Sub-lethal effects of six neonicotinoids on avoidance behavior and reproduction of earthworms (Eisenia fetida)

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\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

Avoidance behavior of earthworms (Eisenia fetida) against six neonicotinoids (NEOs) (acetamiprid, dinotefuran, clothianidin, thiacycloprid, nitenpyram, imidacloprid) was studied following the protocol of ISO. The results showed obvious avoidance behavior of E. fetida against the tested insecticides, and the medium effective concentration for avoidance behavior (EC\textsubscript{50}) of the six pesticides was 0.14, 0.55, 0.91, 7.87, 1.32 and 0.77 mg/kg, respectively. Compared to the acute toxicity, avoidance behavior was more sensitive as an indicator of soil contamination with NEOs. Chronic toxicity of above six NEOs to E. fetida was also evaluated; cocoon production, hatchability, cocoon weight and adult weight were all affected in the test. Cocoon production and hatchability were more sensitive than cocoon weight and adult weight. The reproduction of earthworms was significantly reduced with a 56 d half-maximal effective hatchability concentration (EC\textsubscript{50}) of 0.37, 0.74, 1.30, 3.57, 1.20 and 0.77 mg/kg (acetamiprid, dinotefuran, clothianidin, thiacycloprid, nitenpyram, imidacloprid), respectively. Most of the tested NEOs were highly toxic to E. fetida. Avoidance behavior and reproduction damage of E. fetida was observed at very low concentrations. The existing levels of pollution with NEOs in soil frequently exceed the lowest observed adverse effect concentrations, which are likely to have negative biological and ecological impacts on earthworms.

1. Introduction

Neonicotinoids (NEOs) have been widely used all over the world since they were registered in the early 1990s, for instance, imidacloprid-containing products alone which has been dominating the insecticide market are registered for use on more than 140 crops in 120 countries (Jeschke and Nauen, 2008). Together with other members in neonicotinoid family, such as clothianidin, acetamiprid, dinotefuran, nitenpyram, thiamethoxam, thiacycloprid, they represent the best-selling class of insecticide on the global market (Jeschke et al., 2011). The acute toxicity of NEOs to mammals is low, however, NEOs are highly toxic to many invertebrates, including non-target aquatic species (Liess and Beketov, 2011; Pestana et al., 2009; Roessink et al., 2013) and pollinators such as bees (Pisa et al., 2015; Tsvetkov et al., 2017). NEOs are reported to have long half-lives in soil typically from a few days to in excess of 1000 days (range 28–1250 for imidacloprid; 7–353 days for thiamethoxam; 148–6931 days for clothianidin; 3–74 days for thiacycloprid and 31–450 days for acetamiprid) (Goulson, 2013), therefore the accumulation could easily happen when used repeatedly (Bonmatin et al., 2015). Hence, concerns have been raised regarding the environmental fate and effects of NEOs, such as soil persistence, effects on pollinators and other non-target invertebrates etc. (Goulson, 2013). The toxicity of NEOs to both target and non-target organisms, such as mammals, birds, fish, insects, annelids etc. have been investigated in many studies (De Cant and Barrett, 2010; Luo et al., 1999; Mota-Sanchez et al., 2006; Renaud et al., 2018). Some of them are proven highly toxic to soil invertebrates (de Lima e Silva et al., 2017) and even show constancy in toxicity for survival and reproduction for three generations (van Gestel et al., 2017).

Earthworms play an important role in agricultural soils to maintain and improve soil structure and fertility (Lee, 1985). Earthworms can be exposed to NEOs by direct contact when applied, contaminated soil, seed etc. Globally, about 60% of NEOs is used as a seed dressing in farming (Jeschke et al., 2011). NEOs can easily bind to soil particles at its second phase of its loss from agricultural soil (Goulson, 2013), which can pose a risk to earthworm survival and behavior changes, and
further cause the disruption of soil fertility maintenance processes. The accumulation and persistence of NEOs in soil can extend the exposure period for earthworms (Broznic et al., 2012; Gupta and Gajbhiye, 2007). A range of different endpoints, including mortality, reproduction, growth, molecular response, behavior, have been investigated. Avoidance behavior has been recognized as a more sensitive endpoint than mortality, growth etc. (Hund-Rinke et al., 2003) and may be as sensitive as reproduction (Van Gestel, 1992). Avoidance behavior of earthworms against heavy metals, nanoparticles has been extensively studied (Brami et al., 2017; Syed et al., 2017), however, there are only a handful of studies about avoidance behavior of earthworm against NEOs (Alves et al., 2013; Dittbrenner et al., 2012, 2011; Capowiez and Berard, 2006), and most of them only studied imidacloprid. The purpose of the present study was to comprehensively investigate the effects of 6 widely used NEOs on avoidance behavior of E. fetida at sub lethal concentrations; meanwhile, the effect of long term exposure to low concentrations of 6 NEOs on E. fetida reproduction was also investigated.

2. Materials and methods

2.1. Test organisms and soil

E. fetida earthworms were originally purchased from Jurong Earthworm Farm (Nanjing, China). The earthworms were acclimatized for 7 days to the artificial climate chamber (20 ± 1 °C with a dark: light ratio of 10:14 h with illumination of 400–800 lx; humidity: 80–85%). Adult earthworms with visible clitellum and an individual wet weight of 300–500 mg were randomly selected for the tests. Artificial soil used for the soil tests consisted of 10% ground sphagnum peat (< 0.5 mm), 20% kaolinite clay (> 50% kaolinite), 70% fine sand (OECD, 1984, 2004) and the water content was adjusted to 30% (OECD, 2004). A small amount of calcium carbonate was added to adjust the pH to 6.0 ± 0.5.

The Predicted Environmental Concentration (PEC) of the studied neonicotinoids in soil was calculated. Single application with 75 (imidacloprid), 120 (clothianidin), 200 (acetamiprid), 300 (nitenpyram), 400 (dinotefuran), 500 (thiacloprid) g active ingredient (a.i.) per hectare, homogenous distribution in the top 5 cm of soil, no crop interception and a soil density of 1.5 kg l⁻¹.

2.2. Test chemicals

Imidacloprid (95.3%), clothianidin (98.0%), acetamiprid (96.8%), nitenpyram (98.0%), thiamethoxam (97.5%), thiacloprid (97.2%) were purchased from Shandong Luba Chemical Co., Ltd. (Shandong, China).

2.3. Avoidance test

Acute toxicity tests were conducted ahead to obtain 7-d LC₅₀ and 14-d LC₅₀ values. The avoidance tests were performed in the artificial soil according to ISO guidelines (ISO, 2008). Tests were conducted at 5 concentrations based on 7-d LC₅₀, which was 0.5%, 1%, 5%, 10% and 20% of 7-d LC₅₀ (Table 1). A stock solution was used to make dilution series and each replicate of the soil was fortified with test chemicals individually. At the beginning of the avoidance and reproduction experiment, the concentration of NEOs in fortified soil was measured, and the results were also listed in Table 1 and 2, respectively. All the treatments were performed with 4 replicates. A two section unit was used for the test, one half of the unit was filled with 300 g contaminated soil, and the other half with 300 g control soil. Then the separator in the middle of the unit was removed and 10 earthworms were placed on the separating line. The units were then closed with transparent perforated lids. The tests were conducted in dark in incubators at 20 ± 1 °C for 48 h. The water content of the soil was maintained at 30%. The earthworms were not fed during the test. At the end of 48 h test period, the control and the contaminated soil sections were carefully separated and the number of earthworms was determined in each section. Individuals found between the sections (on the separating line) were counted as 0.5 for each side. Dead earthworms were classified as escaped animals. A control with clean soil (without pesticides) on both sides of the units was also carried out in the test.

Dual-control experiment was carried out before the formal test at two concentrations of 0.5% and 20% of 7-d LC₅₀, control with clean soil was also carried out at the same time. The distribution of earthworms in clean soil and contaminated soil was studied.

2.4. Reproduction test

Based on the 14d-LC₅₀ of acute toxicity tests, four different concentrations (5%, 10%, 20% and 50% of 14d-LC₅₀) were set to study the effect of NEOs on reproduction of E. fetida (Table 2). The procedure of dosing soil was the same as described in avoidance test. Each treatment was performed in 4 replicates. Ten adult earthworms (300–500 mg with a clitellum) were selected for reproduction test according to OECD 222 guideline (OECD, 2004). A total of 500 g soil treated with tested chemicals was placed in a 1-L glass jar and 10 adult earthworms were added to each jar. Controls were prepared similarly but without tested soil.

Table 1

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>7 d-LC₅₀ (mg/kg)</th>
<th>Nominal/Measured concentrations (mg/kg, soil dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5%</td>
<td>1%</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>4.0</td>
<td>0.02/0.002</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>6.0</td>
<td>0.03/0.02</td>
</tr>
<tr>
<td>Nitenpyram</td>
<td>8.0</td>
<td>0.04/0.017</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>1.2</td>
<td>0.006/0.003</td>
</tr>
<tr>
<td>Dinotefuran</td>
<td>3.0</td>
<td>0.015/0.003</td>
</tr>
<tr>
<td>Thiacloprid</td>
<td>120</td>
<td>0.60/0.57</td>
</tr>
</tbody>
</table>

The values of measured concentrations are means of three replicates.

Table 2

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>14d-LC₅₀ (mg/kg)</th>
<th>Nominal/Measured concentrations (mg/kg, soil dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>3.6</td>
<td>0.18/0.15</td>
</tr>
<tr>
<td>Dinotefuran</td>
<td>3.0</td>
<td>0.15/0.096</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>5.5</td>
<td>0.275/0.24</td>
</tr>
<tr>
<td>Nitenpyram</td>
<td>8.0</td>
<td>0.40/0.26</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>1.2</td>
<td>0.06/0.036</td>
</tr>
<tr>
<td>Thiacloprid</td>
<td>12.0*</td>
<td>0.60/0.57</td>
</tr>
</tbody>
</table>

* data from the preliminary test; the values of measured concentrations are means of three replicates.