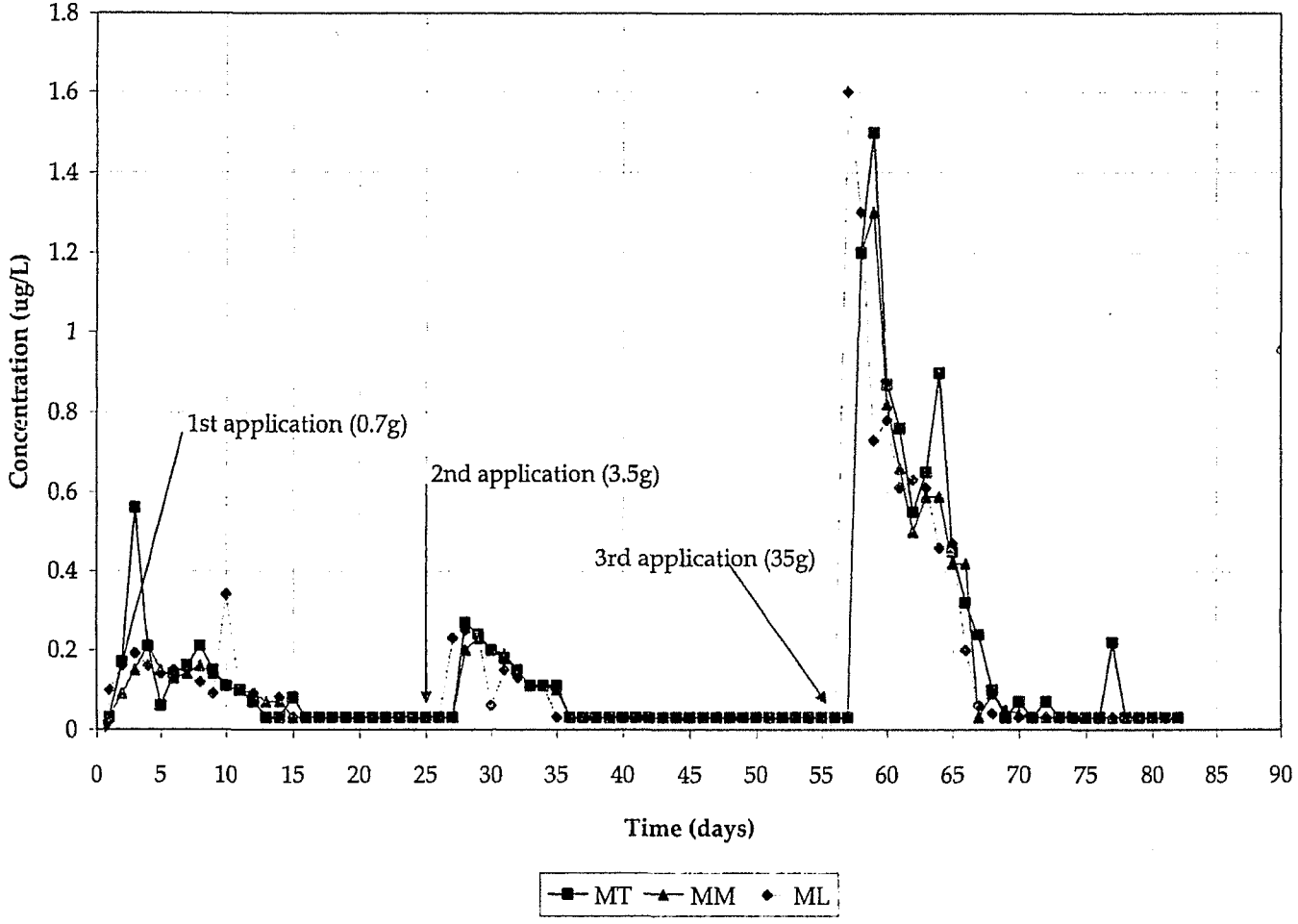

Figure 4.21 Methoprene concentration in the model catch basin in the first batch of laboratory experiments.

The second dose was approximately 5 times the first dose, yet concentrations in the tanks were similar to the first trial, but had lower peak concentrations. This was hypothesized to be attributed to the high variation in length of the individual methoprene pellets, as they all had similar measured diameters, but an irregular distribution of length, which could affect the physical breakdown of the pellets. The third dose was 50 times the first dose, and showed a much more elevated concentration of methoprene, peaking at 1.6 µg/L.

Exponential trend lines were fitted to the methoprene concentration curves for each spout, for each trial, beginning at the first peak in concentration. The decay rates range from $0.045d^{-1}$ to $0.1829d^{-1}$ (Table 4.2) and the reaction order for all curves was 1st order exponential decay.

Table 4.2 Exponential regression equations and R-squared values for methoprene concentration decay curves in 1st batch of lab tank experiments.

	1st trial - O.7g			*2nd trial – 3.5g*			*3rd trial – 35g*		
	MT	MM	ML	MT	MM	ML	MT	MM	ML
Decay rate (day^{-1})	0.0983	0.045	0.0983	0.0686	0.0627	0.0586	0.1765	0.1766	0.1829
R-squared	0.695	0.8288	0.731	0.6029	0.5606	0.5192	0.7436	0.7563	0.8102

Published values for the decay of methoprene in water indicate a half-life greater than 4 weeks, and a daily release rate from solid formulations of less than 4 µg/L (Wellmark International 2003; USEPA 2001). Light increases the decay rate of methoprene, as its half-life is less than 10 hours by photolysis.

4.2.2. Methoprene concentration and water depth

Preliminary results from the field study and lab tank experiment suggested that there may be a relationship between water volume and the rate of

dissolution and decay of methoprene. In order to observe this interaction, a second batch of trials was conducted using the mosquito control dose of 0.7g, and two different depths of water, 450 mm and 600 mm.

In general, the concentration curves for the different water depths were similar and exhibited multiple peaks (Fig 4.22). Both tanks maintained methoprene concentrations above 0.5 μg/L until the 23rd day when the concentration in the tank with the greatest volume was 0.44 μg/L at the lower tap. However, the tank had concentrations of 1.8 μg/L from the lower tap at day 25 of the trial. All samples had concentrations above detection limits for the duration of the trial. Some samples on day 11 and on days 20, 21 and 22 were lost due to instrument failure in the analytical lab.

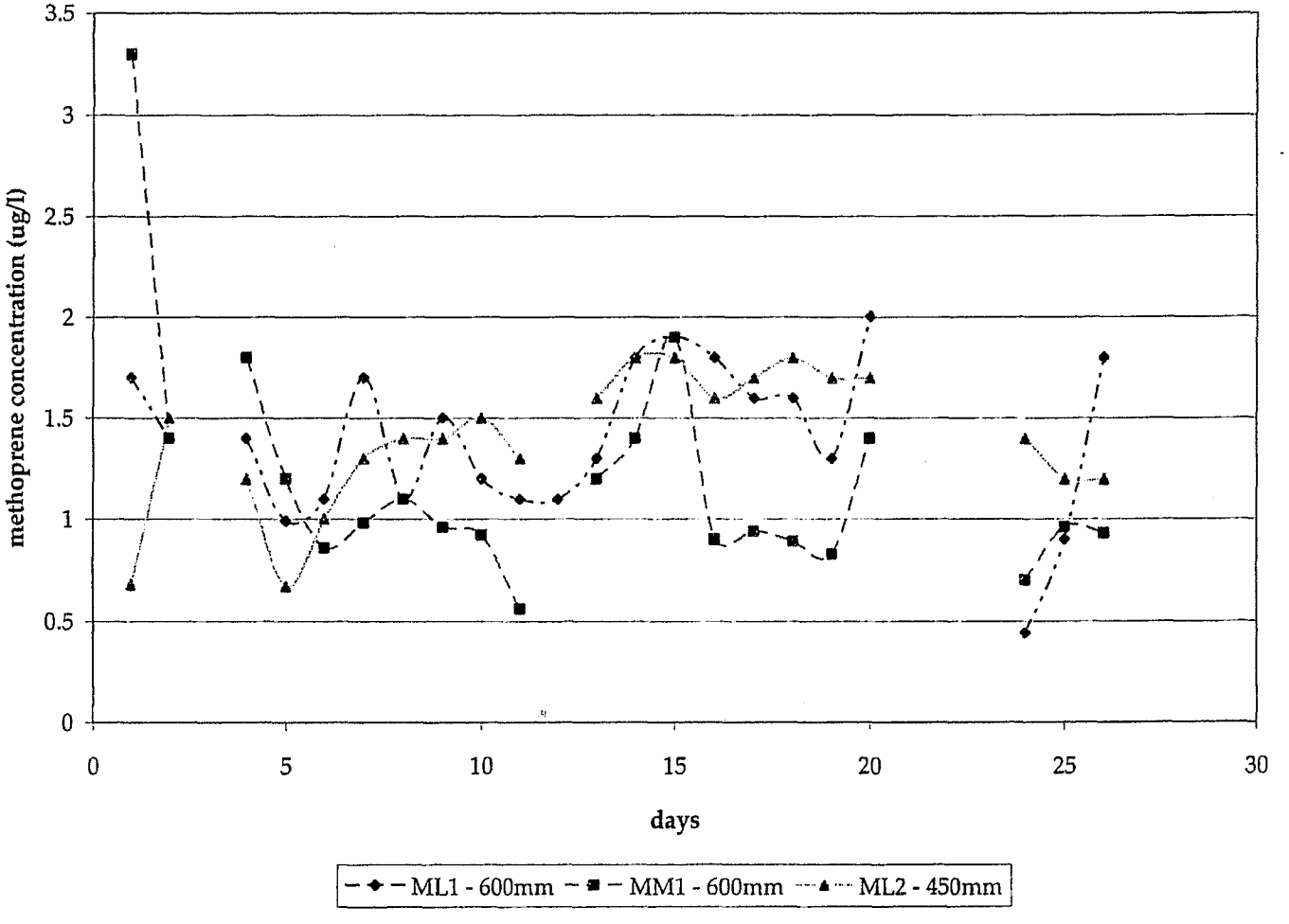

Figure 4.22 Methoprene concentration in model catch basin at two water depths.

4.2.3. Methoprene concentration and pellet size

During the first batch of lab experiments, the second trial, which was conducted with 5 times the mass of pellets of the first trial, showed a concentration curve that did not peak as high as the first trial, and did not maintain a sustained concentration for any longer. This suggested that the random distribution of pellet length may affect the total surface area of the pellet sample and either increase or decrease the dissolution rate of the pellets according to available surface area. In order to observe this relationship, a third batch of trials was conducted using two samples of pellets, both 0.7 g, one with all long pellets, and one with all short pellets. Both tanks had the same water depth, 600 mm.

Although both tanks had curves with multiple peaks, a definite trend in the concentration curves demonstrates that the sample of short pellets dissolves more rapidly and sustains a lower concentration of methoprene over the duration of the experiment (Fig 4.23). It suggests that longer pellets dissolved more slowly, and maintained a higher methoprene concentration over the duration of the experiment. The balance between decay and dissolution is shifted as dissolution is slowed down by the size of the pellets, and decay is balanced by a sustained release of methoprene. The longer pellets maintained a concentration of 0.5 μg/L or higher for most of the experiment, while the shorter pellets were below that concentration for the greater part of the trial.

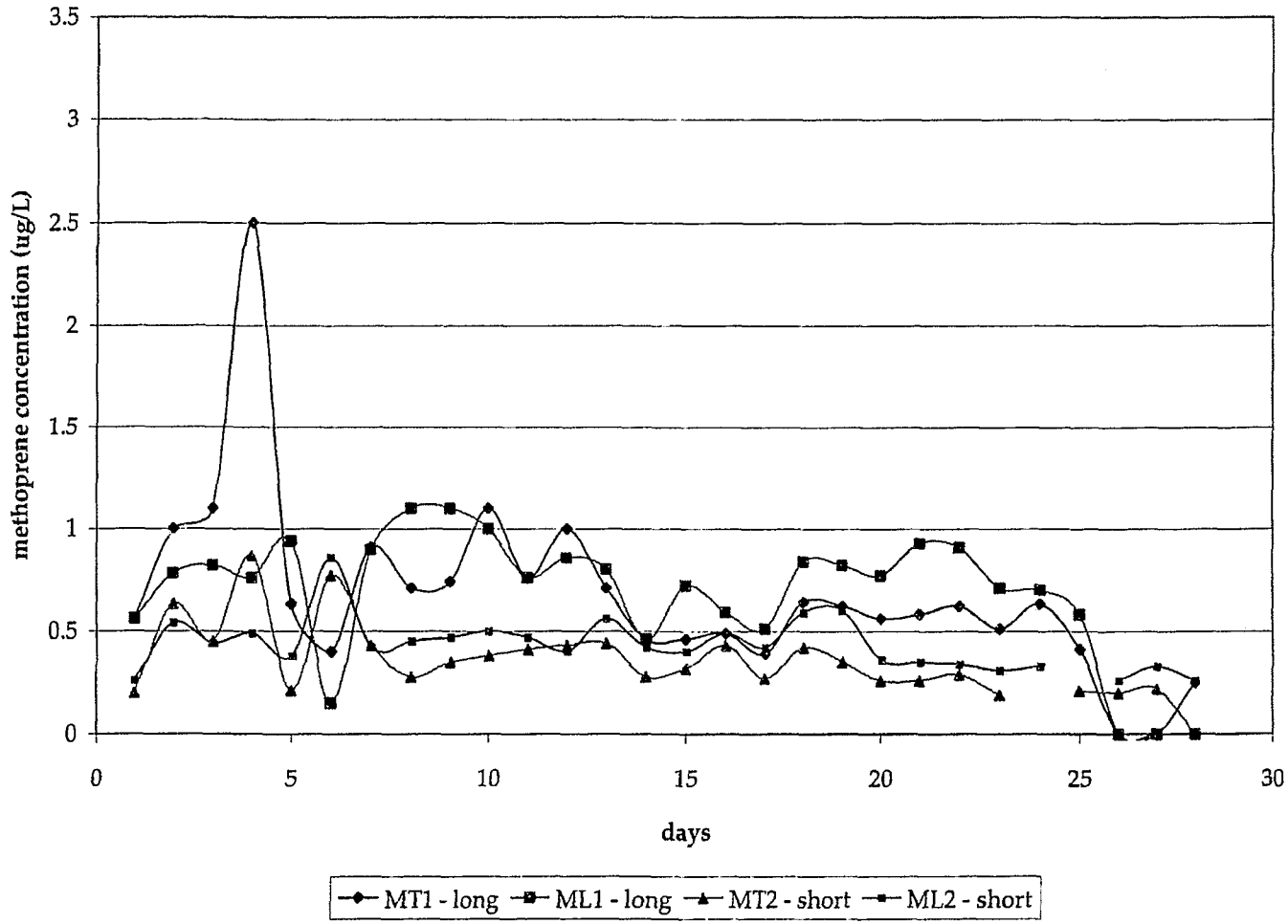

Figure 4.23 Methoprene concentration in model catch basin with two samples of different pellet sizes (gaps in plot lines due to lost samples during laboratory analysis).

4.3 Discussion

The field study has demonstrated that there are several environmental conditions affecting the concentration of methoprene in a catch basin, including rainfall and water volume. There are four major factors affecting the concentration of methoprene over time in the catch basins; the first two could be observed in the lab tank experiment, and the second two in the field study. These factors are:

- dissolution: when the pellets physically break down
- degradation: when the methoprene chemical decays into secondary products
- dilution: the chemical is diluted in the catch basin water
- flushing: which happens during rain events

The last is the factor which may have ecological implications for other invertebrates and amphibians downstream.

These physical characteristics, as well as the chemical composition of each catchbasin, also affect the frequency of larvae presence observations. It is clear that rainfall has some effect on the peak concentration of methoprene in catch basins and that rain contributes to flushing of the chemical through the storm sewer system and into downstream waters, which means that it is important to determine how much methoprene is being added to natural waters. Rain may have also been a factor in determining the presence of mosquito larvae in the catch basins; however, the current data do not confirm a correlation. The chloride concentration is probably the more likely inhibitory factor and W1 had the fewest larvae observations, and had the highest chloride concentration. The presence of malathion in catch basin W1 may also have affected the larvae population.

In the quiescent conditions of the laboratory tank, methoprene was present at detectable levels for approximately 7 days longer than in the catch basins. This may be due to different water levels, flushing and changes in temperature. The second batch of laboratory trials showed that varying water levels had less impact on the concentration of methoprene than did the size of the pellets.

In general, the shapes of the concentration curves over time are similar for the catch basins and the laboratory tank, exhibiting two peaks before decreasing to non-detectable levels. This shape suggests a balance between the decay and dissolution of the methoprene pellets. The rises in concentration late in each trial for some of the catch basins are not seen in the laboratory tank, and may therefore be attributable to some hydrodynamic properties of the catch basins. The lower concentrations of methoprene in the catch basins may also be attributable to microbial activity and changes in temperature not present in the laboratory conditions. The next step in this research is to improve the mass-balance model of this process, in order to refine knowledge of the release of methoprene into the environment.

CHAPTER 5

5. Conclusions and Recommendations

Filling in the data gaps in our knowledge is essential in order to assess the risk balance crucial to public health decisions regarding West Nile virus. As the virus becomes a yearly concern, pesticide application will occur regularly and must be concurrent with environmental reviews, including monitoring for short and long-term effects.

The scope of the study outlined specific undertakings that were implemented to meet the study's objectives. The scope of this study involved the characterization of the concentration of methoprene in the field in three study catch basins and the storm sewer outfall of the Newtonbrook sewershed over three 30-day periods. It also involved the determination of the dissolution and degradation of methoprene over time in a model catch basin in the laboratory. Finally, the scope included the characterization of the total outputs of methoprene from an urban sewershed. These tasks were successfully performed and the following conclusions are made.

There are four major factors affecting the concentration of methoprene over time in the catch basins; the first two could be observed in the lab tank experiment, and the second two in the field study. These are dissolution, degradation, dilution, and flushing. Flushing is the factor which may have ecological implications for other invertebrates and amphibians downstream and may reduce methoprene concentrations to residual levels, reducing efficacy. It is clear that rainfall has some effect on the peak concentration of methoprene in catch basins and that rain contributes to flushing of the chemical through the storm sewer system and into downstream waters. Nevertheless, the release of methoprene during rain in other situations may pose some environmental

impact in the receiving waters if the dosage is high and the flushing is severe, and the time elapsed from the application to the rainfall. This study has shown that the storm sewer outfall did not release methoprene at detrimental concentrations during the study period; however, not all rain events were sampled. This would have been helpful in September when there were more rain events, and events of greater magnitude than during July and August.

The laboratory lab tank experiments showed that in addition to the hydrodynamic and chemical environment of the catch basin, methoprene concentration is affected by dilution and the physical degradation of pellets. The physical degradation of the pellets is related to the size of the pellets which determines the total surface area available for breakdown and release of methoprene. The experiments demonstrated that the size distribution of the pellet sample is more important in determining methoprene concentration than water volume.

A simulation model attempted to characterize the concentration of methoprene released from catch basins over time. However, the model did not take into account the decay of methoprene over time, and overestimated the output mass of methoprene. It did illustrate a simplistic relationship between land use and runoff volume, and the process of flushing from the storm sewer system and its effect on methoprene concentration in catch basins. However, while the model predicted a decrease in methoprene concentrations after a rain event, field data indicated that methoprene concentrations tended to rise after a rain event. The model will need to be adjusted to include a decay function and hydrodynamic activity within the catch basin such as resuspension of sediments. In its current form, the model is not an appropriate tool for predicting outgoing storm sewer concentrations of methoprene.

Continued and more intricate monitoring, more vigilant rainfall and in situ temperature recording and sampling of the storm sewer outfall during rain events and during drought periods would be necessary to adequately assess the output concentrations of methoprene. A greater number of catchbasins over a spectrum of environments and land use would be helpful in determining the methoprene output for an entire sewershed. Further analyses of the field and laboratory results could include ANOVA to determine the variability of the methoprene curves. This could determine how significant the concentration gradient in the model catch basin in laboratory is, and would help estimate the differences in methoprene release due to differences in catch basins.

Further studies on the efficacy of methoprene in controlling larval emergence in these catch basins, as well as concurrent analyses of methoprene metabolites in the daily methoprene samples, the interaction between Altosid pellets and catch basin sediments, and other factors in the degradation of methoprene such as temperature, ultraviolet radiation and microbial activity, would be useful in outlining the advantages and disadvantages of the mosquito control program. An improved simulation model which includes chemical decay factors and the integration of more complex GIS functions would assist in monitoring the input and output of methoprene in the urban environment.

The Newtonbrook storm sewer system drains into Newtonbrook Creek, which is a tributary of the Don River, which flows into Lake Ontario. These are important ecological features for the Toronto region, and they are under intense urbanization pressure. In order to maintain functioning ecosystems to insure the lasting environmental and human health, it is important to monitor human impacts. Responsible implementation of a widespread urban mosquito control program using a chemical larvicide such as methoprene requires monitoring of long and short-term environmental effects. This research is one element in the

planning of mosquito control programs in the Greater Toronto Area, and the achievement of the best balance between effective public health initiatives and ecological health.

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