Environmental Pollution 264 (2020) 114793

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Uptake and physiological effects of the neonicotinoid imidacloprid and its commercial formulation Confidor® in a widespread freshwater oligochaete☆

Valeska Contardo-Jara [a, *], Mark O. Gessner [a, b]

[a] Technische Universität Berlin, Department of Ecology, Ernst-Reuter-Platz 1, 10587, Berlin, Germany
[b] Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Department of Experimental Limnology, Alte Fischerhütte 2, 16775, Stechlin, Germany

ARTICLE INFO

Article history:
Received 20 January 2020
Received in revised form
30 April 2020
Accepted 9 May 2020
Available online 11 May 2020

Keywords:
Aquatic invertebrate
Environmental exposure concentration
Bioaccumulation
Biotransformation
Oxidative stress

ABSTRACT

The neonicotinoid imidacloprid (IMI) is one of the most extensively applied neuro-active insecticides worldwide and continues to enter surface waters in many countries despite a recent ban for outdoor use in the EU. Yet little is known about ecotoxicological effects on non-target benthic freshwater species exposed to environmentally relevant concentrations of IMI and its marketed products. The aim of the present study was to narrow this gap by assessing effects of pure IMI and its commercial formulation Confidor® on the aquatic oligochaete *Lumbriculus variegatus*, a key species in freshwater sediments. To this end, we determined dose-response relationships in 24 h toxicity tests, bioconcentration during 24 h and 5 d of exposure to 0.1, 1 and 10 μg IMI L^{-1}, and physiological stress responses by measuring glutathione S-transferase, glutathione reductase and catalase activity in the same conditions. Maximum neonicotinoid concentrations reported from the field were lethal to *L. variegatus* within 24 h (LC_{50} of 65 and 88 μg IMI L^{-1} in pure form and as active ingredient of Confidor®, respectively). At sub-lethal exposure concentrations, tissue content of IMI significantly increased with exposure time. The observed bioconcentration factors (BCFs) were far above the water octanol coefficient (K_{OW}), indicating a potentially large underestimation of IMI bioaccumulation when based on K_{OW}. Activities of biotransformation and antioxidant enzymes indicated attempts of *L. variegatus* to counter xenobiotic-triggered oxidative stress to very low IMI and Confidor® concentrations. Together, our data add significantly to growing evidence that the continued proliferation of neonicotinoids require increased efforts in environmental risk assessment, especially in view of species-specific differences in sensitivities to the insecticide and possibly to additives of commercial formulations.

© 2020 Elsevier Ltd. All rights reserved.

1. Introduction

The massive introduction of novel chemicals into the environment is one of the main drivers of environmental change causing ecosystem degradation at the global scale (Steffen et al., 2015; Johnson et al., 2020). Pesticides pose a particular threat, due to their adverse biological effects by design and large-scale application in agriculture. The proliferation of these agrochemicals has indeed been increasing dramatically (Bernhardt et al., 2017; Sharma et al., 2019), although Carson (1962) focused attention on the strong negative consequences of excessive pesticide application for wildlife already almost six decades ago. In response, the chemical industry has strived to develop increasingly effective pesticides with modes of action and behaviour in natural environments that minimize potential harm to non-target organisms. This, however, has not prevented the acceleration of pesticide production and application at the global scale (Bernhardt et al., 2017).

A new class of effective synthetic insecticides supposed to minimize harm reached the market in the 1990s. Possessing contact, stomach and systemic activity, these compounds known as neonicotinoids alter neurotransmission by irreversibly binding to the nicotinic acetylcholine receptors (nAChR) of insects (Buckingham et al., 1997; Jeschke et al., 2013). As a result, nerve impulse transmission is disrupted, causing uncoordinated body movements, paralysis and ultimately death within 24–48 h upon contact and ingestion (Bai et al., 1991). One of the most extensively

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.
* Corresponding author. Ernst-Reuter-Platz 1, 10587, Berlin, Germany.
E-mail address: v.contardojara@tu-berlin.de (V. Contardo-Jara).

https://doi.org/10.1016/j.envpol.2020.114793
0269-7491/© 2020 Elsevier Ltd. All rights reserved.

used neonicotinoids is imidacloprid (IMI). Introduced around 2004 (Pollack, 2011), this insecticide is today among the top ten agrochemicals worldwide (Simon-Delso et al., 2015). Application trends vary among countries, depending on legal restrictions, including a recent ban in the EU, but the global market continues to grow, with new commercial products containing IMI being permanently introduced (Simon-Delso et al., 2015).

Since neonicotinoids such as IMI are effective against numerous common pest species (Jeschke et al., 2013), they have been widely applied in crop and ornamental plant protection, urban pest control, veterinary treatment, and fish farming. Applications range from foliar spraying to seed dressing and pilling, soil treatment, trunk injection or stem brushing of trees, drenching of flower bulbs, and more (Simon-Delso et al., 2015). Most popular in crop protection is the prophylactic coating of seeds. This approach results in uptake of the active substance by roots and subsequent translocation to all plant parts during germination and growth, making the entire plant toxic to insects over its whole lifecycle (van der Sluijs et al., 2013). However, while highly effective in crop protection, this mode of application also releases huge amounts of neonicotinoids (up to 80%) into the environment (van der Sluijs et al., 2013), thus posing considerable risks to non-target species and ecosystems.

Negative effects on pollinators were attributed to IMI exposure soon after introduction of the nicotinoid to the market (Bryden et al., 2013, Sanchez-Bayo et al., 2016). Like other neonicotinoids, IMI yet continues to be promoted as a pesticide that is less toxic to non-target organisms than older classes of insecticides, despite clear evidence that the presumed selectivity and environmental safety is lower than initially assumed. Non-target insects, plankton, fish and mammals are affected, with effects on oxidative stress and DNA damage observed, and impacts demonstrated on growth, feeding, locomotion and survival (Muzinic and Zeljezic, 2018). Moreover, bioaccumulation of IMI has been found in various aquatic invertebrate species, including the mayfly *Isonychia bicolor* (Camp and Buchwalter, 2016). As a consequence, the European Food Safety Authority (EFSA, 2013) has stipulated a revaluation of toxicological thresholds and restriction requirements, and the US Environmental Protection Agency (EPA) is to revaluate the ecological risk potential as well (EPA, 2019).

Although neonicotinoids are primarily applied in terrestrial environments (Kreutzweiser et al., 2007; Bonmatin et al., 2015), large quantities enter freshwaters where they are relatively soluble and can persist for weeks, especially in the dark (Tisler et al., 2009, Morrissey et al., 2015). Entry pathways include spray and dust drift during application; improper use in farms, farmyards and urban areas; and transport with runoff, especially when lawns, road embankments and street trees are treated (Smitley et al., 2010; Bonmatin et al., 2015). Average and maximum neonicotinoid concentrations in diverse freshwater bodies near croplands range from 0.1 to 1 and 1–100 μg L^{-1}, respectively (Morrissey et al., 2015), as shown by a compilation of monitoring data from 29 locations on five continents reported from programs suggesting that neonicotinoid contamination of freshwaters has become serious. This conclusion is corroborated by more recent reports on IMI, the most widespread neonicotinoid, from Canadian surface waters (maximum of 10.4 μg L^{-1}; Struger et al., 2017) and tributaries of the Great Lakes (0.15 μg L^{-1}; Hladik et al., 2018). Similarly, IMI concentrations of up to 4.5 μg L^{-1} have been found in groundwater wells (Bradford et al., 2018) and up to 40 ng L^{-1} even in tap water (Klarich et al., 2017). Finally, sediment contamination has recently been reported (Tisler et al., 2009, Morrissey et al., 2015), with concentrations ranging from 0.11 to 7.16 μg IMI kg^{-1} dry mass in South China and Belize (Bonmatin et al., 2019; Zhang et al., 2019).

Both acute and chronic toxicity of IMI has been demonstrated for multiple aquatic taxa (Morrissey et al., 2015). While sensitivity varies among organisms, with LC_{50} ranging from 0.25 to 2500 μg IMI L^{-1} (Morrissey et al., 2015), short-term exposure at concentrations above 0.2 μg IMI L^{-1} and long-term exposure exceeding 0.035 μg L^{-1} have been found to harm aquatic invertebrates. However, little is known about adverse effects of IMI on organisms in sediments such as the aquatic oligochaete *Lumbriculus variegatus*, which is a useful test organism to close this gap but has only been used in a single study to assess effects of nicotinoids (Sardo and Soares, 2010). The species is widely distributed in freshwater sediments throughout the northern hemisphere, and as an often abundant and effective deposit-feeder, it is an important component of benthic food webs, serving as prey for secondary consumers (Krezoski and Robbins, 1985). Importantly, *L. variegatus* shows moderate sensitivity to environmental contaminants (Leppänen and Kukkonen, 1998; Contardo-Jara and Wiegand, 2008, Vehniäinen and Kukkonen, 2015). This facilitates assessments of sublethal effects, including activity changes of enzymes involved in the biotransformation of pesticides, or in defense mechanisms against oxidative stress to prevent damage of proteins, lipids, and DNA (van der Oost et al., 2003; Rodrigues et al., 2019).

In the European Union, the authorization of plant protection products involves two stages. First, active substances are approved across the EU after an extensive review has been carried out by the EFSA and the competent authorities of the member states. A positive assessment is a prerequisite for approval of commercial plant protection products by the member states in a second step, reflecting the recognition that the specific formulation of a plant protection product influences toxicity of a particular pesticide. For example, Confidor® SL200, which contains IMI, might aggravate adverse effects of pure IMI (Tisler et al., 2009). Examples include the inhibition of luminescence in a bacterium (*Vibrio fischeri*) and growth of a green alga (*Desmodesmus subpicatus*), immobility and activity changes of selected detoxification enzymes in water fleas (*Daphnia magna*), as well as lethal and sublethal malformations during embryonic development of zebrafish, *Danio rerio* (Tisler et al., 2009). These findings highlight the need to test specifically the toxicity of commercial products containing IMI, in addition to evaluating the pure substance.

The aim of the present study was to determine the impact of Confidor® in comparison to pure IMI at environmentally relevant concentrations (i.e. low μg per litre range) on a representative freshwater invertebrate associated with sediments. For this purpose, we (1) established LC_{50} values for the aquatic oligochaete *L. variegatus* in acute toxicity tests, (2) examined IMI and Confidor® uptake by the worms in one-day and five-day exposure trials, (3) determined bioconcentration factors at different exposure concentrations and durations, and (4) tested for induced oxidative stress and detoxification responses by using established biomarkers for sublethal effects of both pure IMI and Confidor®.

2. Material and methods

2.1. Test substances

An analytical standard of IMI (CAS no. 138261-41-3) was purchased from Sigma-Aldrich to prepare a stock solution of 1.25 mg mL^{-1} in 25% EtOH. Dilution with artificial freshwater resulted in a final EtOH concentration of <0.002%, which is ecotoxicologically negligible. The concentration of IMI in Confidor®, a widely used commercial granulate dispersing in water (Bayer AG, Leverkusen, Germany), was quantified by HPLC-MS/MS (see below) over a concentration range of 1–1000 μg L^{-1}. The average IMI content in Confidor® of 77 ± 8% (SD, n = 3) was used to calculate the dilution required to achieve very similar concentrations of IMI

in exposure scenarios with the pure substance vs Confidor® (see below).

2.2. Rearing of Lumbriculus variegatus

A culture of *L. variegatus* was purchased from the aquarium trade (PM-Aquaristik, Berlin, Germany). The animals were placed in 10-L glass tanks containing artificial fresh water (AFW, i.e. deionized water reconstituted with 100 mg L^{-1} sea salt, 200 mg L^{-1} $CaCl_2$ and 103 mg L^{-1} $NaHCO_3$) and reared at 20 °C and a photoperiod of 14:10 h. They were fed daily with 10 mg of ground algal-based fish food (JBL Spirulina Premium Flakes). Pre-soaked paper towels served as substratum. The AFW was renewed once a week. Criteria for selecting worms for the experiment were size (20 ± 2 mm), uniformly red colour and immediate twitching upon contact. Worms were not fed during the experiment.

2.3. Experimental design

Short-term lethal concentration experiment: To establish LC_{50} values, worms were exposed for 24 h to 11 concentrations of pure IMI or Confidor® dissolved in AFW (0.1, 1, 5, 10, 20, 40, 60, 80, 100, 120, 1000 μg IMI L^{-1}). These concentrations were chosen to cover a range of five powers of ten, including the range of environmentally relevant concentrations, and higher resolution in the concentration range where preliminary tests had indicated lethal effects to occur. Each of three replicates per concentration consisted of 20 worms in 150 mL of exposure medium. The number of dead worms was recorded at the end of the experiment and expressed as % mortality. LC_{50} values were calculated based on the mean IMI concentrations measured in the exposure medium at the beginning and end of the experiment.

Uptake and enzyme activity experiment: To assess the bioconcentration factor of IMI in *L. variegatus* as well as potential enzymatic changes of the worms' biotransformation and antioxidant system affected by IMI exposure, worms were exposed to three different IMI concentrations applied as either pure substance or in Confidor® (0.1, 1, 10 μg L^{-1} IMI in AFW) for 24 h and 5 d. Exposure medium was not renewed during the experiment. Five replicates, each consisting of 20 worms in 150 mL of exposure medium, were prepared for each exposure concentration, including controls in AFW without IMI, and both exposure times. Ten worms per replicate were pooled for enzyme activity measurements. The remaining 10 worms were pooled to determine IMI uptake. All worms were transferred to AFW for 1 h to clear guts and remove IMI from the epidermis before shock-freezing the samples in liquid nitrogen and storing them at −80 °C for later IMI analysis and enzyme assays. In order to check the stability of the exposure solution, IMI concentrations were immediately measured by HPLC-MS/MS at the beginning of the experiment and after one and five days of exposure.

2.4. Analytics

IMI extraction: The pooled worm samples were subjected to five freeze (−20 °C) and thaw (20 °C) cycles before they were homogenized in 10 vol of MeOH (ca. 100 mg sample in 1 mL MeOH). After 2 h of ultra-sonication at 30 °C, samples were centrifuged (10 min at 10,600 *g* and 4 °C) and the resulting supernatant directly injected into the HPLC-MS/MS for IMI quantification. A set of samples spiked with IMI was subjected to the same procedure to assess compound stability during the extraction procedure, which was found to be high given recovery rates of 96 ± 5% (mean ± SD, N = 5).

HPLC-MS/MS: IMI in exposure medium and tissue extracts was quantified according to Iturburu et al. (2017). Chromatographic separation of IMI was achieved on a Phenomenex 2.5 μm Synergi-fusion RP column (2.0 × 50 mm) connected to an Alliance 2695 UHPLC and a Micromass Quattro micro™ mass spectrometer (Waters), using a step gradient from 100% solvent A (0.1% formic acid in H_2O) to 75% solvent B (0.1% formic acid in acetonitrile) (Iturburu et al., 2017). The flow rate was 0.25 mL min^{-1} and the sample injection volume was 10 μL. Temperature of the column oven was set to 25 °C. The positive ion MRM mode was used for mass spectrometric detection with a collision energy of 25 V and a mass transfer of 256 m/z to 175 and 209 m/z (MS/MS). Quantification was based on the total ion count of both product ions. Calibration was liner between 0.1 and 1000 μg L^{-1} (R^2 = 0.999, LOD = 0.05 μg L^{-1} IMI). Separate calibration curves (IMI in AFW and IMI in MeOH) were prepared to measure concentrations in exposure medium and tissue extracts, respectively. Furthermore, to evaluate any matrix effects, an additional calibration curve was prepared in a methanolic worm extract, which yielded no differences to the calibration curves prepared in fresh MeOH. Multiple blanks run before, in between and after analysing samples were never found to be contaminated. All analytical steps were subject to standard quality control in terms of accuracy, precision and recovery rate.

2.5. Enzyme preparation and measurements

The activities of detoxification and oxidative stress enzymes in *L. variegatus* were selected as physiological endpoints to assess sublethal effects of IMI exposure. Pooled samples of 10 worms were homogenized in ice-cold 0.1 M sodium phosphate buffer (pH 6.5, 20% glycerol) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 1.4 mM dithioreythriol (DTE). A centrifugation step (10 min at 4 °C and 10,600 *g*) ensured removal of cell debris. The supernatant was used to determine protein content according to Bradford (1976) and spectrophotometric measurements of enzyme activities. The activity of glutathione S-transferase (GST, EC 2.5.1.18) was determined with reduced glutathione (GSH) added to an aliquot of the supernatant and 1-chloro-2,4-dinitrobenzene (CDNB) used as substrate (Habig et al., 1974). Kinetics were followed at 340 nm for 3 min at 30 °C. The activity of catalase (CAT, EC 1.11.1.6) was determined according to Claiborne (1985), using H_2O_2 as substrate. The decrease of H_2O_2 was followed at 240 nm for 5 min at 30 °C. The activity of glutathione reductase (GR, EC 1.8.1.7) was determined according to Carlberg and Mannervik (1985) using glutathione disulfide as substrate. The decrease in NADPH added to the reaction was followed at 340 nm for 10 min at 30 °C. Determination of protein content and enzyme activities were performed in triplicate for each experimental replicate.

2.6. Data analysis

Statistical analysis of the data was performed using fully factorial analyses of variance (ANOVA) with exposure time, exposure concentration and application form as factors (SYSTAT 6). Additionally, two-way ANOVAs (GraphPad Prism 6™) using only exposure time and exposure concentration as factors were calculated individually for each application form (i.e. pure IMI and Confidor®) to test for significant differences in enzyme activities between controls and worms exposed to IMI. Bonferroni tests were chosen for pairwise comparisons when the overall effects of IMI concentration were significant. Finally, Student's *t*-test was used to test for differences in the application form (i.e. IMI as an ingredient of Confidor® or as a pure substance) for each exposure concentration and time point. The significance level of all statistical tests was set at $p = 0.05$. LC_{50} values were derived from a logistic regression

analysis performed with TableCurve 2D (Systat Software, Inc.).

3. Results

3.1. Lethal concentrations

All worms survived exposure to concentrations below 40 μg L^{-1} IMI for 24 h (Fig. 1). Exposure to 40, 60, 80 and 100 μg L^{-1} caused death in 3, 32, 90 and 100% of the worms exposed to pure IMI and in 2, 23, 72 and 85% of the worms exposed to Confidor®. No worms survived at 120 and 1000 μg L^{-1} of pure IMI or IMI in Confidor®, respectively. The lethal concentration at which 50% of the organisms died (LC_{50}) was calculated at 65.3 ± 2.8 μg L^{-1} of pure IMI and 87.8 ± 2.2 μg L^{-1} of IMI in Confidor® (means and 95% confidence intervals; Fig. 1).

3.2. Bioaccumulation

IMI concentrations and the total volume of the exposure medium containing worms remained constant over the entire exposure time of 5 d. The mean concentrations measured were i) 0.10 ± 0.02 μg L^{-1} for both pure IMI and IMI in Confidor®, ii) 0.87 ± 0.09 and 1.03 ± 0.12 μg L^{-1} and iii) 9.8 ± 0.7 and 13.1 ± 1.1 μg L^{-1} for target concentrations of 0.1, 1 and 10 μg L^{-1}, respectively.

IMI was never detected in worms not exposed to IMI. Exposure time, exposure concentration and application form (pure IMI or IMI in Confidor®) all had a significant influence on the IMI concentration of worm tissue, but did not produce significant interactions (Fig. 2). Tissue concentrations consistently increased between 1 and 5 d ($p < 0.0001$), and with increasing exposure concentration ($p < 0.0001$). Application form also has a significant effect ($p = 0.04$), since tissue concentrations after 5 d were slightly higher in worms exposed to Confidor® compared to worms exposed to pure IMI. Specifically, worms exposed to 0.1 μg L^{-1} of pure IMI or IMI in Confidor® accumulated 6.6 ± 2.9 and 7.0 ± 4.9 ng IMI g^{-1} animal fresh mass (FM) and 19.1 ± 3.3 and 23.9 ± 6.5 ng IMI g^{-1} FM, respectively, after 1 and 5 d of exposure (Fig. 2). At 1 μg L^{-1}, the increase was fourfold, from 24.5 ± 4.7 to 104 ± 33 ng IMI g^{-1} FG (pure IMI) and from 34.8 ± 7.8 to 126 ± 11 ng IMI g^{-1} FM (IMI in Confidor®), respectively (Fig. 2). Exposure to 10 μg L^{-1} led to tissue concentrations of 254 ± 34 and 258 ± 38 ng IMI g^{-1} FM (pure IMI) and of 732 ± 65 and 946 ± 76 ng IMI g^{-1} FM (Confidor®) after 1 and 5 d, respectively (Fig. 2).

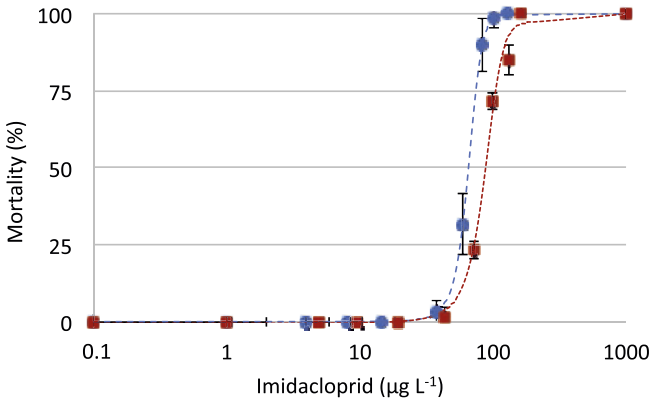

Fig. 1. Percent mortality of *Lumbriculus variegatus* after 24 h of exposure to increasing concentrations of pure IMI (blue circles) and IMI as active ingredient of the commercial formulation Confidor® (red squares). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The bioconcentration factor (BCF) calculated based on mean measured exposure concentrations of IMI also increased substantially between 1 and 5 d for all exposure concentrations ($p < 0.0001$), although it decreased with increasing exposure concentration ($p < 0.0001$; Fig. 3). The stronger increase of the BCF at lower exposure concentrations led to a significant interaction effect between exposure time and concentration ($p < 0.0001$). The BCF in worms exposed to the lowest IMI concentration (0.1 μg L^{-1}) increased between 1 and 5 d from 66 to 190 and from 70 to 240 for pure IMI and Confidor®, respectively (Fig. 3). Exposure to 1 μg L^{-1} of pure IMI or IMI in Confidor® led to an increase over time from 28 to 119 and from 34 to 123, respectively. Exposure to 10 μg L^{-1} of pure IMI or IMI in Confidor® led to an increase from 26 to 75 and from 20 to 72, respectively. The differences between IMI applied as pure substance or in Confidor® were never significant.

3.3. Enzyme activities

After 1 d, the activity of GST was significantly increased in *L. variegatus* exposed to 0.1 μg L^{-1} IMI applied as pure substance or in Confidor® (Fig. 4, Table 1), and to 10 μg L^{-1} of pure IMI (Fig. 4). After 5 d, GST activity was significantly increased at 1 and 10 μg L^{-1} pure IMI. This pattern resulted in a significant interactive effect of exposure time and concentration on GST activity ($p = 0.0001$), and both factors also had independent effects ($p = 0.03$). Application form, in contrast, did not have a significant influence.

The activity patterns of GR, which catalyses the reduction of glutathione disulphide to glutathione, were similar to those of GST (Fig. 5). The activity was significantly higher in *L. variegatus* exposed for 1 d to 0.1 μg L^{-1} of IMI applied as pure substance or in Confidor®, or to 1 μg L^{-1} of IMI in Confidor®, but became similar to the activity of control worms after 5 d (Table 1). Worms exposed for 1 d to 1 μg L^{-1} showed a significantly higher GR activity when exposed to IMI in Confidor® as opposed to the pure substance (*t*-test, $p = 0.016$). Exposure time and concentration thus had significant main ($p < 0.001$) and interactive effects ($p = 0.049$). Overall, application form had no significant influence on GR activity.

The activity of the antioxidant enzyme CAT, responsible for the detoxification of hydrogen peroxide as a reactive oxygen species, was significantly higher in *L. variegatus* after 1 d of exposure to 1 μg L^{-1} IMI in Confidor®, but not at the other concentrations (Fig. 6, Table 1). This was exclusively due to the higher CAT activity when the worms were exposed to IMI in Confidor® as opposed to the pure substance (*t*-test, $p < 0.0001$). As a result, application form ($p < 0.001$), in addition to exposure time ($p < 0.0001$), also affected CAT activity, and there was also an interactive effect of application form and time ($p < 0.001$). After 5 d, average CAT activities in worms exposed to IMI were invariably lower than in control worms, with differences between application forms no longer being apparent.

4. Discussion

Our data show that at maximum concentrations measured in the field (1–100 μg L^{-1}; Morrissey et al., 2015), IMI can be lethal within 24 h of exposure to a widespread non-target species in freshwater sediments, the blackworm *L. variegatus*. The LC_{50} values we determined (65 and 88 μg L^{-1} of IMI as pure substance and in Confidor®, respectively) are consistent with data obtained in 96 h exposure assays (45 μg L^{-1}) by Raby et al. (2019), and are also within the range of values for other non-target freshwater species (Morrissey et al., 2015): the amphipod *Gammarus pulex* (LC_{50} of 350 μg L^{-1}), the opossum shrimp *Americamysis bahia* (34–159 μg L^{-1}), and larvae of the non-biting midge *Chironomus riparius* (20 μg L^{-1}), all of which are arthropods, whereas *L. variegatus* is an annelid. The similarity among these four species in terms of sensitivity to IMI in

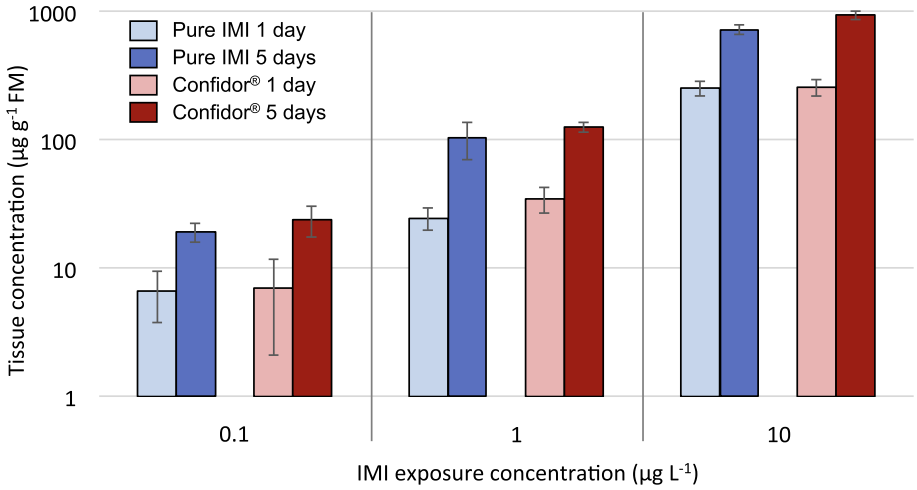

Fig. 2. IMI tissue concentration in *Lumbriculus variegatus* after exposure to IMI as pure substance (blue shading on the left) or as active ingredient in Confidor® (red shading on the right) after one (light shading) or five (dark shading) days of exposure. Data are means and standard deviations of five independent replicates (n = 5) each comprising 10 worms. Exposure time ($p < 0.0001$), exposure concentration ($p < 0.0001$) and application form (pure IMI or IMI in Confidor®; $p = 0.04$) all had very consistent effects on tissue concentration, with none of the interactions being significant ($p > 0.36$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

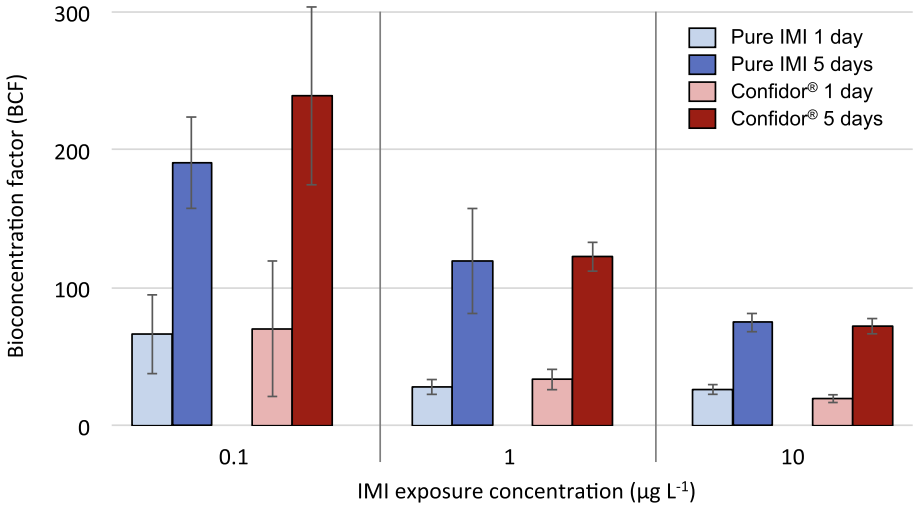

Fig. 3. Bioconcentration factor (BCF) for IMI in *Lumbriculus variegatus* after exposure to IMI as a pure substance (blue shading on the left) or as active ingredient in Confidor® (red shading on the right) after one (light shading) and five (dark shading) days. Data are means and standard deviations of five independent replicates (n = 5), each comprising 10 worms. Exposure time and exposure concentration had significant main and interactive effects on the BCF ($p < 0.0001$ in all cases). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the tens of micrograms per litre range indicates that reliance on standard acute toxicity tests with *Daphnia* (LC_{50} of 65–133 mg L^{-1}; Sanchez-Bayo and Goka, 2006) could severely underestimate the toxicity of IMI (i.e. by a factor 1000). Clearly, this widely used neonicotinoid can affect freshwater species beyond non-target arthropods at environmentally relevant concentrations and thus poses greater risks to freshwaters than is currently recognized.

This conclusion holds although IMI does not classify as a bioaccumulative substance. The classification is independent of whether our bioconcentration factors (BCF; critical threshold = 2000 according to EU regulations) or the widely applied water octanol coefficient (K_{OW}; critical threshold of log $K_{OW} = 5$, i.e. $K_{OW} = 10^5$) are used as the criterion. The BCFs of IMI in our study (20–70 and 70–240 after 1 and 5 days of exposure, respectively) greatly exceed the K_{OW} of 3.7 (log K_{OW} = 0.57) determined by Pestana et al. (2009), whereas the ratio of the critical thresholds (BCF:K_{OW}) is only $2000:10^5 = 0.02$. This large discrepancy suggests that estimates of bioaccumulation potential based on K_{OW} alone involve considerable risk. Furthermore, our distinctly larger BCFs at low exposure concentrations point to the need to cover a realistically broad concentration range when assessing bioaccumulation potential.

The similar BCFs we observed between IMI applied as pure substance and Confidor® (irrespective of exposure concentration or duration) matches results of acute toxicity tests (24 and 48 h) with both a bacterium (*Vibrio fischeri*) and zebrafish (*Danio rerio*) (Tisler et al., 2009). Differences in effects on the water flea *Daphnia magna* also were absent in the short term, emerging only after 21 d of exposure (Tisler et al., 2009). A green alga (*Desmodesmus subspicatus*) proved more sensitive to Confidor® in the same study. Overall, however, the data available to date suggest that information on the behaviour of pure IMI provides generally realistic

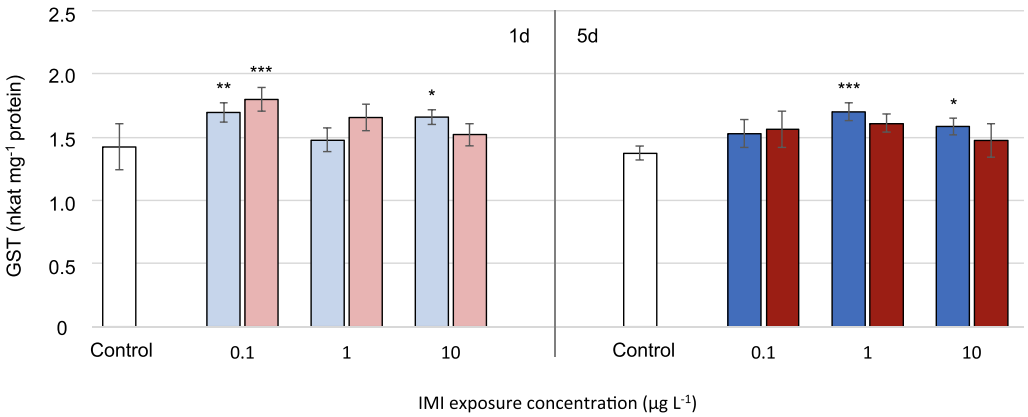

Fig. 4. Glutathione S-transferase (GST) activity in *Lumbriculus variegatus* after exposure for one (left side, light shading) or five days (right side, dark shading) to IMI as a pure substance (blue shading) or as active ingredient in Confidor® (red shading). Data are means and standard deviations of five independent replicates (n = 5), each comprising 10 worms. Exposure time ($p = 0.032$) and exposure concentration ($p = 0.028$) had a significant main effect and the interaction was also significant ($p = 0.0001$). Asterisks indicate significant differences compared to the controls (two-factorial ANOVA, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Results of full-factorial ANOVAs of five endpoints measured to assess effects of imidacloprid (IMI) on the freshwater oligochaete *Lumbriculus variegatus*: IMI uptake, bioaccumulation assessed as bioconcentration factor (BCF), glutathion S-transferase (GST), glutathione reductase (GR), and catalase (CAT). The tested factors are exposure time (Time), IMI exposure concentration (Conc) and application form (Form), which refers to the comparison of IMI as a pure substance or in Confidor®.

Endpoint	Source of variation	Sum of squares	df	Mean squares	*F*-ratio	*P*
Uptake	Time	4.469	1	4.469	273.8	<0.0001
	Conc	25.535	2	12.767	782.3	<0.0001
	Form	0.075	1	0.075	4.59	0.038
	Time * Conc	0.015	2	0.008	0.461	0.63
	Time * Form	0.014	1	0.014	0.852	0.36
	Conc * Form	0.025	2	0.013	0.768	0.47
	Time * Conc * Form	0.022	2	0.011	0.664	0.52
BCF	Time	134763	1	134763	163.2	<0.0001
	Conc	88850	2	44425	53.8	<0.0001
	Form	1111	1	1111	1.35	0.25
	Time * Conc	22651	2	11325	13.7	<0.0001
	Time * Form	889	1	889	1.077	0.30
	Conc * Form	2366	2	1183	1.433	0.25
	Time * Conc * Form	1547	2	773	0.937	0.40
GST	Time	0.046	1	0.046	4.91	0.032
	Conc	0.072	2	0.036	3.85	0.028
	Form	0.000	1	0.000	0.045	0.83
	Time * Conc	0.206	2	0.103	10.96	0.0001
	Time * Form	0.040	1	0.040	4.21	0.046
	Conc * Form	0.107	2	0.053	5.68	0.006
	Time * Conc * Form	0.054	2	0.027	2.89	0.066
GR	Time	0.013	1	0.013	16.70	0.0002
	Conc	0.015	2	0.008	9.72	0.0003
	Form	0.002	1	0.002	2.58	0.12
	Time * Conc	0.005	2	0.003	3.25	0.049
	Time * Form	0.003	1	0.003	3.30	0.076
	Conc * Form	0.003	2	0.002	2.04	0.14
	Time * Conc * Form	0.006	2	0.003	3.47	0.041
CAT	Time	15235	1	15235	34.0	<0.0001
	Conc	939	2	469	1.048	0.36
	Form	6874	1	6874	15.4	0.0003
	Time * Conc	3637	2	1818	4.06	0.025
	Time * Form	6964	1	6964	15.6	0.0003
	Conc * Form	3213	2	1606	3.59	0.037
	Time * Conc * Form	1480	2	740	1.65	0.20

bioaccumulation estimates also for Confidor®. This recognition is important because the high BCFs and a marked concentration increase in *L. variegatus* between 1 and 5 d of exposure in our study indicate that IMI could be enriched in body tissues during prolonged exposure even when environmental concentrations are low (e.g., 0.1 µg L^{-1}).

In contrast to our results on the LC_{50} and BCF of pure IMI and Confidor®, which were consistent, differences were apparent in the enzymatic reaction of *L. variegatus* to IMI exposure. The pattern of CAT and GR activities we observed suggests that ingredients of Confidor® other than IMI affected the physiology of the worms in our study, and implies greater oxidative stress by exposure to

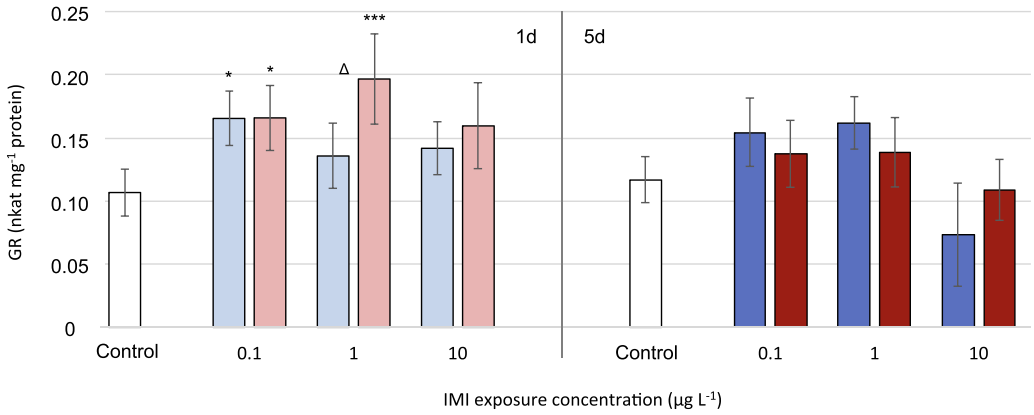

Fig. 5. Glutathione reductase (GR) activity in *Lumbriculus variegatus* after exposure for one (left side, light shading) or five days (right side, dark shading) to IMI as a pure substance (blue shading) or as active ingredient in Confidor® (red shading). Data are means and standard deviations of five independent replicates (n = 5), each comprising 10 worms. Exposure time ($p = 0.0002$) and concentration ($p = 0.0003$) both had a significant main effect and also produced a significant interaction ($p = 0.049$). Asterisks indicate significant differences to the controls (two-factorial ANOVA, $*p < 0.05$, $***p < 0.001$). Worms exposed to 1 µg L^{-1} also showed a significantly higher GR activity when exposed to IMI in Confidor® as opposed to the pure substance (*t*-test, $p = 0.016$), as indicated by a triangle. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

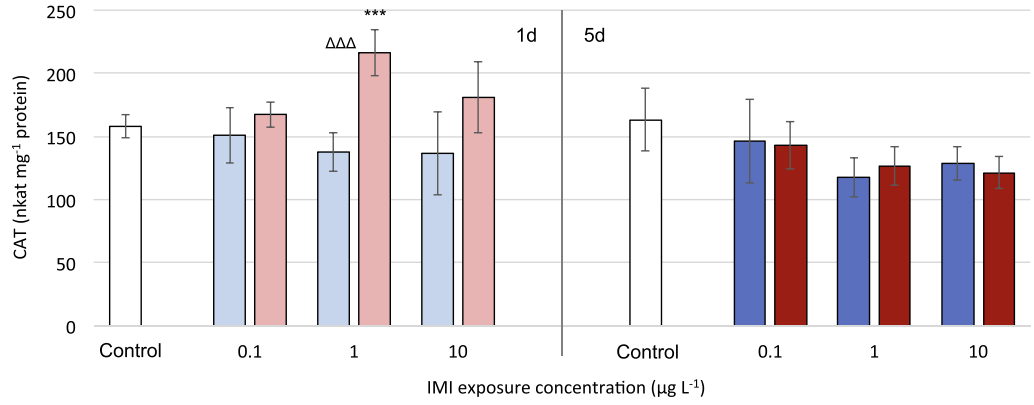

Fig. 6. Catalase (CAT) activity in *Lumbriculus variegatus* after exposure for one (left side, light shading) or five days (right side, dark shading) to IMI as a pure substance (blue shading) or as active ingredient in Confidor® (red shading). Data are means and standard deviations of five independent replicates (n = 5), each comprising 10 worms. Exposure time ($p < 0.0001$) and application form ($p = 0.0003$) both had significant individual effects on CAT activity (global ANOVA), in addition to a significant interaction effect ($p = 0.0003$). Asterisks indicate significant differences compared to the controls (two-factorial ANOVA, $***p < 0.001$). In addition, worms exposed to IMI in Confidor® as opposed to the pure substance showed a significantly higher CAT activity at 1 µg L^{-1} (*t*-test, $p < 0.0001$), as indicated by triple triangles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Confidor® than to pure IMI. This outcome contrasts with the results of a recent study on oysters which found no consistent differences in toxicity on various physiological responses (CAT, GST, acetylcholinesterase, fatty acid composition) between pure IMI and a commercial IMI-containing formulation other than Confidor® (Spectrum 200SC) (Ewere et al., 2019). The basis of these differences between studies remains unknown, not least because the manufacturers have not released the composition of their commercial products and both the test organisms and endpoints partly differed between the studies.

Nonetheless, one mechanism underlying neonicotinoids toxicity to non-target species appears to be oxidative stress, as highlighted in a comprehensive review summarizing effects on mammals, birds, fish and terrestrial invertebrates (Wang et al., 2018). In freshwater invertebrates, decreased levels of reduced glutathione (GSH) in larvae of the non-biting midge *Chironomus riparius*, as well as slightly induced lipid peroxidation at concentrations as low as 0.063 µg IMI L^{-1}, indicated oxidative stress specifically induced by IMI after 10 d of exposure (Chandran et al., 2018). Our enzyme data on *L. variegatus* imply an immediate antioxidant response (partly significant increases in GST and especially GR activity after 1 d), which diminished for GR after longer exposure (5 d). This temporal pattern suggests either acclimatization or exhaustion of the enzymatic response capacity, and the lack of a further increase in GST activity and a tendency towards lower activity of GR suggests the latter mechanism could indeed have played a role. However, the overall pattern of enzymatic activities we measured is not clear-cut, nor did a clear relationship emerge between enzymatic responses to IMI exposure and the level of bioconcentration. This could mean that the scope for physiological responses of *L. variegatus* within the narrow concentration range (10–100 µg L^{-1}) that we found between no effect and permanent damage and death could be very limited.

Besides exposure to necotinoids released into surface waters by

spray drift or runoff from agricultural land, freshwater organisms can be affected if they ingest any contaminated food, particularly when large amounts of contaminated plant or soil organic matter enter surface waters (Englert et al., 2017a, 2017b, 2018). This scenario is highly likely in water bodies receiving crop residues and soil particles in agricultural catchments. Moreover, this food-mediated pathway could expose not only detritus feeders to the contaminant. Predators would also be affected if the contaminant accumulates in their prey such as *L. variegatus* (see above). Uptake of IMI and other neonicotinoids by bees and other terrestrial insects ingesting pollen, nectar, leaves or other plant parts suggest that this exposure and biomagnification route *via* food can indeed be important for non-target species in terrestrial environments (Bonmatin et al., 2015; Botias et al., 2016). In the present study, ingestion of contaminated food and transfer and enrichment of IMI within food chains was not specifically addressed. However, our data on IMI uptake, bioaccumulation, and physiological responses of an abundant detritus-feeding worm living in freshwater sediments indicate that such food-mediated effects are equally plausible for freshwater benthic species and food chains.

5. Conclusion

In conclusion, our data add to growing evidence that the continued proliferation of neonicotinoids, some of which eventually enter freshwaters, require increased attention in environmental risk assessment. Despite being marketed as selective and environmentally safe, neonicotinoids clearly cause adverse effects on non-target sediment biota in freshwaters at environmentally relevant concentrations. This calls for a revision of admission procedures for neonicotinoids based on information from assays including non-target sediment organisms to complement the established standard tests with fish, crustaceans and insects. The annelid *L. variegatus* used in the present study would be a useful model candidate. As our bioconcentration (BCF) data show, these assessments must also consider the potential for bioaccumulation as a function of exposure concentration and, possibly, for biomagnification in food chains. Moreover, assessments need to take into account that exposure in field situations will be accompanied by the presence of a range of other chemicals, including other pesticides. For mixtures of neonicotinoids, such effects are likely to be additive because modes of action are similar or identical (Morrissey et al., 2015), whereas synergistic effects could occur for mixtures containing chemicals that elicit other cellular processes than neonicotinoids. Thus, future tests on effects of insecticides also need to consider realistic mixtures with environmentally relevant contaminants. Finally, these risk assessments need to include indirect contaminant effects resulting from inter- and intraspecific species interactions that have potential repercussions at the population, community and ecosystem level in both freshwaters and other environments (e.g. Gessner and Tlili, 2016).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114793.

References

Bai, D., Lummis, S.C.R., Leicht, W., Breer, H., Sattelle, D.B., 1991. Actions of imidacloprid and a related nitromethylene on cholinergic receptors of an identified insect motor neurone. Pestic. Sci. 33, 197–204.

Bernhardt, E.S., Rosi, E.J., Gessner, M.O., 2017. Synthetic chemicals as agents of global change. Front. Ecol. Environ. 15, 84–90.

Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A.D., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environ. Sci. Pollut. Res. Int. 22, 35–67.

Bonmatin, J.M., Noome, D.A., Moreno, H., Mitchell, E.A.D., Glauser, G., Soumana, O.S., van Lexmond, M.B., Sanchez-Bayo, F., 2019. A survey and risk assessment of neonicotinoids in water, soil and sediments of Belize. Environ. Pollut. 249, 949–958.

Botias, C., David, A., Hill, E.M., Goulson, D., 2016. Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target insects. Sci. Total Environ. 566, 269–278.

Bradford, M.M., 1976. Rapid and sensitive method for quantification of microgram quantities of protein utilizing principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Bradford, B.Z., Huseth, A.S., Groves, R.L., 2018. Widespread detections of neonicotinoid contaminants in central Wisconsin groundwater. PloS One 13, e0201753.

Bryden, J., Gill, R.J., Mitton, R.A.A., Raine, N.E., Jansen, V.A.A., 2013. Chronic sublethal stress causes bee colony failure. Ecol. Lett. 16, 1463–1469.

Buckingham, S.D., Lapied, B., LeCorronc, H., Grolleau, F., Sattelle, D.B., 1997. Imidacloprid actions on insect neuronal acetylcholine receptors. J. Exp. Biol. 200, 2685–2692.

Carlberg, I., Mannervik, B., 1985. Glutathione-reductase. Methods Enzymol. 113, 484–490.

Camp, A.A., Buchwalter, D.B., 2016. Can't take the heat: temperature-enhanced toxicity in the mayfly *Isonychia bicolor* exposed to the neonicotinoid insecticide imidacloprid. Aquat. Toxicol. 178, 49–57.

Contardo-Jara, V., Wiegand, C., 2008. Biotransformation and antioxidant enzymes of *Lumbriculus variegatus* as biomarkers of contaminated sediment exposure. Chemosphere 70, 1879–1888.

Carson, R., 1962. Silent Spring. Hougthon Mifflin, New York, NY, USA.

Chandran, N.N., Fojtova, D., Blahova, L., Rozmankova, E., Blaha, L., 2018. Acute and (sub)chronic toxicity of the neonicotinoid imidacloprid on *Chironomus riparius*. Chemosphere 209, 568–577.

Claiborne, A., 1985. Catalase activity. In: Greenwald, R.A. (Ed.), CRC Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, FL, USA, pp. 283–284.

EFSA, European Food Safety Authority, 2013. Conclusion on the peer review of the pesticide risk assessment for bees for the active substance Imidacloprid. EFSA J. 11, 3068.

Englert, D., Zubrod, J.P., Neubauer, C., Schulz, R., Bundschuh, M., 2018. UV-irradiation and leaching in water reduce the toxicity of imidacloprid-contaminated leaves to the aquatic leaf-shredding amphipod *Gammarus fossarum*. Environ. Pollut. 236, 119–125.

Englert, D., Zubrod, J.P., Link, M., Mertins, S., Schulz, R., Bundschuh, M., 2017a. Does waterborne exposure explain effects caused by neonicotinoid-contaminated plant material in aquatic systems? Environ. Sci. Technol. 51, 5793–5802.

Englert, D., Bakanov, N., Zubrod, J.P., Schulz, R., Bundschuh, M., 2017b. Modeling remobilization of neonicotinoid residues from tree foliage in streams — a relevant exposure pathway in risk assessment? Environ. Sci. Technol. 51, 1785–1794.

EPA, United States Environmental Protection Agency, 2019. Schedule for Review of Neonicotinoid Pesticides. https://www.epa.gov/pollinator-protection/schedule-review-neonicotinoid-pesticides. last accessed 10.01.2020.

Ewere, E.E., Reichelt-Brushett, A., Benkendorff, K., 2019. Imidacloprid and formulated product impacts the fatty acids and enzymatic activities in tissues of Sydney rock oysters, *Saccostrea glomerata*. Mar. Environ. Res. 151, 104765.

Gessner, M.O., Tlili, A., 2016. Fostering integration of freshwater ecology with ecotoxicology. Freshw. Biol. 61, 1991–2001.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Hladik, M.L., Corsi, S.R., Kolpin, D.W., Baldwin, A.K., Blackwell, B.R., Cavallin, J.E., 2018. Year-round presence of neonicotinoid insecticides in tributaries to the Great Lakes, USA. Environ. Pollut. 235, 1022–1029.

Iturburu, F.G., Zomisch, M., Panzeri, A.M., Crupkin, A.C., Contardo-Jara, V., Pflugmacher, S., Menone, M.L., 2017. Uptake, distribution in tissue, and genotoxicity of imidacloprid in the freshwater fish *Australoheros facetus*. Environ. Toxicol. Chem. 36, 699–708.

Jeschke, P., Nauen, R., Beck, M.E., 2013. Nicotinic acetylcholine receptor agonists: a milestone for modern crop protection. Angew. Chem. Int. Ed. 52, 9464–9485.

Johnson, A.C., Jin, X., Nakada, N., Sumpter, J.P., 2020. Learning from the past and considering the future of chemicals in the environment. Science 367, 384–387.

Klarich, K.L., Pflug, N.C., DeWald, E.M., Hladik, M.L., Kolpin, D.W., Cwiertny, D.M., LeFevre, G.H., 2017. Occurrence of neonicotinoid insecticides in finished drinking water and fate during drinking water treatment. Environ. Sci. Technol. Lett. 4, 168–173.

Kreutzweiser, D., Good, K., Chartrand, D., Scarr, T., Thompson, D., 2007. Non-target effects on aquatic decomposer organisms of imidacloprid as a systemic insecticide to control emerald ash borer in riparian trees. Ecotoxicol. Environ. Saf. 68, 315–325.

Krezoski, J.R., Robbins, J.A., 1985. Vertical-distribution of feeding and particle-selective transport of CS-137 in lake-sediments by lumbriculid oligochaetes. J. Geophys. Res. Oceans 90, 1199–2006.

Leppänen, M.T., Kukkonen, J.V.K., 1998. Relationship between reproduction, sediment type, and feeding activity of *Lumbriculus variegatus* (Muller): implications for sediment toxicity testing. Environ. Toxicol. Chem. 17, 2196–2202.

Morrissey, C.A., Mineau, P., Devries, J.H., Sánchez-Bayo, F., Liess, M., Cavallaro, M.C.,

Liber, K., 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. Environ. Int. 74, 291–303.
Muzinic, V., Zeljezic, D., 2018. Non-target toxicity of novel insecticides. Arh. Hig. Rada. Toksikol. 69, 86–102.
Pestana, J.L.T., Alexander, A.C., Culp, J.M., Baird, D.J., Cessna, A.J., Soares, A.M.V.M., 2009. Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. Environ. Pollut. 157, 2328–2334.
Pollack, P., 2011. Fine Chemicals: the Industry and the Business, Second ed. John Wiley and Sons, Hoboken, NJ, USA.
Raby, M., Maloney, E., Poirier, D.G., Sibley, P.K., 2019. Acute effects of binary mixtures of imidacloprid and tebuconazole on 4 freshwater invertebrates. Environ. Toxicol. Chem. 38, 1093–1103.
Rodrigues, C., Bio, A., Guimarães, L., Fernandes, V.C., Delerue-Matos, C., Vieira, N., 2019. Assessing the ecological status of fluvial ecosystems employing a macroinvertebrate multi-taxon and multi-biomarker approach. Environ. Monit. Assess. 191, 503.
Sanchez-Bayo, F., Goka, K., 2006. Influence of light in acute toxicity bioassays of imidacloprid and zinc pyrithione to zooplankton crustaceans. Aquat. Toxicol. 78, 262–271.
Sanchez-Bayo, F., Goulson, D., Pennacchio, F., Nazzi, F., Goka, K., Desneux, N., 2016. Are bee diseases linked to pesticides? - A brief review. Environ. Int. 89–90, 7–11.
Sardo, A.M., Soares, A.M.V.M., 2010. Assessment of the effects of the pesticide imidacloprid on the behaviour of the aquatic oligochaete *Lumbriculus variegatus*. Arch. Environ. Contam. Toxicol. 58, 648–656.
Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G.P.S., Handa, N., Kohli, S.K., Yadav, P., Bali, A.S., Parihar, R.D., Dar, O.I., Singh, K., Jasrotia, S., Bakshi, P., Ramakrishnan, M., Kumar, S., Bhardwaj, R., Thukral, A.K., 2019. Worldwide pesticide usage and its impacts on ecosystem. SN Appl. Sci. 1, 1446.
Simon-Delso, N., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Chagnon, M., Downs, C., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C.H., Liess, M., Long, E., McField, M., Mineau, P., Mitchell, E.A.D., Morrissey, C.A., Noome, D.A., Pisa, L., Settele, J., Stark, J.D., Tapparo, A., Van Dyck, H., Van Praagh, J., Van der Sluijs, J.P., Whitehorn, P.R., Wiemers, M., 2015. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ. Sci. Pollut. Res. Int. 22, 5–34.
Smitley, D.R., Rebek, E.J., Royalty, R.N., Davis, T.W., Newhouse, K.F., 2010. Protection of individual ash trees from emerald ash borer (Coleoptera: Buprestidae) with basal soil applications of imidacloprid. J. Econ. Entomol. 103, 119–126.
Steffen, W., Richardson, K., Rockstrom, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., de Vries, W., de Wit, C.A., Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B., Sorlin, S., 2015. Planetary boundaries: guiding human development on a changing planet. Science 347, 736.
Struger, J., Grabuski, J., Cagampan, S., Sverko, E., McGoldrick, D., 2017. Factors influencing the occurrence and distribution of neonicotinoid insecticides in surface waters of southern Ontario, Canada. Chemosphere 169, 516–523.
Tisler, T., Jemec, A., Mozetic, B., Trebse, P., 2009. Hazard identification of imidacloprid to aquatic environment. Chemosphere 76, 907–914.
van der Sluijs, J.P., Simon-Delso, N., Goulson, D., Maxim, L., Bonmatin, J.M., Belzunces, L.P., 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. Curr. Opin. Environ. Sustain. 5, 293–305.
van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
Vehniäinen, E.-R., Kukkonen, J.V.K., 2015. Multixenobiotic resistance efflux activity in *Daphnia magna* and *Lumbriculus variegatus*. Chemosphere 124, 143–149.
Wang, X., Anadon, A., Wu, Q.H., Qiao, F., Ares, I., Martinez-Larranaga, M.R., Yuan, Z.H., Martinez, M.A., 2018. Mechanism of neonicotinoid toxicity: impact on oxidative stress and metabolism. Annu. Rev. Pharmacol. 58, 471–507.
Zhang, C., Tian, D., Yi, X.H., Zhang, T., Ruan, J.J., Wu, R.R., Chen, C., Huang, M.Z., Ying, G.G., 2019. Occurrence, distribution and seasonal variation of five neonicotinoid insecticides in surface water and sediment of the Pearl Rivers, South China. Chemosphere 217, 437–446.