Ecological risks of imidacloprid to aquatic species in the Netherlands: Measured and estimated concentrations compared to species sensitivity distributions

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**HIGHLIGHTS**
- Use of neonicotinoids could because of declines of insect’s biomass.
- Calculated risk implied that imidacloprid concentrations exceed acute toxicity levels.
- Imidacloprid concentration estimations by SimpleBox were within the range measured.
- SimpleBox validation allows application in regions where measurements are limited.

**ABSTRACT**
Recent declines of insects’ biomass have been a major point of interest. While several causes, including use of neonicotinoids like imidacloprid, have been suggested, scientific underpinning is limited. The aim of our study was to assess the potential risk of imidacloprid for freshwater fauna in the Netherlands and to validate the SimpleBox model to allow application elsewhere. To this end, we compared imidacloprid concentrations estimated from emissions using the SimpleBox model to measurements obtained from monitoring databases and calculated the ecological risk based on measured concentrations for aquatic fauna. Imidacloprid concentration estimations were within the range measured, opening opportunities for application of SimpleBox to regions where measurements are limited. Aquatic insects were found to be most sensitive to imidacloprid while amphibians and fish are least sensitive to imidacloprid. In particular, the ecological risk of measured imidacloprid concentration in the Netherlands was 1%, implying that concentrations frequently exceed levels that are lethal in short-term experiments. Hence, based on lab toxicity data, the present study suggests that imidacloprid concentrations can be high enough to explain insect decline observed in the same areas.

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1. Introduction
Recent declines of insects’ biomass have been a major point of interest in biology, because of adverse effects on ecosystem functioning (Conrad et al., 2006; Walpole et al., 2009; Ollerton et al., 2014; Hallmann et al., 2017). In particular, insects play a central role in processes such as pollination (McGregor, 1976; Ockinger and Smith, 2007; Ollerton et al., 2011), grazing and decomposition (Mattson and Addy, 1975; Lövei and Sunderland, 1996; Losey and Vaughan, 2006; Yang and Gratton, 2014) and act as food sources for higher trophic levels (Losey and Vaughan, 2006). Despite various efforts, insect decline could not directly be linked to specific factors (Colla and Packer, 2008; Shortall et al., 2009; Brooks et al., 2012; Dennis et al., 2017; Hallmann et al., 2017). However, potential causes include pesticides, especially neonicotinoids, which have been identified and suspected as key factors responsible for the global pollinator decline affecting biodiversity conservation, ecosystem services and agriculture (Chagnon et al., 2015; Leather, 2018; Nakanishi et al., 2018).

In particular, imidacloprid is one of the most widely used pesticides to protect crops from pest insects like aphids, leafhoppers, and whiteflies (Blacquiere et al., 2012). Imidacloprid is transported...
rapidly through plant tissues after application, protecting growing shoots (Sur and Stork, 2003). Distribution across plant tissues makes imidacloprid very effective for all kinds of herbivorous attacks (Goulson, 2013). Imidacloprid acts as a neurotoxicant, especially on insects, because it is chemically related to the nicotinic acetylcholine receptor (nAChR) agonists nicotine and epibatidine (Tomizawa and Casida, 2005; Jeschke and Nauen, 2008; Matsuda et al., 2020). The binding of imidacloprid to nAChRs elicits a continuous electric impulse that results in hyperactivity and over-stimulation of the neurons (Mehlhorn et al., 1999; Matsuda et al., 2020). This process eventually leads to the death of the neuron. When more chemical molecules bind to other nAChRs it leads to damage and eventually death of the organism (Matsuda et al., 2001; Tomizawa and Casida, 2005; Jeschke and Nauen, 2008 Tennekes and Sánchez-Bayo, 2013).

Imidacloprid has a high water solubility and relatively low half-life in soil (Bonmatin et al., 2015). Therefore, it has the potential to accumulate in soils and to leach to surface water and groundwater (Wettstein et al., 2016; Hladik et al., 2018). Within an agricultural context, imidacloprid poses a risk of water contamination due to spray drift, leaching and runoff (Gupta et al., 2002; Haith, 2010; Thuyet et al., 2012; Sánchez-Bayo and Hyne, 2014). In this way, non-target freshwater organisms are exposed to imidacloprid and adversely affected. The abundance of freshwater macroinvertebrates correlated negatively with increased measured concentrations of imidacloprid (Van Dijk et al., 2013). For instance, Yamamuro et al. (2019) found that neonicotinoid application to catchments since 1993 coincided with an 83% decrease in average zooplankton biomass in spring, causing the smelt harvest to collapse from 240 to 22 tons in Lake Shinji, Shimane Prefecture, Japan.

Worldwide, imidacloprid concentrations ranged from 0.001 to 320 μg.L⁻¹ and the highest concentration was found in the Netherlands (Van Dijk et al., 2013; Morrissey et al., 2015). However, systematic inventories on other regions is scarce. Although, there are many reports of survey and water contamination by neonicotinoids (Muenze et al., 2015; Hladik et al., 2018; O’Brien et al., 2016). Recently, several mesocosm studies have addressed the toxicity of imidacloprid to the aquatic environment (Roessink et al., 2016). Landscape properties are, e.g., the surface area of compartments and several physical properties (wind speed, temperature, etc.). Physicochemical properties are molecular weight, solubility, diffusion rates, vapour pressure, partition ratios and degradation rates. SimpleBox is a generic model, in which the default setting of the regional and continental scales of the model matches the European Union procedures for the evaluation of the substances (Brandes and Den Hollander, 1996). SimpleBox is operated as Microsoft Excel spreadsheet.

### 2. Methods

#### 2.1. Fate

##### 2.1.1. SimpleBox: the model

SimpleBox is a multimedia environmental fate model used to calculate steady state concentrations based on data from emissions. SimpleBox has three spatial scales (regional, continental and global) and three climate zones, reflecting arctic, temperate and tropical climatic zones of the Northern hemisphere (Supporting information, Figs. S1 and S3; Brandes and Den Hollander, 1996). For the present study, SimpleBox 4.0 was used (Schoorl et al., 2016). The Netherlands was represented at the regional scale, including compartments for air, water, sediment and soil (Supporting Information, Figs. S2 and S3; Schoorl et al., 2016). The distribution of a chemical over these boxes is calculated using data on emissions (tons/year), landscape properties, and physico-chemical properties of the substance (Brandes and Den Hollander, 1996; Schoorl et al., 2016). Landscape properties are, e.g., the surface area of compartments, classification of the compartments and several physical properties (wind speed, temperature, etc.). Physicochemical properties are molecular weight, solubility, diffusion rates, vapour pressure, partition ratios and degradation rates. SimpleBox is a multimedia environmental fate model used to calculate steady state concentrations based on data from emissions. SimpleBox has three spatial scales (regional, continental and global) and three climate zones, reflecting arctic, temperate and tropical climatic zones of the Northern hemisphere (Supporting information, Figs. S1 and S3; Brandes and Den Hollander, 1996). For the present study, SimpleBox 4.0 was used (Schoorl et al., 2016). The Netherlands was represented at the regional scale, including compartments for air, water, sediment and soil (Supporting Information, Figs. S2 and S3; Schoorl et al., 2016). The distribution of a chemical over these boxes is calculated using data on emissions (tons/year), landscape properties, and physico-chemical properties of the substance (Brandes and Den Hollander, 1996; Schoorl et al., 2016). Landscape properties are, e.g., the surface area of compartments, classification of the compartments and several physical properties (wind speed, temperature, etc.). Physicochemical properties are molecular weight, solubility, diffusion rates, vapour pressure, partition ratios and degradation rates. SimpleBox is a generic model, in which the default setting of the regional and continental scales of the model matches the European Union procedures for the evaluation of the substances (Brandes and Den Hollander, 1996). SimpleBox is operated as Microsoft Excel spreadsheet.

#### 2.1.2. Data collection

Data on the physico-chemical properties of imidacloprid were obtained from two different databases (Table 1) (EC, 2006; Rosenbaum et al., 2008). Emissions of imidacloprid to air, soil and surface water in the Netherlands were obtained from the

<table>
<thead>
<tr>
<th>Chemical Properties</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>256</td>
<td>g.mol⁻¹</td>
</tr>
<tr>
<td>Melting temperature</td>
<td>144</td>
<td>°C</td>
</tr>
<tr>
<td>Vapour pressure (25 °C)</td>
<td>2.24 × 10⁻³</td>
<td>Pa</td>
</tr>
<tr>
<td>Solubility (25 °C)</td>
<td>6.10 × 10⁻¹</td>
<td>mg.L⁻¹</td>
</tr>
<tr>
<td>Partition coefficient (log Kow)</td>
<td>0.57</td>
<td>–</td>
</tr>
<tr>
<td>Degradation rate (air)</td>
<td>1.14 × 10⁻⁸</td>
<td>s⁻¹</td>
</tr>
<tr>
<td>Degradation rate (water)</td>
<td>1.34 × 10⁻⁵</td>
<td>s⁻¹</td>
</tr>
<tr>
<td>Degradation rate (sediment)</td>
<td>1.49 × 10⁻⁸</td>
<td>s⁻¹</td>
</tr>
<tr>
<td>Degradation rate (soil)</td>
<td>4.61 × 10⁻⁸</td>
<td>s⁻¹</td>
</tr>
</tbody>
</table>

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2.1.3. Data treatment

We used an emission scenario on a regional scale in SimpleBox with no changes in the settings of the model (“all default”). Only emissions via surface water were included. For each of the 21 water boards in the Netherlands, we obtained the emissions of 2005 and 2016 from the Emission Registration database (Rijksoverheid Emissieregistratie, 2018) as well as their surface area (Waterschappen, 2019). The mean precipitation and mean air temperature were obtained from KNMI (2018) (Table 2). The degradation rate was taken from UseTox (Rosenbaum et al., 2008).

The estimated concentrations in freshwater were compared with imidacloprid measurements between 2005 and 2016 (Informatiehuis Water, 2018). The dataset of the measured concentrations of imidacloprid in surface water (lakes, rivers, streams and channels) consisted of 31,661 data points, of which 21,864 below detection limit (years 2000–2016). Monitoring was carried out by water boards according to a protocol from Rijkswaterstaat (Faber et al., 2011). Measurements below detection limit (< d.l.) were included as actual values (d.l.), as half the detection limit (d.l./2) or excluded (without d.l.) completely (MacDougall and Crummett, 1980).

2.2. Effect

2.2.1. Data collection

LC50 toxicity data on aquatic species were obtained from the United States Environmental Protection Agency (US EPA) ECOTOX database (EPA US, 2018). To supplement the set, data was obtained from the RIVM e-toxbase (RIVM, 2018) and literature as well (Smit et al., 2015). In total, we retrieved 142 LC50 values for amphibians, fish, nematodes, crustaceans and insects (Supporting Information, Table S1).

2.2.2. Data treatment

Of all LC50 data collected, only those with short-term test durations (< 4 days) were included.

Geometric mean values were determined if multiple toxicity values were available for a species (Smit et al., 2015). SSDs were constructed to assess the differences in sensitivity to imidacloprid. An SSD is a statistical distribution describing the variation between species’ sensitivity to an environmental stressor (Posthuma et al., 2002; Del Signore et al., 2016). The mean and the standard deviation of an SSD represent the average and variation in sensitivity of species to the chemical, respectively. The standard deviation of the SSD is inversely proportional to the effect of a change in the environmental factor on the fraction of species affected, known as the potentially affected fraction (PAF) (Posthuma et al., 2002). At least five species were considered necessary to derive an SSD (Del Signore et al., 2016).

A lognormal distribution was fitted to the original data (Del Signore et al., 2016) using the Fitdistplus package in R (Delignette-Muller and Dutang, 2015; R Core Team, 2018). The 2.5% and 97.5% confidence intervals were derived using a bootstrapping function with 1000 iterations. Separate SSDs were constructed for different taxonomic groups, which formed subsets: 1) insects, 2) crustaceans, and 3) amphibians and fish. The Shapiro-Wilk Normality Test was used to test if the data were log-normally distributed (R Core Team, 2018). Subsequently, SSDs were constructed for each subset using the aforementioned method to assess differences in sensitivity between the groups. To determine if the subsets differed significantly, the ks.test, the Kolmogorov-Smirnov test of the equality of two sample cdfs, function was used in R (R Core Team, 2018). Finally, the hazardous concentration for 5% (HC5) of the species was calculated (de Hoop et al., 2011).

Because of insufficient data we could not construct SSDs based on chronic and sub-lethal toxicity levels. Yet, typical extrapolations factors for acute-chronic (3.2) and lethal-sublethal (2.5) for aquatic arthropods, mainly crustaceans (Hendriks et al., 2005), suggests that the median value of the SSDs (HC50) for chronic sublethal effects is about a factor of 3.2–2.5 – 8 smaller than for acute lethal across chemicals. An additional factor of 2–3 may be needed to arrive at no-effect levels (Hendriks et al., 2005). Imidacloprid in particular, induced sublethal and no effect at 1/60 and 1/230 of acute levels in 7 aquatic arthropods (Roessink et al., 2013). In view of the uncertainties, we will therefore calculate risks at 1, 1/10 and 1/100 of the LC50.

2.3. Ecological risk

2.3.1. Data collection

The SSD derived for acute LC50s of aquatic organisms and the concentrations measured in surface water (Informatiehuis Water, 2018) were used to calculate the ecological risk. Concentration measurements of 2005, 2010, 2015 and 2016 were used to cover the period of interest.

2.3.2. Data treatment

The ecological risk ER is the probability that species are exposed to an environmental concentration of imidacloprid exceeding their effect concentrations (LC50) (van Straalen, 2001; Fedorenkova et al., 2012). This probability corresponds to the area under the curve (AUC) of the probability density function (PDF) of the measured concentrations and the cumulative density function (CDF) according to the method described by Fedorenkova et al. (2012). This probability can be expressed as

$$ Pr(x_1 > x_2) = \int_{-\infty}^{\infty} (1 - CDF_{x_1}(x)) \times PDF_{x_2}(x) \, dx $$

where $x_1$ is a random variable of the logarithm of field concentrations, and $x_2$ is a random variable of the logarithm of species sensitivity concentrations (Fedorenkova et al., 2012).

The measured water concentrations of imidacloprid were log10-transformed. The sample mean ($\mu$) and standard deviation ($\sigma$) for the nation-wide concentrations were calculated based on the log10-transformed data, and were used to derive a normal distribution. The environmental risk was calculated by multiplying the normal

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean participation (mm yr$^{-1}$)</th>
<th>Mean air temperature ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>785</td>
<td>10.7</td>
</tr>
<tr>
<td>2010</td>
<td>803</td>
<td>9.1</td>
</tr>
<tr>
<td>2015</td>
<td>831</td>
<td>10.9</td>
</tr>
<tr>
<td>2016</td>
<td>757</td>
<td>10.7</td>
</tr>
</tbody>
</table>
distributed data from the SSD values by the environmental concentrations.

3. Results

3.1. Fate

National emissions of imidacloprid were available for air, water and soil for the years 2005, 2010, 2015 and 2016 with comparable emissions into air and water, and low emission into soil (Fig. 1). Concentrations were estimated with SimpleBox for 2005 and 2016 according to emissions obtained from different water boards. For both years, concentrations estimated with SimpleBox were largely within a factor of 2 of the measurements, which mostly resulted in an underestimation compared to the measured concentrations. Accounting for detection limits in different ways did not affect the outcome, except when using < d.l. as d.l. in 2005. (Fig. 2).

3.2. Effect

The LC50 for different species ranged from 1.42 to 1.93 $10^5$ mg L$^{-1}$ (Fig. 3). SSDs were log-normally distributed (Shapiro-Wilk Normality Test; Supporting Information, Table S5). The mean and standard deviations of the SSDs differed significantly between taxonomic groups (Supporting Information, Table S6). Therefore, a distinction between the taxonomic groups was made (Fig. 3). Insects were found to be most sensitive to imidacloprid while amphibians and fish were least sensitive to imidacloprid (Fig. 3). This sensitivity to imidacloprid was also reflected in the calculated HC5 (Fig. 3).

3.3. Ecological risk

At the LC50 level, the calculated ER for the measured imidacloprid concentrations was <1% for amphibians and fish and 1% for insects between 2005 and 2016 (Table 3). For crustaceans and insects, a decline in ER over the years was calculated. The ER was the highest for insects (Fig. 4). The calculated ER for the estimated imidacloprid concentrations was <1% for all taxonomic groups. At the LC50/100 level, the ER for insects decreased from 19% in 2005 to 8% in 2016 based on measured imidacloprid concentrations (Table 4).

4. Discussion

4.1. Fate

The concentrations estimated with SimpleBox were largely within a factor of two of the environmental concentrations of imidacloprid observed. Predicted concentrations for organic substances in the study of Zijp et al. (2014) were within one order of magnitude. Meanwhile, predicted concentrations of polycyclic aromatic hydrocarbons and hexachlorobenzene in the study of Armitage et al. (2007) differed a factor three of measured concentrations. Our finding of a factor two is in between both outcomes. Emission data were taken from the database as such. Obviously,
uncertainties in these data may also induce deviations between modelled and estimated concentrations. For instance, it has been suggested that imidacloprid, rather than to air, is largely emitted to water and soil, running off or leaching afterwards (Goulson, 2013; Sánchez-Bayo and Goka, 2006; Sánchez-Bayo and Hyne, 2014). Consequently, we used the weighted average of half-lives in the different water types. Furthermore, imidacloprid is recalcitrant to hydrolysis, which causes half-lives in environmental waters longer than a month at neutral pH (Moya et al., 1998; Sánchez-Bayo and Hyne, 2014). Hence, also pH should be taken into account when predicting with SimpleBox.

The close relationship between expected and measured concentrations shows that SIMPLEBOX allows for useful first estimates in areas for which monitoring data are lacking. In such cases, emissions via effluents of wastewater treatment plants (ECHA, 2012) or estimations based on pesticide use in agriculture may serve as input.

### 4.2. Effects

The different taxonomic groups of the present study indicated differences in sensitivity to imidacloprid. Our analysis showed the same findings as Morrissey et al. (2015), namely that the LC50s of imidacloprid for insects was significantly lower than the LC50s for crustaceans. Amphibians and fish were least sensitive to imidacloprid, as expected since neonicotinoids are developed to specifically affect insects (Matsuda et al., 2001). All daphnids were relatively tolerant except Ceriodaphnia dubia. In general, water fleas may therefore not be representative for other invertebrate taxa for imidacloprid or other neonicotinoids (Beketov and Liess, 2008; Daam et al., 2013; Hayasaka et al., 2013; Sánchez-Bayo et al., 2016). C. dubia is recognized as a popular choice of test organisms for ecotoxicology studies (Blaise and Férard, 2005; PAKRASHI et al., 2013).

Usually, one aims to protect as many species as possible to maintain the integrity of the aquatic ecosystem and its services (Sánchez-Bayo et al., 2016). Although our SSDs cover sensitive species groups (Smit, 2014), the dataset is limited and does not meet the requirements of at least 10 data points from different species covering at least eight specified taxonomic groups for a generic SSD suggested by Posthuma et al. (2002).

As chronic data were scarce, acute lab toxicity (LC50s) was used for the construction of the SSDs. Vijver and van den Brink (2014) also mentioned the lack of chronic toxicity data of imidacloprid in their study. On average, population increase rates and densities in lab experiments decreased towards zero with exposure levels increasing towards the LC50 (Hendriks et al., 2005). In addition, field-based environmental ranges of species occurrences may provide a more realistic representation of sensitivity in naturally occurring environmental conditions compared with tolerances obtained in laboratory tests (Leung et al., 2005; Kwok et al., 2008; Strujs et al., 2011). Leung et al. (2005) demonstrated that field data-derived criteria are either equally (PAHs) or stricter (Cd) than current lab-quality standards. The tolerance of highly sensitive species found in the field was different from that of common laboratory test species (Leung et al., 2005). The field tolerance of species to neonicotinoids has been observed to be larger than those expected by laboratory tests (Sánchez-Bayo and Goka, 2006; Stoughton et al., 2008; Pestana et al., 2009). The reason for that discrepancy is that chronic toxicity of neonicotinoids prevails over the acute toxicity of these compounds (Stoughton et al., 2008; Pestana et al., 2009; Roessink et al., 2013). Despite these uncertainties, SSDs are some of the most universal and effective approaches for deriving water
thresholds to protect biodiversity (Posthuma et al., 2002; Del Signore et al., 2016). As insufficient chronic toxicity data were available in literature, we based ER primarily on acute toxicity data. Imidacloprid is known to have a much larger impact under chronic exposure to low concentration than under high pulse exposures (Tennekes and Sánchez-Bayo, 2013; Roessink et al., 2013).

4.3. Ecological risk

Over the period of 2005–2016, ecological risk of measured imidacloprid concentration decreased from 1% to <1% at the LC50 level from 19% to 8% at the LC50/100 level insects in the Netherlands. These outcomes were based on geometrically averaged concentrations monitored throughout the year. However, concentrations of imidacloprid are variable throughout the year. Therefore, some species-based studies used concentrations that are much higher than in the Netherlands. Chronic levels will be substantially higher. Hence, we recommend to obtain more chronic data to properly evaluate risks of neonicotinoids. Additionally, these estimations should be underpinned by empirical studies embedded in modelling context, kinetically covering fluctuations in concentrations and population densities. Nevertheless, the present study demonstrates with lab toxicity data that imidacloprid concentrations can be high enough to at least partly explain insect decline observed in the same areas.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

N.W. Thunnissen: Data curation, Writing - original draft, Visualization. L.S. Lautz: Data curation, Writing - review & editing. T.W.G. van Schaijk: Data curation. A.J. Hendriks: Conceptualization, Writing - review & editing, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at 10.1016/j.chemosphere.2020.126604

References


