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Selective bioaccumulation of neonicotinoids and sub-lethal effects in the earthworm *Eisenia andrei* exposed to environmental concentrations in an artificial soil

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HIGHLIGHTS

- Exposure of earthworms to low concentrations of multiclass organic contaminants.
- Sub-lethal effects and organic contaminants bioaccumulation were evaluated.
- A clear selective bioaccumulation of neonicotinoid insecticides was observed.
- DNA damage in adult earthworms exposed to neonicotinoid insecticides is reported.
- Results suggest a new point of entry of neonicotinoids into the food-chain.

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ABSTRACT

In this study, we evaluated the bioaccumulation of neonicotinoid insecticides in the earthworm *Eisenia andrei* exposed to environmental concentrations (<200 ng g^{-1} dry weight, nominal concentration) in an artificial soil. We tested the selectivity for neonicotinoids by exposing earthworms to 7 neonicotinoids alone and in more complex mixtures of 54 pesticides then 69 organic contaminants (OCs) (54 pesticides and 15 pharmaceuticals). We applied long-term (56-day) toxicity tests to further evaluate the effect of OCs on earthworms. We monitored adult survival, adult DNA damage using a comet assay on earthworm coelomocyte cells, and reproduction performance (i.e. number of cocoons and number and dry weight of juveniles). A selective bioaccumulation of neonicotinoid insecticides in adult and juvenile earthworms was found. This bioaccumulation is concomitant with a significant increase in adult DNA damage and significant effects on reproduction when earthworms were exposed to neonicotinoid insecticides alone. This study reveals a new potential point of entry of neonicotinoid insecticides into the wildlife food chain and also shows that *E. andrei* reproduction could be affected by long-term exposure to environmental concentrations of OCs.

1. Introduction

Conventional agricultural practices can contaminate soil ecosystems with a large number of organic contaminants (OCs). Pesticides are introduced into soils through direct application as pest control chemicals and as seed coatings. Pesticides, pharmaceuticals (PhCs) and personal care products are introduced into soils by way of contaminated fertilizing materials, such as biosolids from municipal wastewater treatment plants (Mompelat et al., 2009; Tadeo et al., 2010) and farm animal wastes (Christian et al., 2003). Agricultural soils are thus contaminated by OCs from a wide array of chemical classes present at very low concentrations (Sanchez-Brunete et al., 2004; Wu et al., 2010). The effects of OCs on soil biota have been documented. However, experiments are often

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carried out under conditions that are not representative of environmental conditions, i.e. single chemicals at high concentration (Yasmin and D'Souza, 2010). An increasing body of literature also highlights the importance of considering long-term exposure to low concentrations, as opposed to acute toxicity tests, when addressing the impact of OCs on soil biota (Eggen et al., 2004; Nash et al., 2004).

Neonicotinoids (NEO) are a family of insecticides that has been incriminated in the bee population collapse (Blacquière et al., 2012; Bonmatin et al., 2014; Van der Sluijs et al., 2013). They are also among the insecticides suspected to be responsible for the decline of several bird populations in agricultural areas (Gibbons et al., 2015; Hallmann et al., 2014). As a result, the use of NEO has been restricted in Europe since 2013. However, they have been and remain intensively used in North America and the use of NEO is rapidly increasing worldwide, and so are the concerns regarding their potential impact on wildlife (Chagnon et al., 2015; Gibbons et al., 2015). Thus, there is a need to better evaluate the effect of NEO exposure under environmentally relevant conditions (i.e. low concentration, complex mixture of OCs) on soil biota.

Earthworms are essential biological organisms in soil where they significantly contribute to soil function. They provide many direct and indirect services to soil ecosystems. They contribute to organic matter decomposition (Cunha et al., 2016), soil structure and organic matter mixing through bioturbation (Lemtiri et al., 2014). They are thus essential to soil fertility and dynamics. Earthworms are also a major food supply to many animals, such as birds (e.g. woodcocks), reptiles (e.g. snakes) and mammals (e.g. moles). Their contamination by OCs can thus be an important vector of contamination for higher levels of the food chain through biomagnification. Earthworms are particularly exposed to OCs in soil, either by dermal contact or ingestion of soil particles for their nutrition. They are thus organisms of choice to estimate bioavailability of OCs in soil (Lanno et al., 2004) and are a reliable bioindicator of environmental health (Lionetto et al., 2002). However, the first and the most frequent studies on the effects of OC exposure on earthworms aimed at establishing LC_{50} for single molecules using 14-day acute toxicity bioassays (e.g. OECD, 1984), using two model earthworm species *Eisenia andrei* and *Eisenia fetida* (Pino et al., 2015; Wang et al., 2012). The OC concentrations used in these tests are often several orders of magnitude higher than concentrations encountered in soils. More recently, research on the effect of OCs on earthworms focused on earthworm reproduction, as reported in numerous studies reviewed by Yasmin and D'Souza (2010). This approach allows assessing sub-lethal effects of OCs on earthworms expressed over longer periods of exposure. However, these standardized 56-day reproduction tests (Environment Canada, 2004; Organization for Economic Co-operation and Development, 2016, 2009) often use single contaminant exposure and have similar biases with regard to OC concentration. Moreover, only a few studies used biomarkers such as enzyme activities or DNA damage to characterize subtle effects of OC exposure on earthworm health (Lin et al., 2014; Wang et al., 2016). The OC bioaccumulation in earthworms is more commonly documented. For example, Kinney et al. (2008) reported that earthworms exposed to soil amended with organic wastes (biosolids and manure) can bioaccumulate PhCs. The accumulation of organochlorine pesticides by earthworms under environmentally relevant conditions was also reported by Miglioranza et al. (1999). However, such studies remain scarce. As a consequence, studies evaluating the effect of exposure to low concentrations of NEO in simple (NEO alone) and complex mixtures of multiclass OCs, on earthworm survival, reproduction, and physiology (e.g. DNA damage) are needed to draw a better picture of the potential impact of NEO on earthworms and the potential of NEO to contaminate the food chain.

The aim of this study was to evaluate the bioaccumulation of NEO insecticides by the earthworm *Eisenia andrei* under environmentally relevant conditions. Earthworms were exposed, in an artificial soil, to environmentally relevant concentrations (<200 ng g^{-1} dry weight (dw), nominal concentration) of 7 NEO alone and 7 NEO included in mixtures of multiclass pesticides (54 pesticides) and OCs (54 pesticides and 15 PhCs). These mixtures were defined to be representative of the multiple sources of OCs in agricultural soils. The selected PhCs correspond to different classes of active ingredients (i.e. anti-inflammatory agents, beta-blocker, antilipemic agents, anticonvulsant, antineoplastics and a stimulant) commonly found in the environment (Aus der Beek et al., 2016). The selected pesticides were among the highest sales in Canada in 2014 (Health Canada, 2014; MDDELCC, 2015) and included fungicides, herbicides, nematicides and insecticides. Bioaccumulation of NEO and other OCs in adult and juvenile earthworms was assessed by quantifying OCs in soil and earthworm tissues. Exposures were made following a reproduction test design, which allowed us to also evaluate the effect of NEO exposure (alone and in a complex mixture of OCs) on adult earthworm survival and reproduction (number of cocoons, number of juveniles, and juvenile dry weight). Finally, sub-lethal effects of NEO exposure on earthworm physiology were evaluated using a genotoxicity test (comet assay).

2. − Materials and methods

2.1. Chemicals and reagents

Analytical standards (pesticides and pharmaceuticals) and formic acid were purchased from Sigma Aldrich (Saint-Louis, MO, USA). Solvents (ethyl acetate, acetonitrile and methanol at liquid chromatography − mass spectrometry (LC-MS) grade), sodium chloride and anhydrous sodium sulphate were purchased from Fisher Scientific (Ottawa, ON, Canada). Anhydrous magnesium sulphate was purchased from VWR (Radnor, PA, USA). Primary secondary amine (PSA) sorbent was purchased from Agilent (Mississauga, ON, Canada). Silica sand was purchased from AGSCO (#140–270, Wheeling, IL, USA), sphagnum peat and kaolin were purchased from VWR (Mississauga, ON, Canada).

2.2. Artificial soils

Agricultural soils are contaminated by a large variety of OCs (Sanchez-Brunete et al., 2004; Wu et al., 2010) which can interfere with tested chemicals. This is particularly true when working under environmentally relevant concentrations. We thus opted for an artificial soil, which has the additional advantage of being compatible with standardized reproduction toxicity tests. Artificial soils were prepared according to the Quebec Ministry of Sustainable Development, Environment and the Fight against Climate Change (MDDELCC) guidelines (CEAEQ, 2012). A first mixture (MI) composed of 70% (g/g) sand (106–250 μm), 22% silt (20–75 μm), 5% air-dried black earth and 3% kaolin was prepared and mechanically homogenized for 1 h. A stock of contaminated livestock peat was produced by soaking 10 g of livestock peat (Magic products, Amherst Junction, US) in 25 mL of a highly concentrated solution of OCs (ranging from 2 to 20 μg mL^{-1}), prepared by spiking Milli-Q water with OC standards. The OC standards were prepared in methanol and then diluted in Milli-Q water to reach <0.1% of organic solvent prior to the addition of peat. For control soils, the same procedure was performed with OC-free diluted methanol solutions. Contaminated livestock peat stocks were then frozen at −80 °C before lyophilisation the next day. Artificial soils were composed of 75% (g/g) MI and 25% livestock peat. The amount of contaminated livestock