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# Occurrence and Fate of Methoprene Compounds in Urban Areas of Southern Ontario, Canada

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Methoprene [isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate] is an insect growth regulator commonly used to control mosquitoes at the larval growth stage. Other applications include control of fleas, cigarette beetles, and fire ants. Common commercial brand names of formulations containing methoprene include Precor™ and Altosid™. With the recent advent of the mosquito-borne West Nile Virus, methoprene is now widely used in urban areas in both the United States and Canada. It is routinely applied to prime mosquito breeding areas in urban environments, including storm water drainage systems (i.e., catch basins).

Methoprene has a water solubility of 1.4 mg/L (25°C, Kidd and James, 1991), and exhibits a relatively high $K_{oc}$ value (23,000, Toxnet, 2003), and as a result binds to suspended particulate matter in the water column. It is reported to degrade quickly in water; Schooley et al. (1975) reported half-lives in pond water of 30 hrs at a concentration of 0.001 mg/L and 40 hrs at a concentration of 0.01 mg/L, while the photolysis half-life of methoprene is less than one day (Quistad et al., 1975). Methoprene degrades by demethylation, hydrolysis, oxidative cleavage, and photodegradation to form a series of metabolites that include methoprene acid and citronellic acid (USEPA, 1991; Quistad et al., 1975; Ross et al., 1994). The primary modes of degradation are photodegradation and degradation by aquatic microorganisms (EXTOXNET, 1996). The methoprene epoxide isomers and methoprene acid are examples of photodegradation and microbial metabolism products, respectively. Therefore, the stability and subsequent half-life of methoprene in an aquatic environment is dictated by a number of factors.

Common formulation types of methoprene include pellets, granules, and briquettes, with corresponding periods of release of the active ingredient typically ranging from 30–90 days. Some newer commercial slow release and briquette formulations release methoprene for up to 150 days. The effective dose for common mosquitoes is roughly 1 μg/L (1 ppb, Antunes-Kenyon and Kennedy, 2001).

Methoprene is generally considered to pose little health risk to humans. Methoprene has an oral LD50 value exceeding 34,000 mg/Kg in rats (Glare and O'Callaghan, 1999), and is classified as a ''practically non-toxic'' class IV compound by the USEPA (1991). A methoprene metabolite (methoprene acid) has been reported to interfere with retinoid-regulated pathways (Schoff and Ankley, 2004; LaClair et al., 1998). Exposure to chemicals that mimic retinoid compounds has been postulated as a factor in an increased prevalence of amphibian malformations in North America (http://www.npwrc.usgs.gov/narcam, Schoff and Ankley, 2004).

As a result of the implementation of mosquito control programs based on methoprene in southern Ontario, Environment Canada and the Ontario Ministry of the Environment initiated monitoring programs in selected areas in 2003 to investigate the occurrence and fate of methoprene and its metabolites in both source areas (i.e.,

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catch basins) and receiving bodies (streams, rivers, and harbors or embayments).

## Materials and Methods

Water samples were collected in 1 L amber glass bottles, which had PTFE lids and were affixed to a fiberglass sampling pole. Bottles and lids were rinsed twice in sample water prior to collection. Samples were collected from the surface water layer in receiving waters (Fig. 1), and from storm water outfalls in the case of storm water sewer samples. Samples were collected during peak flow (wet weather) periods. Wet weather sampling was conducted at catch basin outfalls at the onset of precipitation. Stream sampling (e.g., Indian and Redhill Creeks) was conducted within 60 min of the onset of precipitation, while sampling in receiving waters (e.g., Hamilton Harbour and Cootes Paradise) were sampled several hours after significant rain events (> 5 mm). Water samples were adjusted to pH 5 and extracted through LC-18 Supelclean (Supelco) solid phase extraction (SPE) cartridges, which were preconditioned with 10 mL dichloromethane (DCM), 10 mL ethyl acetate and then 10 mL Type I water at pH 5. Samples were loaded at a flow rate of 5 mL/min and the cartridges subsequently dried for 10 min before being eluted with 10 mL of 50/50 DCM/ethyl acetate. The extract was then split into two equal fractions, for acidic and neutral metabolite analysis.

For analysis of acid derivatives, the extracts were exchanged into acetone and evaporated to dryness under nitrogen, then reconstituted with 4 mL of acetone, 200 $\mu$L 5% pentafluorobenzylbromide (PFBBr), and 30uL 30% $K_2CO_3$. The mixture was allowed to react for three hours at 60°C. When complete, the extracts were solvent exchanged into trimethylpentane and eluted through 0.7g 5% deactivated silica gel to 10 mL 5% methanol/toluene. The extracts were then analysed by GC-NICIMS in SIM mode. Quantitation was achieved using four-level multipoint ($r^2 >$ 0.990) external standard calibration.

For analysis of neutral derivatives, the extracts were concentrated to 3 mL under nitrogen and passed through 2 g mini-columns containing $Na_2SO_4$ to remove water. The column was then rinsed with 4 mL of DCM. The collected extract was exchanged into trimethylpentane and concentrated to 1 mL for clean-up on a 0.7g 5% silica gel column. The sample was eluted with 8 mL of DCM and then concentrated to a final volume of 1 mL for GC-EIMS analysis. The target compounds were calculated using an external standard method and a four-level multipoint ($r^2 > 0.985$) calibration.

Analyses were performed using a Hewlett Packard 6890 gas chromatograph with a Hewlett Packard 5973 Mass Selective Detector. The GC-MS was equipped with a 30 m × 0.25 mm i.d. HP5-MS analytical column with a 0.25 micron film thickness (Agilent) and a pulsed-splitless injector (25 psi for 1 minute and then purged at 100 mL/min, gas saver 20 mL/min at 3.0 min). The oven was temperature-programmed from 80°C (held for 3 minutes) to 160°C at 6°C/min, then to 200°C at 2°C/min, and then 285°C at 30°C/minute and held for 10 min. Total run time was 49 minutes. Helium was used as the carrier gas and gave a column head pressure of 11.7 psi. The ions monitored for acidic metabolites in SIM mode were m/z 267 and 235 for methoprene acid. The ions monitored for neutral compounds were m/z 109 and 98 for methoprene, and m/z 191 and 95 for the methoprene epoxide isomers.

One laboratory blank and two laboratory spikes were processed with every batch of twelve samples. No methoprene or target derivatives were detected in the laboratory

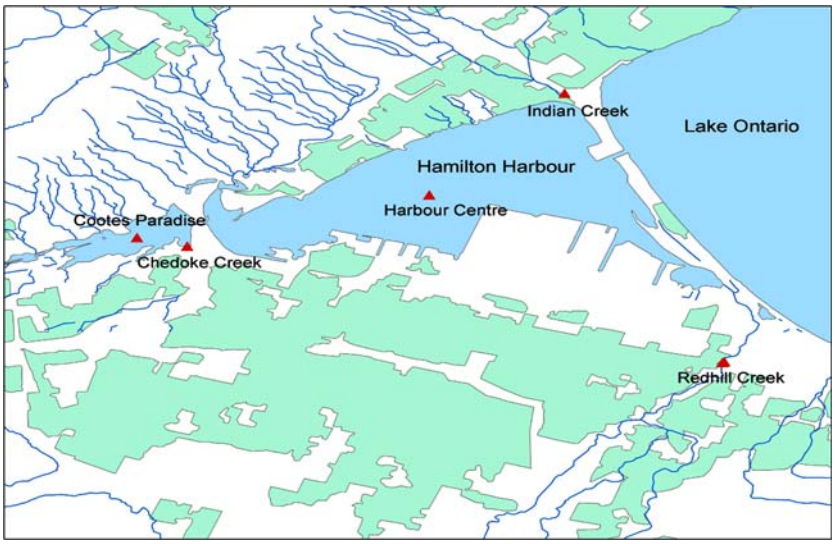


**Fig. 1** Hamilton Harbour methoprene sampling sites

**Table 1** Summary of methoprene data for receiving waters in the Hamilton Harbour area of western Lake Ontario

| Location | Indian Creek | Redhill Creek | Hamilton Harbour | Cootes Paradise |
|---|---|---|---|---|
| Number of sites/ samples | 1/6 | 1/5 | 4/13 | 2/13 |
| Number of detections | 1 | 0 | 0 | 0 |
| Maximum concentration | 100 ng/L | NA | NA | NA |

blanks. Spike recovery and percent relative standard deviation (%RSD) ranged from 78% to 112%, and 5% to 16%, respectively.

## Results and Discussion

Hamilton Harbour and its tributaries were sampled for this study due to the nature of its watershed; Hamilton is located at the western end of Lake Ontario, and is the seventh largest city in Canada (population of roughly 500,000). The harbour has a relatively small surface area (40 $km^2$), but receives discharges from a watershed of roughly 900 $km^2$, much of which is highly urbanized. The Hamilton storm water system is characterized by roughly 40,000 catch basins. In 2003, methoprene was applied three times to those catch basins; total methoprene usage in Hamilton was 130 kg (MOE, 2003). Ottawa, the nation's capital, is located at the eastern end of the province of Ontario and is the fourth largest city in Canada (population of roughly 1,000,000). Pinecrest Creek in Ottawa is classified as having the most urbanized watershed in the city with 25 storm sewers discharging over a length of roughly 4.5 km. Ottawa has roughly 90,000 catch basins. As with Hamilton, methoprene was applied three times to catch basins in 2003, with total usage of approximately 125 kg (MOE, 2003). Total permitted usage of methoprene in the Province of Ontario in 2003 was 1254 kg (MOE, 2003).

Water samples were collected from two tributaries (Redhill Creek and Indian Creek) of Hamilton Harbour (Fig. 1), four sites in open water areas of Hamilton Harbour, the Cootes Paradise area at the western end of Hamilton Harbour, and six sites in a stream (Pinecrest Creek) in the eastern city of Ottawa, Ontario in 2003.

Methoprene was generally not detected in receiving waters. Methoprene was detected in only 1 of 37 samples collected in the Hamilton Harbour area (100 ng/L, Table 1), and in 1 of the 14 samples from the Ottawa stream (650 ng/L). Of the 51 total surface water samples collected in the study, only 1 sample exceeded the Ontario Ministry of Environment draft Interim Provincial Water Quality Objective (IPWQO) of 200 ng/L. However, the draft IPWQO is well below the reported benchmarks for protection of amphibians (1,600 ng/L), invertebrates (10,000 ng/L), and fish (80,000 ng/L).

Catch basins and their associated outfalls were typically sampled during precipitation events in 2004 in order to assess concentrations of methoprene and metabolites in waters being flushed from these source areas into receiving waters. Our analytical methodology was originally developed only for analysis of methoprene; the analyte suite was later expanded to include both the neutral (methoprene epoxide A, methoprene epoxide B) and acid derivatives (methoprene acid).

The relative concentrations of methoprene and its derivatives in effluent at a catch basin outfall in Burlington during two selected precipitation events in 2004 are shown in Fig. 2. The most prevalent compounds in waters being flushed from the catch basins were methoprene and its acid derivative microbial metabolite, methoprene acid. With the exception of significant concentrations of the photodegradation product methoprene epoxide A in the first two sampling intervals in the first event (Fig. 2A), the neutral derivatives were present only at trace concentrations, or not detected. In both cases, concentrations of methoprene compounds showed a trend toward decreasing concentrations over the course of the precipitation event.

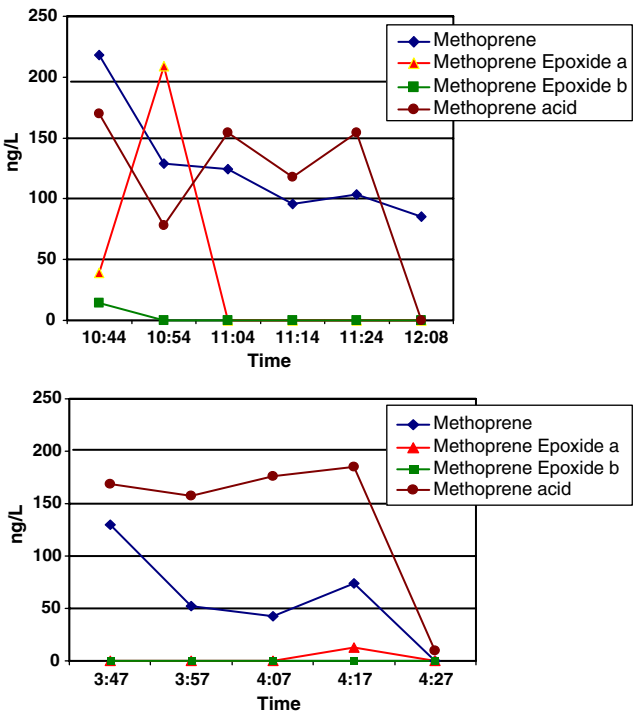


**Fig. 2** Time series of methoprene and methoprene derivative concentrations in effluents from catch basins in Hamilton, ON

In the second event (Fig. 2B), the dissolved methoprene compounds were essentially flushed from the system over a period of roughly 40 min. With the exception of the first sample in the first sampling event (Fig. 2A), methoprene concentrations in all samples were below the Ontario draft IPWQO of 200 ng/L. Concentrations of methoprene prior to the precipitation event were not measured; however, a series of catch basins in Ottawa were sampled during dry weather periods over a range of 7–16 days after methoprene application. The concentrations of methoprene detected in the catch basin ranged from 840 ng/L to 4,350 ng/L; concentrations in waters at the outfalls of these catch basins during periods of ambient flow ranged from undetectable to 1,520 ng/L. Maximum concentrations of methoprene, using stipulated application rates, are on the order of 10,000 ng/L (Antunes-Kenyon and Kennedy, 2001). These methoprene concentrations are also similar to those reported by Ross et al. (1994) in post-application studies of methoprene in freshwater microcosms using both liquid and briquette formulations.

The methoprene concentrations in the Ottawa catch basins were similar to those observed in a study conducted by the Ontario Ministry of the Environment (MOE, 2003); peak concentrations of methoprene in catch basins immediately after application were roughly 4,000 ng/L, which decreased to approximately 2,000 ng/L within seven days after application.

The results of our studies of methoprene and its metabolites in Ontario catch basins and receiving waters provide two clear conclusions:

1. Dissolved methoprene and its derivatives are quickly flushed from catch basins during precipitation events, and concentrations in outfall effluents show marked concentration decreases over relatively short time periods, and;
2. Methoprene is generally not detected in receiving waters; of the 51 receiving water samples analyzed in this study, only one exceeded the most stringent benchmark of 200 ng/L.

Although concentrations of methoprene did not generally exceed the strictest guideline levels, concentrations of metabolites, particularly methoprene acid, sometimes exceeded those of methoprene. Therefore, the metabolites should be considered in any assessment of potential environmental impacts of methoprene application.

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