Canadian Water Quality Guidelines for the Protection of Aquatic Life

METHOPRENE

Methoprene (or *S*-Methoprene) (CAS Registry Number 40596-69-8; IUPAC name isopropyl (2E, 4E, 7S)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) is a synthetic active ingredient used in various insecticide products registered for use in Canada. It is an amber or pale yellow liquid with a faint fruity odour (Kidd and James 1991). There are two forms of methoprene, the (R or cis) and (S or trans) isomers, of which (S)-methoprene has the greatest insecticidal activity (Henrick et al. 2002).

Methoprene is classified as an insect growth regulator, as defined by its mechanism of action. Methoprene interferes with maturation and reproduction in insects by mimicking the activity of the insect's juvenile hormone (JH). The JH is responsible for controlling the growth of insect larvae. Concentrations of JH normally decrease to very low levels during the third and fourth larval instar stages, allowing for adult emergence from pupae. If the mimic JH, methoprene, is present during development and is ingested and/or absorbed through the larvae's exoskeleton, developmental delays in the physical structures necessary for pupation and adult emergence will result. Insect mortality during larval and pupal moults will occur (Staal 1975). Methoprene, unlike the natural insect JH, is not affected by degenerative enzymes (JH-specific esterase), which normally break down the hormone (Weirich and Wren, 1973). By suppressing esterase activity, methoprene extends hormonal activity beyond its normal limits (Downer et al. 1976, Sawby et al. 1992). JH mimics, such as methoprene, are not directly toxic to target organisms. Exposure to these insecticides leads to developmental abnormality which in turn impairs the survival of the insect. Methoprene has been reported to reduce the survival of many different insect species including, but not limited to, true flies (Diptera), moths and butterflies (Lepidoptera) and beetles (Coleoptera) (Glare and O'Callaghan 1999). Methoprene has also been shown to affect the survival of some mite species.

Methoprene has been registered for use as an insecticide in Canada since 1977 and is registered for use in 53 products, including manufacturing concentrates and formulated pesticides (PMRA 2006). Trade and other names used for methoprene-based pesticides include, but are not limited to, Altosid, Apex, Hartz, Kabat, Ovex, Precor, Pre-Strike, Raid, Vet-Kem, and Zodiac. The various methoprene-based pesticides registered in Canada are used for a wide variety of terrestrial purposes including: general insecticide products for domestic use; flea and tick control on household pets; insect control for mushroom cultures, as well as insect control for stored tobacco. Methoprene is also registered for use as the restricted product Altosid and the domestic product Pre-Strike for use in aquatic applications for the control of floodwater mosquitoes that are vectors for the transmission of serious diseases (e.g., West Nile Virus) (PMRA 2006). Methoprene is not produced in Canada, but rather imported from the United States. The total amount of *S*-Methoprene (active ingredient in all Altosid products used for aquatic applications) imported into Canada in 2005 was 330.61 kg (S-Methoprene) (L. Goczan, Manager, Canadian Division, Wellmark International, pers. comm.). The breakdown by various formulas is Altosid XR Briquets 47.71 kg, Altosid Pellets 147.9 kg, and Altosid Granules 135.00 kg (L. Goczan, Manager, Canadian Division, Wellmark International, pers. comm.). Use data compiled from Ontario Ministry of the Environment 2005 West Nile virus larviciding permits indicate that approximately two thirds of imported S-methoprene was used in Ontario for aquatic applications (S. Bowerman, Pesticide Regulatory Scientist, Standards Development Branch, Ontario Ministry of Environment, pers. comm.). The remaining one third was most likely used in other provinces, e.g., Quebec, for larviciding activities.

Table 1. Water quality guidelines for methoprene for the protection of aquatic life (Hall 2006).

Aquatic life	Guideline value ($\mu g\ a.i. \cdot L^{-1}$)	Target Organism Management value ($\mu g\ a.i. \cdot L^{-1}$)
Freshwater	0.09*	0.53*
Marine	NRG+	NRG+

*Interim guideline.

+No recommended guideline.

For aquatic application against adult mosquito emergence, methoprene is available in granule (1.5% active ingredient

or a.i.), briquette (2.1% a.i.), pellet (4.25% a.i.), and liquid (20% a.i.) formulations. Typical application rates for restricted use methoprene granules range from 5.6 to 22.4 $kg \cdot ha^{-1}$ water surface area whereas application rates for domestic use methoprene granules range from 2.5 mL per 32 sq ft to 500 mL per 3200 sq ft water surface area. Pellet application rates range from 2.8 to 11.2 $kg \cdot ha^{-1}$. Liquid larvicide application rates are 73 $mL \cdot ha^{-1}$. Application rates depend on such factors as the target mosquito species, water depth, vegetation cover, size of the catchment, as well as presence/absence of aquatic pollution (PMRA 2006).

The use of methoprene in floodwater and permanent water sites for the prevention of adult mosquito emergence has led to concern over potential impacts to aquatic life in receiving water bodies. The restricted product Altosid, is to be used only by those who are certified and/or licensed by Provincial agencies (e.g., public health officials, mosquito abatement officials and other trained personnel of public mosquito programs) (PMRA 2006). The liquid formulation is used to instantly alter mosquito larval development and subsequent emergence of adults, while the granules, pellets and briquets can provide control for 21 days, 30 days or several months, respectively (PMRA 2006). The granules, liquid larvicide and pellets are registered for application to floodwater sites such as pastures, fields, snow pools, fresh water swamps and marshes, woodland pools and meadows, dredging soil sites, drainage areas, ditches, waste treatment and settling ponds, water-holding receptacles (e.g., tires) and other natural and man-made depressions (PMRA 2006). In addition to floodwater sites, the pellets can also be applied to permanent water sites such as ornamental ponds and fountains, flooded crypts, transformer vaults, abandoned swimming pools, tree holes, other artificial water holding containers, storm drains, catch basins, roadside ditches, cesspools, septic tanks, and waste treatment settling ponds (PMRA 2006). The briquets are to be applied only in storm sewers and catch basins (PMRA 2006). The domestic product, Pre-Strike®, is registered for use in standing water wholly confined to the property of the user where there is no existing outflow beyond the property as well as in containerized water (e.g., bird baths, flower planters, discarded tires, ornamental ponds, rain barrels) (PMRA 2006).

The most predominant form of insect JH (JH-III) is synthesized by mosquitoes (Edgar et al. 2000). Other forms of insect JH include JH-0, JH-I and JH-II, which all differ from each other in the pattern of additional ethyl or methyl group substitutions on the carbon chain (Wheeler & Nijhout 2003). Methoprene has a molecular weight and chemical structure similar to the natural insect JH found in mosquitoes (JH-III). The difference between the two is that methoprene has a higher potency and greater field stability than naturally occurring JH (Glare and O'Callaghan 1999). Methoprene has the molecular formula $C_{19}H_{34}O_3$ and a molecular weight of 310.48 (Kidd and James 1991). It is only slightly soluble in water, with a solubility of 1.4 $mg \cdot L^{-1}$ at 25°C and, with a vapour pressure of 3.15 mPa at 25°C, may volatilize from surface waters (Kidd and James 1991). The log octanol-water partitioning coefficient (log K_{ow}) is reported as being 5.50, and the soil organic carbon partition coefficient (K_{oc}) is estimated to be 23,000 (HSDB 2002). Based on this large Koc value, methoprene is not expected to leach in soil. Several studies have indicated that the half-life of methoprene in soil ranges from 10 to 14 days, even when applied at an extremely high rate of 1 pound per acre (Glare & O'Callaghan 1999, US EPA 1991), where the processes of degradation include photolysis and biodegradation. For this reason, methoprene should not persist in soil or contaminate groundwater.

When methoprene is added to water, it is only slightly soluble, and has been reported to remain in the upper layers of water following application (Glare & O'Callaghan 1999). A laboratory based study was conducted to assess the distribution of methoprene in a plastic tank, designed to represent a typical catch basin in Ontario, with three spouts located at different heights of the tank (J. Li, Dept. of Civil Engineering, Ryerson University, Toronto, Ontario, pers. com.). Methoprene pellets were added to the water, with water samples being collected daily from each spout. Overlap of the plotted methoprene concentration data points indicated that methoprene was evenly distributed throughout the tank. Methoprene has been shown to degrade rapidly in water through both photolytic and microbial processes, which can result in detoxification of the methoprene product. Under normal environmental conditions, methoprene does not appear to be susceptible to hydrolysis (US EPA 1991). The half-life of methoprene in water has been shown to range from less than one day (Quistad et al. 1975) to up to two days to one week (Glare and O'Callaghan 1999; Madder and Lockhart 1980). In the presence of sunlight, in both sterile and non-sterile pond water, methoprene transforms into four major and 40 minor products (LaClair et al. 1998). The major breakdown product is methoxycitronellic acid, with minor amounts of the biologically inactive (R)-methoprene isomer, methoxycitronellal, methoprene acid and methoprene epoxide (LaClair et al. 1998). Many of these degradation products are further oxidized and degraded.

A laboratory study conducted by Degitz et al. (2003) evaluated the concentrations of various methoprene break-down products over time. At the end of the 7-day assessment, the only product present at initial (Day 0) concentrations was methoxycitronellic acid. Since this was a controlled laboratory exposure, with no influence of plant / sediment / biota uptake or influence of UV light, the resultant zero loss of product could have been underestimated. The presence of methoxycitronellic acid as the dominant break-down product was also confirmed in a field study conducted to characterize the degradation of *S*-methoprene in pond water. Results indicated that after a 7 day period, the only significant component remaining was methoxycitronellic acid (Henrick et al. 2002).

Salinity does not impact the rate of methoprene degradation, which occurs at approximately the same rate in both salt and fresh waters maintained in the dark (US EPA 1991). Temperature has been shown to influence methoprene degradation rates. Degradation proceeds more rapidly at 20 degrees Celsius (half-life of 10-35 days) compared to degradation at 4.5 degrees Celsius (half-life $\geq$ 35 days) (US EPA 1991). Reduced recovery of methoprene in surface water samples polluted by sewage and dairy effluent (as well as higher temperature and light intensity) was documented by Schafer and Dupras (1973). Overall, methoprene has a generally short persistence in water. The presence of the insecticide may be extended when the pellet, briquette or granular form are used to ensure slow release for consistent mosquito larval exposure (e.g., pseudo-persistence). Data specific to factors that modify methoprene toxicity were difficult to find. One study conducted by Schoeppner (1977) found no reduction in methoprene effectiveness in the presence of high organic matter.

Analytical methods for measuring methoprene in water generally use gas chromatography-mass spectrometry, but may differ in their methods for extraction and detection. Specific methods include those used by Environment Canada's National Laboratory for Environmental Testing (Ed Sverko 2006, National Laboratory for Environmental Testing, Environment Canada, Burlington, Ontario, pers. com.) and the Ontario Ministry of the Environment (Yang 2003), with detection limits of 0.02 and 0.005 $\mu g \cdot L^{-1}$, respectively.

Initiated in 2003, a methoprene catch basin application program was conducted by municipalities in southern Ontario in response to West Nile Virus human health concerns. Methoprene was applied to sewer catch basins 1 to 3 times from May to September. Environmental monitoring of methoprene concentrations in water was co-ordinated by Environment Canada and the Ontario Ministry of the Environment. Sampling was conducted at catch basins, in the receiving water as well as in treated drinking water during the application period (OMOE 2003; Struger et al. 2004). In 2003, sixteen samples were collected at catch basins during both dry and wet events, of which 8 showed detection of methoprene (detection limit of 0.02 $\mu g \cdot L^{-1}$) , with the highest concentration being 4.35 $\mu g \cdot L^{-1}$ (Struger et al. 2004). Fifty one receiving water samples were also analyzed, with only 2 indicating detection of methoprene, with the highest concentration being 0.65 $\mu g \cdot L^{-1}$ (Struger et al. 2004). Surface water monitoring for methoprene was conducted through Ontario's Provincial Water Quality Monitoring Network (PWQMN) in 2003, 2004 and 2005. A total of 157, 134 and 45 surface water samples were collected, respectively. All samples were below the detection limit of 0.005 $\mu g \cdot L^{-1}$ (OMOE, Environmental Monitoring and Reporting Branch, PWQMN, unpublished data). Sampling and analysis of both raw and treated drinking water through Ontario's Drinking Water Surveillance Program (DWSP) resulted in no detections above the detection limit (0.005 $\mu g \cdot L^{-1}$) since monitoring began in 2003 (L. Poff, Pesticide Management, Ontario Ministry of the Environment, pers. comm.). Manitoba has also monitored for methoprene in surface water. A total of approximately 40 samples have been collected in rivers, creeks and storm drains within the City of Winnipeg (and just downstream) and concentrations were always below detection in all samples (< 0.05 $\mu g \cdot L^{-1}$) (N. Armstrong, Water Quality Monitoring Specialist, Water Science and Management Branch, Manitoba Water Stewardship, pers. comm.).

Field application rates of 1.0 $\mu g \cdot L^{-1}$ have been reported to be adequate to eradicate the common target mosquitoes without producing lethality to most non-target aquatic biota (Glare and O'Callaghan 1999). Methoprene has been reported to be moderately toxic to warm-water, freshwater fish (e.g., bluegill sunfish, *Lepomis macrochirus*) and slightly toxic to cold-water, freshwater fish (e.g., rainbow trout, *Oncorhynchus mykiss*) (US EPA 1991). Methoprene toxicity to plants is low to negligible, (Glare and O'Callaghan 1999, Miura and Takahashi 1973). Methoprene is practically non-toxic to birds and mammals (Glare & O'Callaghan 1999). Since all insects synthesize JH, predominantly of the form JH III, there has been concern raised regarding the impacts of methoprene on non-target aquatic insects located in freshwater swamps and marshes. A three year field study was conducted to determine the long-term effects of methoprene applied to wetlands at recommended

application rates of 5-10 kg·ha^{-1} (Hershey et al. 1998; Niemi et al. 1999). Three-week sustained release granules were applied once every three weeks for a total of six treatments per trial. Observed effects included significant declines in aquatic insect density and biomass, but these effects were not noted until the second and third year of treatment. It should be noted that the three years previous to the start of this wetland field-trial had been affected by drought (e.g., various test wetlands had been completely dried out). There may not have been enough time for recolonization of these dried-up wetlands to occur. As well, the use of 3-week sustained release granules, with application every 3 weeks, would result in a constant exposure of methoprene. This would also make it difficult for insects to re-colonize the drought-affected wetlands. Methoprene water concentrations were not measured during this field-trial. A controlled laboratory-based study was conducted by Miura and Takashi (1973) that tested the effects of methoprene on 15 insect species, the results of which are described in this factsheet under the heading 'Freshwater Life'. Overall, these field- and laboratory-based studies do not provide conclusive evidence of the potential for permanent wetland ecosystem disruption following treatment with methoprene. Following PMRA (2006) product label instructions should ensure that wetland ecosystem function will remain intact.

Most concern lies in the potential effects of methoprene in the development of frog deformities. Methoprene is similar structurally and behaviourally to retinoids, such as retinoic acid, which are biochemical metabolites of vitamin A (Antumes-Kenyon and Kennedy 2001). Retinoids regulate gene expression and play a role in vertebrate limb development. Several studies have induced frog deformities following exposure to methoprene. One study that was included as a data point in Figure 1, and has the lowest vertebrate chronic effect concentration (32 µg a.i.·L^{-1}), is the *Rana sphenocephala* (southern leopard frog) 90-d teratogenesis study (Sparling 2000). Six experimental wetlands were treated with a nominal sunlight-exposed methoprene concentration of 32 µg a.i.·L^{-1}, with another six wetlands used as the controls. Following exposure, southern leopard frogs collected from methoprene-sprayed wetlands had a significantly greater frequency of limb deformities compared to frogs collected from control wetlands. Sparling (2000) does indicate that these data are inconclusive since numerous cases of amphibian malformations have been reported in areas with no known methoprene application, suggesting that there may be other factors responsible in the onset of malformations. Several other studies have induced frog deformities following exposure to methoprene. LaClair et al. (1997) examined the effects of sunlight and methoprene exposure to the African clawed frog *Xenopus laevis.* Exposure to methoprene and its breakdown products resulted in significant frog malformations following exposure to concentrations of 15,000 µg·L^{-1}, a concentration greater than 15,000 times the normal application rate. Degitz et al. (2001) also used *Xenopus laevis* as the test organism. The effects of methoprene and its breakdown products (methoprene acid, methoprene epoxide, methoxy-citronellal, and methoxycitronellic acid) were examined at exposure concentrations ranging from 100 to 30,000 µg·L^{-1}. Methoprene acid was the most potent of the test products, inducing malformations at concentrations ≥ 1250 µg·L^{-1}, three orders of magnitude higher than expected methoprene concentrations in the field, following application. Field monitoring studies have indicated that aquatic methoprene concentrations typically do not exceed 10 µg·L^{-1} which suggests that methoprene break-down products are not likely to cause adverse effects on amphibians (Degitz et al. 2001). To date, amphibian malformations as a result of methoprene exposure in the natural environment has not been proven (Meteyer et al. 2000a,b). No studies have been found which investigate the toxicity of methoprene breakdown products to fish, invertebrates or aquatic plants.

Water Quality Guideline Derivation

The CCME guiding principle states that Canadian Water Quality Guidelines (CWQG) for the protection of aquatic life "are set at such values as to protect all forms of aquatic life and all aspects of the aquatic life cycles" (CCME 1991). However, methoprene is intentionally applied to water to control mosquito populations. A guideline for methoprene which would abide by this guiding principle would not be useful in areas where methoprene is applied to eradicate mosquitoes (e.g., freshwater marshes and wetlands). Currently, there is no guidance on how to deal with the issue of setting a guideline for a substance intentionally used for aquatic organism control. Given the uniqueness of this substance, several options were explored by Environment Canada's National Guidelines and Standards Office (Gatineau, QC), on how to set a CWQG for this substance. It was decided that two separate values would be established. The first is a CWQG value that is protective of all aquatic life (including mosquitoes). This guideline value is science-based and is adhering to CCME's Guiding Principle. The second is a Target Organism Management Value which is set without inclusion of the target species data (mosquitoes). This value is also a science-based value but has been modified by policy considerations, and is no

longer adhering to the Guiding Principle. Neither value should be considered as more valid. The difference between the values is the manner in which they are to be implemented, each one having a particular purpose. The value which is implemented would be dictated by the level of protection which is desired at the site of concern (e.g., allow effects on mosquitoes or not). For more information, see the scientific supporting document (Hall and Fletcher 2006).

Freshwater Life

Freshwater toxicity data used in the derivation of the 1) interim water quality guideline for methoprene, and 2) target organism management value are presented in Fig. 1. Several of the non-target acute and chronic vertebrate and invertebrate studies, as well as all but one of the acute target invertebrate studies, used low active ingredient methoprene (5-15%) in the exposures (date points denoted by an open diamond). All other studies used high active ingredient methoprene (≥ 65%). Data points for bioassay exposures using low active ingredient methoprene to assess effects were included in the derivation graph (Fig. 1) since low active ingredient products are typically used in the field (granules 1.5%, briquettes 2.1%, pellets 4.25%, liquid 20%).

The most sensitive chronic fish study was conducted by Ross et al. (1994). The authors exposed newly spawned eggs and fry of the fathead minnow (*Pimephales promelas*) to varying methoprene concentrations for 37 days. The lowest observable effect concentration (LOEC) was 92 µg·a.i. L^{-1} based on decreased fry length and weight. The no observable effect concentration (NOEC) was 53 µg a.i.·L^{-1}. This was based on the finding of no significant reductions in egg hatchability, fry survival or total survival at any of the test concentrations, when compared to controls. Rainbow trout (*Oncorhynchus mykiss*), at the embryo life stage, appear to be slightly less sensitive, with a static exposure 7-day EC_{25} and EC_{50} for embryo survival of 270 and 650 µg a.i.·L^{-1}, respectively (Stantec 2004). Complete chemical analyses of methoprene species and test water used for the bioassays were provided. However, concentrations of methoprene were shown to have significantly declined throughout the experiment, with considerable variability evident between test concentrations as well as within test replicates. This variability was potentially due to the length of time taken between sample collection and analysis (up to one month). Methoprene is known to degrade rapidly in water in the presence of light. Prolonged sample storage prior to analysis may have contributed to accelerated methoprene degradation. As a result, the presented EC_{25} and EC_{50} values are based on nominal concentrations. In a study of swimming behaviour, Ellgaard et al. (1979) observed no alteration of locomotor activity in either the goldfish (*Carassius auratus*) or the mosquitofish (*Gambusia affinis*) to a two week exposure to 2000 µg a.i.·L^{-1} of methoprene.

Static acute toxicity testing with fish exposed to methoprene was conducted by Johnson and Finley (1980). Rainbow trout (*Oncorhynchus mykiss*; weight 0.6 g), a cold-water species, and, three warm-water species, fathead minnow (*Pimephales promelas*; 0.7 g), channel catfish (*Ictalurus punctatus*; 1.2 g), and bluegill sunfish (*Lepomis macrochirus*; 0.6 g), were continuously exposed to measured concentrations of methoprene in aquatic bioassays maintained at pH 7.2-7.5, alkalinity of 30-35 mg·L^{-1} and hardness of 40 to 50 mg·L^{-1} $CaCO_3$. The reported 96-h LC_{50} values were 1600 µg a.i.·L^{-1} (95% CI - 1.0-2.4) for rainbow trout and >10,000, > 100,000 and 2900 µg a.i.·L^{-1} for fathead minnow, channel catfish and bluegills, respectively. Other experimental details for the bioassays were not provided. In a second study by McKague and Pridmore (1978), the 96-h LC_{50} methoprene concentrations for rainbow trout and coho salmon (*Oncorhynchus kisutch*) were 106,000 and 86,000 µg a.i.·L^{-1}, respectively. This difference in rainbow trout LC_{50} data between studies could be due to potential differences in the analytical method and/or instrumentation used to detect methoprene in the water samples. Differences in analytical methods could have resulted in varying measured methoprene concentrations (e.g., methoprene stock solutions dissolved in acetone, ethanol, iso-octane or water).

In a study conducted by the US EPA (HSDB 2002), methoprene residues were shown to have accumulated in the muscle tissue of bluegill sunfish (bioconcentration factor of 457) and crayfish (bioconcentration factor of 75). A chemical is considered to be bioaccumulative when the bioconcentration factor (BCF) is equal to or greater than 1000 (or Log $K_{ow} \geq 4.00$) (OMOE 1992). These data indicate that methoprene is not bioaccumulative. No data were found indicating that methoprene combined with UV exposure results in increased methoprene sensitivity in fish species, as is hypothesized with amphibians.

Chronic invertebrate studies were carried out by Gelbic et al. (1994) in which the aquatic bug *Ilyocoris cimicoides* was exposed to methoprene in the laboratory during the fifth and last nymphal instar before transforming into an adult. At concentrations of 210 and 100 µg a.i.·L^{-1}, 67%

and 30% of the nymphs died, respectively. The duration of the moult from the fourth instar to the adult stage decreased by 5 days in exposed larvae (mean = 10.5 ± 3 days) compared to controls (16.6 ± 6 days) at a methoprene concentration of 210 µg a.i.·L^{-1}, and by two days (14.6 ± 2 days) at 100 µg a.i.·L^{-1}. In addition, the number of morphologically deformed adults progressively increased with increasing concentration (16, 19, 22 and 30 deformed adults at 21, 100, 210 and 1000 µg a.i.·L^{-1}, respectively), compared to controls. All of the individuals (n = 30) died at 1000 µg a.i.·L^{-1} of methoprene. Chu et al. (1997) exposed the water flea *Moina macrocopa* to methoprene for two weeks in the laboratory. Survival, longevity and fecundity of this freshwater crustacean were reduced at 1000 µg a.i.·L^{-1} and higher concentrations. The authors concluded that if concentrations of methoprene do not exceed 1000 µg a.i.·L^{-1} in the natural environment, this insect growth regulator (IGR) will not cause detrimental effects to populations of *Moina macrocopa*. At very low levels of methoprene (5 µg·L^{-1}), reproductive performance was enhanced and can influence reproduction in the water flea *Moina macrocopa.* Reproduction and survival tests were conducted on *Ceriodaphnia dubia* exposed to different methoprene concentrations in the laboratory (Stantec 2004). The 7-day mean nominal IC_{25} and IC_{50} (IC = inhibitory concentration) values for reproduction were 45 (8.8 - 49, 95% C.I.) and 64 µg a.i.·L^{-1} (54 - 77, 95% C.I.), respectively. In terms of reproduction, the lowest observable effect concentration (LOEC) was 82 µg a.i.·L^{-1} and the no observable effect concentration (NOEC) was 41 µg a.i.·L^{-1}. No significant mortality was observed at any of the tested methoprene concentrations, which ranged from 0 to 78 µg a.i.·L^{-1} (Stantec 2004). The side swimmer *Hyalella azteca* was exposed to methoprene at different concentrations to determine growth and survival. The mean 14-day nominal IC_{25} and IC_{50} values for growth were 115 (13.8 - 202, 95% C.I.) and 215 µg a.i.·L^{-1} (96 - 298, 95% C.I.), respectively. The LOEC and NOEC for growth were 310 and 150 µg a.i.·L^{-1}, respectively. The LC_{50} for survival exceeded the highest test concentration used of 310 µg a.i.·L^{-1} (Stantec 2004). Methoprene has been reported to alter male/female ratios in freshwater zooplankton. Exposure of the zooplankton species *Daphnia pulex* to methoprene concentrations of 100 µg a.i.·L^{-1} produced a decrease in the incidence of all male broods and an increase in the incidence of all female broods, compared to controls (Peterson et al. 2001). The authors conclude that 100 µg a.i.·L^{-1} is at the upper concentration for methoprene which is applied to the natural environment and may affect reproduction in *Daphnia*. Because methoprene has been reported to bind with retinoid X receptor in mammalians, *D. pulex* bioassays with methoprene were also conducted with 9-*cis*-retinoic acid and all-*trans*-retinoic acid, each at a concentration of 1000 µg·L^{-1}. Zooplankton exposure to the latter two chemicals produced no observable effects on reproduction. Static long-term laboratory tests were conducted by Miura and Takashi (1973) using the mothfly larvae *Pericoma* sp., the midge larvae *Chironomus stigmaterus*, and the shorefly larvae *Brachydeutera argentata*. The respective 20-, 12- and 21-day LC_{50} concentrations were 110, 11 and 11 µg a.i.·L^{-1}. (The lowest chronic effect concentration of 11 µg a.i.·L^{-1} was selected as the critical endpoint for derivation of the target organism management value for methoprene. See the last paragraph for guideline derivation).

Miura and Takahashi (1973) studied the short-term effects of methoprene for many freshwater invertebrates. The 24-h LC_{50} values reported from these experiments were 960 µg a.i.·L^{-1} for *Daphnia magna* (water flea), 1100 µg a.i.·L^{-1} for *Eulimnadia* (clam shrimp), 1300 µg a.i.·L^{-1} for *Notonecta unifasciata* (backswimmer), 1600 µg a.i.·L^{-1} for *Cypricercus* (seed shrimp) and 4900 µg a.i.·L^{-1} for *Cyclops* (copepod). The 96-h LC_{50} values were 1760 for *Corisella decolor* (water boatman) and 5320 µg a.i.·L^{-1} for *Triops longicaudatus* (tadpole shrimp). The 24-h LC_{50} for *Hyalella azteca* (sideswimmer) was 1330 µg a.i.·L^{-1}. The 48-h LC_{50} for *Paramecium* (protozoan) was 1330 µg a.i.·L^{-1} and the 48-h LC_{50} for *Laccophilus* (predacious water beetle) was 2130 µg a.i.·L^{-1}. For water fleas (*Moina macrocopa*) the 48-hour LC_{50} test for (*S*)-methroprene exposure was reported as 6800 µg a.i.·L^{-1} (Chu et al. 1997).

Algae and diatoms are not particularly sensitive to methoprene. Secondary data were available for five algal species. Green algae (*Pithaphora oedogonia, Hydrodictyon reticulatum, Spirogyra*), a blue-green alga (*Anacystis*) and a diatom (*Diatoma vulgare*) were exposed to methoprene concentrations of 110 µg a.i.·L^{-1} for one week (Miura and Takahashi 1973). No detectable adverse effect was observed for these algal species.

As the target organisms, mosquitoes are very sensitive to methoprene (Glare and O'Callaghan 1999). The most sensitive mosquito species studied was *Aedes nigromaculis*, with a 24-h LC_{50} of 0. 1 µg a.i.·L^{-1} (Miura and Takashi 1974). The least sensitive species studied was *Culex quinquefasciatus* with a 14-d LC_{50} of 100 µg a.i.·L^{-1} (Mulla and Darwazeh 1979). Canadian mosquito species had methoprene sensitivities intermediate to these high and low values. The 24-h LC_{50} values for *Culex tarsalis* ranged from 3 µg a.i.·L^{-1} (Miura and Takashi 1974) to 4 µg a.i.·L^{-1} (Schaefer and Wilder 1972). The

24-h LC_{50} values for *Aedes triseriatus* ranged from 0. 93 to 3.4 µg a.i.·L^{-1} (Wells et al. 1975). Only one chronic study was found for methoprene impacts on mosquitoes. Ali et al. (1995) reported a 7 to 10-d LC_{50} of 2.3 µg a.i.·L^{-1} for laboratory-reared third and fourth instars of the tiger mosquito, *Aedes albopictus.* (This chronic study was used in the derivation of the interim Canadian water quality guideline. See the second last paragraph for guideline derivation). According to the CCME (1991) protocol, it is preferable to use a LOEC from a chronic study for guideline derivation, as opposed to an LC_{50}. In addition to the LC_{50} datapoint, Ali et al. (1995) also supplied LC_{90} (8.1 µg a.i.·L^{-1}) and slope (2.29) data. It was decided that calculating a lower effect value from the data given in the original paper was warranted, and therefore an LC_{20} (representative of a LOEC) was calculated using probits. The calculated LC_{20} was 0.90 µg a.i.·L^{-1} with a resultant slope of 2.26, which is close to the slope of 2.29 provided in the study. The equation of the line fit to the LC_{50} and LC_{90} data was y = 2.264x + 11.017).

It is important to note that Canadian Water Quality Guidelines (CWQGs) are intended to provide protection to freshwater life from all anthropogenic stressors, including pesticides. Methoprene is used to control the emergence of adult mosquitoes in constructed water sites (e.g., ornamental ponds and fountains, abandoned swimming pools, storm drains, catch basins) where non-target organisms would not reside. It is also permitted for use in floodwater sites (e.g., snow pools, water holding receptacles, drainage areas, freshwater swamps and marshes), but is restricted from being applied directly to surface waters such as lakes, rivers, streams and ponds. Since application of methoprene is allowed in areas with minimal non-target organism presence (e.g., ornamental ponds) as well as in freshwater swamps and marshes (with substantial non-target organism presence), two values were derived, 1) a CWQG value where the protection of mosquito larvae was taken into consideration, and 2) a Target Organism Management Value, where the protection of mosquito larvae was not considered.

The interim water quality guideline for methoprene for the protection of freshwater life is 0.09 µg a.i.·L^{-1} (Table 1, Fig. 1). The development of this value took into consideration data relevant to both non-target and target (mosquito) organisms. It was derived by multiplying the Canada-resident tiger mosquito species (*Aedes aIbopictus)* 7 to 10-day LC_{20} end-point of 0.9 µg a.i.·L^{-1} (Ali et al. 1995) by a safety factor of 0.1 CCME 1991). The CCME (1991) guideline derivation protocol states that the most sensitive chronic LOEC is to be multiplied by a safety factor of 0.1 to arrive at the guideline value. The rationale for using this chronic value to set the interim guideline, as opposed to the lower *Aedes triseriatus* 24-hour LC_{50} of 0.9 µg a.i.·L^{-1} (Wells et al. 1975) is that guidelines are preferably derived using chronic study data (CCME 1991). In addition to this, this study was only one of two that used high active ingredient methoprene (96% a.i.) for the exposure to address effects. This value would be protective of all Canadian mosquito species, as seen in Figure 1. The reason an interim guideline was set, as opposed to a full guideline, was due to data quality. Almost all of the study data were classified as secondary, with the exception of two data points, which were classified as primary (Fig. 1). In order to set a full guideline, all data included in the minimum data set must be primary. This guideline is to be used to protect all species, including mosquitoes, as guidelines do not distinguish between intentional and unintentional exposure to a contaminant. A guideline is (among other applications and uses) to be used for assessing whether a particular measured environmental concentration of a contaminant has the potential to cause harm. While proper methoprene application is intended to control mosquitoes, improper application can occur (e.g. too many applications per year at greater concentrations than recommended), and potential effects need to be assessed. Excluding mosquito species effect concentration data from the guideline derivation process would not allow for this important use of the guideline.

The target organism management value for methoprene for the protection of all organisms, except mosquitoes, is 0.53 µg a.i.·L^{-1} (Table 1, Fig. 1). This value was derived without taking into consideration any of the target organism (mosquito) data. The management value was set using the lowest chronic non-target organism effect concentration, as found in the literature. Two organisms had the exact same effect concentrations, being the *Chironomus stigmaterus* and *Brachydeutera argentata* 12- and 21-day LC_{50} of 10.6 µg a.i.·L^{-1} (Miura and Takashi 1973). This effect concentration was multiplied by a safety factor of 0.05 (CCME 1991) in order to obtain the final management value. The CCME (1991) guideline derivation protocol states that a safety factor of 0.05 (or 20) should be applied to LC_{50} data for chemicals that are deemed to be non-persistent ($t_{1/2}$ in water < 8 weeks). Since there is no protocol for target organism management value derivation, the protocol for safety factor application in guideline derivation was adopted in this case. This guideline would be applicable in areas where methoprene is applied intentionally for the

eradication of target mosquito species, but where all non-target organisms would need to be protected. In other words, this guideline would be used to ensure that all forms of non-target aquatic life and all aspects of non-target aquatic life cycles would remain protected. There has been some concern related to the effects of low methoprene concentration exposures on endocrine disruption in the crustacean *Daphnia magna* and the rotifer *Brachionus calyciflorus*. Olmstead and LeBlanc (2003) investigated the effects of methoprene on the production of male offspring in *Daphnia* under exposure conditions ideal for maintaining parthenogenetic (asexual) reproduction, during which only female offspring are produced (e.g. increased daylight, increased food, no overcrowding). Adult female daphnids carrying embryos in their brood chambers were exposed to methoprene. The methoprene EC50 value for stimulating oocytes to develop into males was 354 µg /L. Templeton and Laufer (1983) found no effects of methoprene on *Daphnia* reproduction and development with treatment concentrations of 9.94 µg a.i. L^{-1}. However, exposure of the rotifer *B. calyciflorus* to methoprene concentrations of 0.1 and 1.0 µg L^{-1} significantly decreased overall population growth following a 96-hour exposure, although the authors state this effect was small (Preston et al. 2000). Methoprene was found to have no significant effect on asexual or sexual reproduction in *B. calyciflorus*, most likely because the low test concentrations used (below the toxic threshold).

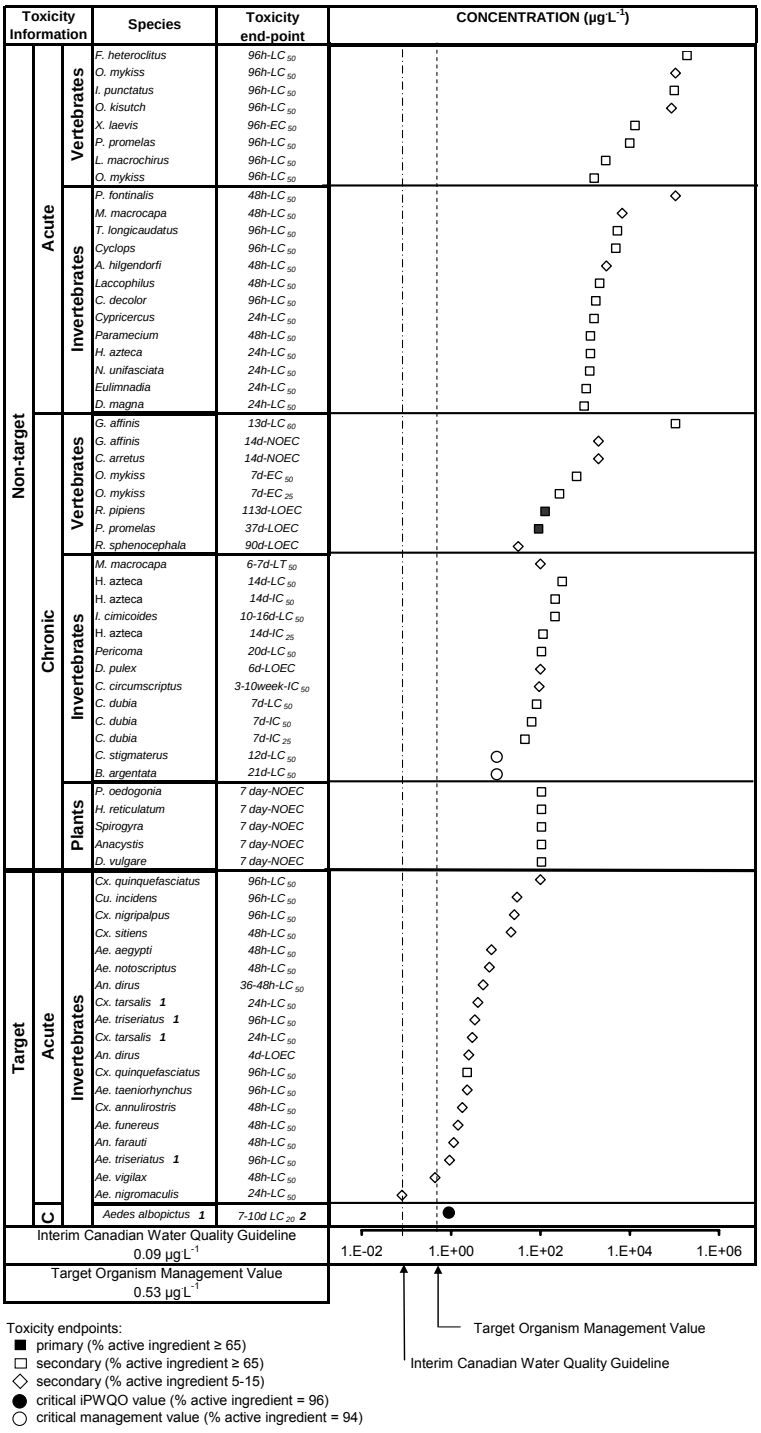

Figure 1. Freshwater non-target and target organism toxicity data used in the derivation of an interim water quality guideline for methoprene. Only non-target organism toxicity data was used in the derivation of the target organism management value.

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