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Sensitivity of the early-life stages of freshwater mollusks to neonicotinoid and butenolide insecticides☆

R.S. Prosser [a, *], S.R. de Solla [b], E.A.M. Holman [a], R. Osborne [c], S.A. Robinson [d], A.J. Bartlett [a], F.J. Maisonneuve [d], P.L. Gillis [a]

[a] Environment and Climate Change Canada, Aquatic Contaminants Research Division, Burlington, Ontario, Canada
[b] Environment and Climate Change Canada, Ecotoxicology and Wildlife Health Division, Burlington, Ontario, Canada
[c] University of Waterloo, Department of Biology, Waterloo, Ontario, Canada
[d] Environment and Climate Change Canada, Ecotoxicology and Wildlife Health Division, Ottawa, Ontario, Canada

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ABSTRACT

Neonicotinoid insecticides can be transported from agricultural fields, where they are used as foliar sprays or seed treatments, to surface waters by surface or sub-surface runoff. Few studies have investigated the toxicity of neonicotinoid or the related butenolide insecticides to freshwater mollusk species. The current study examined the effect of neonicotinoid and butenolide exposures to the early-life stages of the ramshorn snail, *Planorbella pilsbryi*, and the wavy-rayed lampmussel, *Lampsilis fasciola*. Juvenile *P. pilsbryi* were exposed to imidacloprid, clothianidin, or thiamethoxam for 7 or 28 d and mortality, growth, and biomass production were measured. The viability of larval (glochidia) *L. fasciola* was monitored during a 48 h exposure to six neonicotinoids (imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid, or dinotefuran), or a butenolide (flupyradifurone). The 7-d LC50s of *P. pilsbryi* for imidacloprid, clothianidin, and thiamethoxam were ≥4000 μg/L and the 28-d LC50s were ≥182 μg/L. Growth and biomass production were considerably more sensitive endpoints than mortality with EC50s ranging from 33.2 to 122.0 μg/L. The 48-h LC50s for the viability of glochidia were ≥456 μg/L for all seven insecticides tested. Our data indicate that neonicotinoid and butenolide insecticides pose less of a hazard with respect to mortality of the two species of mollusk compared to the potential hazard to other non-target aquatic insects.

1. Introduction

Neonicotinoid and butenolide insecticides are used in soil/foliar spray and seed treatments for the protection of crop plants against insect pest species. Neonicotinoids act as agonists of the nicotinic acetylcholine receptor (nAChR) on the post-synaptic surface of neurons in the central nervous system of arthropods. Butenolides, such as flupyradifurone, similarly act as insect nAChR agonists, but unlike neonicotinoids are not susceptible to the development of insect resistance due to overexpression of the detoxification enzyme, CYP6CM1 (Nauen et al., 2015). The use of neonicotinoids has increased substantially in the last two decades due to their selective effectiveness against insects and their relatively low toxicity to mammals (Jeschke et al., 2011). Flupyradifurone has been registered for use in Canada, United States and Australia as of 2015, following a joint review by the respective regulatory agencies of these countries (USEPA, 2015). In 2008, neonicotinoids comprised 80% of the market for seed treatments and 24% of the global market share for insecticides (Jeschke et al., 2011), and neonicotinoid use has continued to grow since 2008 (Simon-Delso et al., 2015). The chemical properties that allow neonicotinoids (hereafter including butenolides) to act as systemic insecticides, which are the reason for their popularity as seed treatments, also increase the potential for movement from the field to surface waters. Neonicotinoids are readily soluble in water, have a limited capacity to sorb to soil particles (i.e., relatively low organic carbon partition coefficient (K_{OC})), and have been shown to be relatively persistent in soil and surface water (CCME, 2007; Banerjee et al., 2008; EFSA, 2008; Tisler et al., 2009; Goulson, 2013; Schaafsma et al., 2015). Surface and sub-surface runoff transport neonicotinoids used in seed treatments from field soil to surface waters

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* Corresponding author.
E-mail addresses: ryan.prosser@canada.ca, rsprosser@gmail.com (R.S. Prosser).

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(Armbrust and Peeler, 2002; Chiovarou and Siewicki, 2008). A number of governmental agencies have developed guidelines on the acceptable concentration of neonicotinoids in surface waters for the protection of aquatic organisms, ranging from 0.0083 to 1.05 μg/L (e.g., CCME (2007), RIVM (2014)). However, several studies have reported concentrations of neonicotinoids in surface waters orders of magnitude greater than guidelines, e.g., maximum concentrations of neonicotinoids reported in surface water ranging from 44.1 to 320 μg/L (Denning et al., 2004; Dunn, 2004; Gibeault-Delisle, 2010; Anderson et al., 2013; Van Kijk et al., 2013; Main et al., 2014). This potential for exposure indicates that neonicotinoids could pose a hazard to aquatic organisms, especially aquatic invertebrates.

A number of studies have observed acute and chronic adverse effects in aquatic invertebrates exposed to environmentally relevant concentrations of neonicotinoids (e.g., Beketov and Liess (2008), Roessnik et al. (2013), Stoughton et al. (2008)). Stoughton et al. (2008) and Roessnik et al. (2013) reported 28-d LC50s (i.e., concentrations that reduce survival by 50%) for *Chironomus tentans, Caenis horaria*, and *Cloeon dipterum* exposed to imidacloprid of 0.91, 0.316, and 0.195 μg/L, respectively. In terms of aquatic invertebrates, the toxicity of neonicotinoids to arthropods has received the greatest attention, given the relatively high selectivity of neonicotinoids to the arthropod nAChR (Anderson et al., 2013; Morrissey et al., 2015). The effect of neonicotinoids on mollusks has received little attention, and mollusks are not represented in species sensitivity distributions used in the risk assessment of neonicotinoids. Tomizawa et al. (2008) have shown that the binding affinity of neonicotinoids to the nAChR can vary significantly among mollusk species; however, they have also shown that neonicotinoids can bind to the nAChR of certain species of mollusks with the same affinity observed with the insect nAChR. Therefore, there is a need for studies examining the toxicity of neonicotinoids to mollusk species. This is especially important considering that numerous populations of freshwater mussels, at risk of extirpation due to a number of pressures (e.g., habitat destruction, larval host decline, poor water quality), are in watersheds where neonicotinoids are regularly applied. Toxicity studies are needed to determine if neonicotinoids in surface waters pose a threat to imperiled freshwater mussel populations.

The objective of this study was to estimate the toxicity of neonicotinoids and a butenolide insecticide, which has the same mechanism of action as neonicotinoids, to the early-life stages of two species of mollusks, *Planorbella pilsbryi* and *Lampsilis fasciola*. A species was chosen from each of the two largest classes within the phylum of Mollusca, i.e., Gastropoda and Bivalvia. The ramshorn snail, *P. pilsbryi*, is a freshwater pulmonate snail found in Canada from southwestern Quebec to central Alberta, and in the northern United States from Massachusetts to Minnesota (Clarke, 1981; Burch, 1982). *P. pilsbryi* is found in lakes, ponds, and the backwaters of streams. Under optimal conditions, snails will produce eggs continually from late spring to early fall (Clarke, 1981), and both eggs and snails provide an important source of food for fish and other aquatic invertebrates in these aquatic systems. The wavy-rayed lampmussel, *L. fasciola*, was historically found throughout the Ohio and Mississippi River basins, the upper Allegheny River in New York, and the watersheds of Lake Erie, Lake St. Clair, Lake Michigan, lower Lake Huron, and Lake Ontario. *Lampsilis fasciola* has significantly declined in the northeastern United States and is now only found in a few rivers in Ontario (COSEWIC, 2016). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has designated *L. fasciola* as a species of special concern (COSEWIC, 2016). Both of these mollusk species are present in watersheds that drain areas where neonicotinoids are used as seed treatments in agriculture. For example, corn and soybeans are grown on more hectares than any other field crop in Ontario (OMAF, 2015), and the majority of corn and soybean seeds sown in Ontario are treated with neonicotinoids (MOECC, 2015). Furthermore, the only Canadian water quality guideline for the protection of aquatic life for neonicotinoids is for imidacloprid (0.23 μg/L; CCME, 2007); the toxicity data for the remaining neonicotinoids and aquatic organisms are too depauperate to derive water quality guidelines. This study examined the effects of neonicotinoid and butenolide exposure to freshwater mussels and snails using traditional endpoints, such as viability, mortality, growth, and biomass production, which can be clearly linked to potential effects on mollusk populations. This research will generate data that can be added to current species sensitivity distributions to improve the characterization of the risk that neonicotinoids may pose to aquatic ecosystems, as well as generate data that can be used to derive water quality guidelines at both the provincial (Ontario) and federal (Canada) level.

2. Materials and methods

2.1. Wavy-rayed lampmussel (Lampsilis fasciola)

Gravid wavy-rayed lampmussels were collected from the Speed River in Ontario, Canada (43°39′09.39″N, 80°37′03.92″W). The mussels were transported to Environment and Climate Change Canada's (ECCC) Aquatic Life Research Facility (ALRF) at the Canada Centre for Inland Waters (CCIW, Burlington, Ontario) in temperature-controlled coolers. Mussels were held in flow-through tanks with water from the City of Burlington that had been dechlorinated using activated carbon beds and sterilized using UV light (chemical properties in Table S1 of the Supplementary Information (SI)). ALRF water was maintained at 14 ± 2 °C and continuously aerated. Mussels were fed Shellfish Diet 1800™ (*Isochrysis*, *Pavlova*, *Thalassiosira*, and *Tetraselmis* sp.) and Nanno 3600™ (*Nannochloropsis* sp.) *ad libitum* (Reed Mariculture Inc., San Jose, CA, USA).

The method used to assess the toxicity of neonicotinoids to the larval life stage, i.e., glochidia, of mussels follows the methods outlined in ASTM (2006) and described in detail in Gillis et al. (2008). Tests were conducted in moderately-hard reconstituted water (MHW) (USEPA, 1994). The chemical properties of the MHW are presented in Table S2 of the SI. Glochidia were isolated from three gravid females and pooled for use in each toxicity test. Glochidia were removed from gravid females by gently flushing the marsupium with a hypodermic syringe filled with water (composed of 60% MHW and 40% ALRF water at 17 °C). The viability of a sub-sample of glochidia (~100–200) from each female was assessed before pooling and using in testing. As per the ASTM (2006) method, glochidia viability was estimated by determining the number of glochidia with open valves and the number with closed valves before and after the addition of a solution of NaCl (~240 mg/L). Viable glochidia will close their valves upon exposure to the NaCl solution. The following equation was then used to calculate glochidia viability.

$$\text{Percent viability} = \left(\frac{\#\text{closed after NaCl} - \#\text{closed prior to NaCl}}{\#\text{closed after NaCl} + \#\text{open after NaCl}}\right) \times 100$$

Only females with glochidial viability ≥90% were used in testing. The viability of pooled glochidia was determined at t = 0 (Table S3).

Solutions containing imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid, dinotefuran, or flupyradifurone (Fig. S1)

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