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Uptake kinetics of pesticides chlorpyrifos and tebuconazole in the earthworm *Eisenia andrei* in two different soils☆

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ABSTRACT

Agriculture is today indispensably connected with enormous use of pesticides. Despite tough regulation, their entrance into soil cannot be excluded and they might enter soil organisms and plants and continue further to terrestrial food chains. This study was conducted to investigate the bioaccumulation of two pesticides currently used in large amounts, the insecticide chlorpyrifos (CLP) and the fungicide tebuconazole (TBZ). Their detailed uptake kinetics in the model earthworm species *Eisenia andrei* were measured in two arable soils differing in organic carbon content (1.02 and 1.93% respectively). According to our results, a steady state was reached after 3–5 days for both pesticides and soils. The values of bioaccumulation factors calculated at the steady state ranged from 4.5 to 6.3 for CLP and 2.2–13.1 for TBZ. Bioaccumulation factors were also calculated as the ratio of uptake and elimination rate constants with results comparable with steady-state bioaccumulation factors. The results suggested that the degradation and bioaccumulation of tested compounds might be influenced by other factors than only total organic carbon (e.g. clay content). The lower K_{oc} and hydrophobicity of TBZ relative to CLP probably led to higher availability of TBZ through pore water exposure. On the other hand, CLP's higher hydrophobicity probably caused an increase in availability by its additional uptake via ingestion. To enable a proper ecological risk assessment of current pesticides in soils, it is necessary to accurately determine their bioaccumulation in soil invertebrates. We believe that our study not only brings such information for two specific pesticides but also addresses key methodological issues in this area.

1. Introduction

The large group of currently used pesticides (CUPs), which includes organophosphate insecticides and conazole fungicides for example, has been used since environmentally problematic organochlorinated pesticides (OCPs) were banned because of their high persistence and toxicity. CUPs are supposed to have lower persistence and environmental mobility than OCPs and due to modern pesticide regulation and legislation they should have only minor environmental effects, particularly on local ecosystems. However, these expectations are not always met: many CUPs show half-lives of months (e.g. pendimethalin, boscalid, quinoxyfen) and even years (e.g. epoxiconazole, flusilazole, diflufenican) (PPDB, 2016) and their repeated and massive use in agriculture can lead to gradual accumulation in soils (pseudo-persistence). Monitoring of arable soils in central Europe revealed that 81% of monitored soils had at least 1 pesticide above 0.01 mg kg^{-1} several months after their last possible application (Hvězdová et al., 2018). Soil can act as a sink for further distribution of CUPs to other environmental compartments by runoff to surface water, leaching to groundwater or by transfer and possible accumulation in plants and soil biota, resulting in distribution of the toxicant into the terrestrial food chain.

Within regulatory risk assessment of pesticides in the EU, there is presently a need for more research on uptake kinetics in in-soil organisms as highlighted by the recent “EFSA Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms” (EFSA, 2017). In this panel, bioaccumulation in soil organisms (earthworms) was considered in order to assess the potential for secondary poisoning in birds and mammals (EFSA, 2009).

Earthworms may constitute up to 80% of the total biomass of the soil fauna (Kabata-Pendias, 2010) and they live in close contact with the soil, have a permeable cuticle, and consume large amounts of

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soil. Due to these characteristics they are an appropriate model organism for bioaccumulation assays (Cortet et al., 1999; Jager et al., 2005; OECD, 2010). The majority of bioaccumulation studies with earthworms are focused on heavy metals and persistent organic pollutants. Only a few studies have focused on CUPs and they are limited only to several representative compounds while there are several hundreds of approved CUPs. The findings of the studies indicate that soil organic matter (SOM) and clay contents in soils (Papini et al., 2006; Wu et al., 2011a; Yu et al., 2006), hydrophobicity (K_{ow}) (Chang et al., 2016; Xu et al., 2014; Yu et al., 2006) and chirality (Chen et al., 2014; Diao et al., 2011; Yu et al., 2012) of the compounds are major factors in CUPs bioaccumulation in earthworms. To our knowledge, there is only one study on uptake of tebuconazole (TBZ) by earthworms (Yu et al., 2012), but it focused on enantioselectivity of TBZ bioaccumulation and it was performed only in one soil. Chlorpyrifos (CLP) bioaccumulation in earthworms has been addressed only in four studies (Yu et al., 2006; Wu et al., 2011a; Lister et al., 2011; Spurgeon et al., 2011), but none of them compared the uptake kinetics of CLP in different soils.

The two main approaches for assessing bioaccumulation process in earthworms are: i) studies with a fixed time of exposure and determination of the bioconcentration factor (BCF), bioaccumulation factor (BAF) or biota-soil accumulation factor (BSAF) (Gevao et al., 2001; Belden et al., 2004; Yu et al., 2006; Wu et al., 2011a) or ii) studies focused on whole uptake kinetics of CUPs by earthworms with calculation of kinetic parameters – uptake (assimilation) and elimination rate constants (Lister et al., 2011; Spurgeon et al., 2011; Yu et al., 2012). For calculation of BCF, BAF or BSAF, it is assumed that an equilibrium between the earthworm body and soil is reached during the exposure as a result of general equilibrium partitioning (EqP) between all phases present (soil solids, pore water, biota). However, the time needed to reach a steady state can differ between species, chemicals or soils. For some compounds and soils, the time necessary to reach such equilibrium is very long e.g. over 28 days for brominated organic compounds (Nyholm et al., 2010). Also, this approach assumes that the uptake curve follows the first-order kinetics and it is invalid for e.g. peak shape curves related to fast degradation of the compound in soil. In those cases, it is not clear whether the steady state was really reached or not, mostly in studies with PAHs (e.g. Jager et al., 2000; Matscheko et al., 2002; Šmídová and Hofman, 2014) but also in CUP studies (Jantunen et al., 2008; Yu et al., 2012; Xu et al., 2014). Therefore, Jager et al. (2000) suggested that BCF should be expressed dynamically as a ratio of uptake and elimination rate constants. As far as we know, this approach has been used in studies of CUPs with the aquatic oligochaete Lumbriculus variegatus (Mäenpää et al., 2003; Jantunen et al., 2008) but also the earthworm *Eisenia fetida* (Yu et al., 2012). Moreover, kinetic data for CUPs are rare with more than one tested soil.

In our experiment, we studied the uptake kinetics of two CUPs (the insecticide chlorpyrifos and the fungicide tebuconazole) in earthworms *Eisenia andrei* in two arable soils of different properties. The aims were to find a sufficient length of exposure to achieve equilibrium in concentration between soil and earthworm, to model the uptake kinetics and to compare two pesticides and two soils. TBZ and CLP were selected as model CUPs because of their very high usage in the European Union. In the Czech Republic, the representative Central Europe country, CLP and TBZ are the third and fifth most used synthetic pesticides respectively, with an average consumption in 2012–2014 of 182 $t\,y^{-1}$ for CLP and 167 $t\,y^{-1}$ for TBZ. The two compounds have distinct properties (Table 1) suggesting their different fate in soil and bioaccumulation. Two contrasting soils (one containing about twice as much total organic carbon (TOC) and clay than the other) were used to see the possible influence of different soil properties on the uptake of compounds by earthworms. We also compared our experimentally obtained BAFs with the BCF model acquired according to the EFSA Guidance document "Risk assessment for birds and mammals" (EFSA, 2009) used to assess pesticide risk assessment at the EU level.

2. Materials and methods

2.1. Experimental soils and test organisms

Two non-contaminated arable soils of fluvisol type, FS1 and FS2, with different TOCs (1.02% and 1.93%) were sampled in the Czech Republic in the summer of 2011. The properties of the studied soils are summarized in Table 2. Soils were air-dried, sieved to a maximum 2 mm fraction and stored in plastic bags in the dark. Both soils were sterilized by gamma irradiation at 25 kGy before the experiment (Bioster Ltd., Czech Republic) to represent the situation of a higher bioavailable fraction caused by limited microbial degradation in order to simulate the worst-case scenario in the soil environment (Šmídová and Hofman, 2014). Sterilized soil was also used in the studies of Yu et al. (2006), Wu et al. (2011a, 2011b). Distilled water was added to reach 50% of the water-holding capacity (WHC) of the individual soils 2 days before contamination. Moist soils were stored in airtight glass vessels in the dark at $20 \pm 2\,^{\circ}C$.

The earthworms (*Eisenia andrei*) were from our laboratory (Research Centre for Toxic Compounds in the Environment, Czech Republic). The substrate for the culture was a mixture of granulated manure (40%), garden substrate (50%), and peat (10%) and was moistened to 60–80% WHC. The pH ranged from 6 to 7 as adjusted by $CaCO_3$. Before the experiment, adult earthworms with developed clitellum and a minimal weight of 250 mg were acclimatized in uncontaminated soils moistened to 50% WHC with added manure 7 $mg\,g^{-1}$ dry weight for 3 days.

2.2. Test chemicals and contamination of soils

Chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate; CAS number 2921-88-2] and tebuconazole [(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol; CAS n. 107534-96-3] were obtained as neat standards from Sigma-Aldrich (Germany) with a purity of >99%.

A spiking solution of CLP and TBZ in acetone was prepared to achieve a nominal concentration in soils of 5 $mg\,kg^{-1}$ soil dry weight ($kg_{soil\text{-}dw}$). Similar concentrations (units to tens of $mg.kg^{-1}$) have frequently been used in other bioaccumulation studies of pesticides (e.g. Yu et al., 2012; Yu et al., 2006; Spurgeon et al., 2011; Wu et al., 2011a, 2011b; Xu et al., 2014; Papini et al., 2006; Belden et al., 2004). When compared to long-term concentrations of pesticides in agricultural soils (Hvězdová et al., 2018), the $mg.kg^{-1}$ levels used in laboratory bioaccumulation studies are 2-3 orders of magnitude higher. However, they might be close to the worst-case scenario in real field top soil: (1) according to the documentation for plant protection products containing TBZ or CLP, the real application doses are 100–350 $g\,ha^{-1}$ for TBZ and 300–2600 $g\,ha^{-1}$ for CLP (CISTA, 2016); (2) considering 5 cm topsoil (relevant for epigeic earthworms) and a soil density of 1.5 $g\,cm^{-3}$ (FOCUS, 1997), the maximum doses correspond to 0.5 and 3.5 $mg\,kg^{-1}$ for TBZ and CLP, respectively; (3) the soil concentration might be higher at field hotspots, e.g. places where the application vehicle turns.

Soils were contaminated according to the procedure of Doick et al. (2003). Briefly, the pesticides in acetone were added to the moist soils (at 50% WHC) in stainless steel bowls and mixed properly. After solvent evaporation in the fume hood, the water loss was measured by weighting of control (non-spiked) soils and