

# Effects of neonicotinoid pesticide pollution of Dutch surface water on non-target species abundance



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## Introduction

Between 1994 and 1996, beekeepers in France noticed greatly increased mortality in honeybees that foraged sunflowers, and discovered that a new pesticide had been introduced as a sunflower seed treatment in 1994. Seed dressing makes spraying crops with pesticides unnecessary because the active substances are spread to all plant tissues when the plant grows. However, the beekeepers suspected this new pesticide, Gaucho<sup>®</sup>, the active substance of which is imidacloprid, was toxic for honeybees (Bonmatin et al., 2005; Maxim & Van der Sluijs, 2007, 2010), and several studies have provided supporting evidence for this link (e.g. CST, 2003; Yang et al., 2008; Maini, 2010).

Pesticides have to be authorised prior to being brought onto the market, and this authorisation includes testing to guarantee that the chemicals are not harmful to pollinators, especially honeybees. But the effects and doses used for spraying and seed dressing differ greatly, and the tests do not represent this: most sprayed pesticides work for a few hours to days only, while pesticides applied via seed dressing remain in all parts of the plant throughout its lifetime (Rortais et al., 2005). The result of seed treatment with imidacloprid should be the protection of crops from harmful insects, with imidacloprid disappearing from the plants before pollinator activity starts, but instead, relatively high levels have been observed in flowering heads and pollen, and additionally, residues remain in the soil and surface water (Bonmatin et al., 2005).

The effects of neonicotinoid pesticides on several other non-target species have also been examined.

Imidacloprid and two other neonicotinoid pesticides, thiamethoxam and thiacloprid, in doses that may be considered safe, can negatively influence foraging behaviour in the bumblebee *Bombus terrestris* (Mommaerts et al., 2010). Imidacloprid was found to be “generally highly toxic” to the one bumblebee species and two wild bee species tested by Scott-Dupree et al. (2009). The three species of stingless bees tested by Valdovinos-Núñez et al. (2009) were also highly vulnerable to neonicotinoid pesticides. Spraying with imidacloprid, thiacloprid and methomyl at the recommended doses caused mortality of up to 100% in larvae and adults of three predacious coccinellid species (Katsarou et al., 2009). Using imidacloprid against wood-boring insects in trees by applying it to the soil can also harm litter-dwelling earthworms if the concentration in litter and upper soil is about 3 mg/kg or higher, which is well within expected concentrations (Kreutzweiser et al., 2008).

All these species are of great importance for nature and for humanity: bumblebees, wild bees and stingless bees are pollinators of wildflowers and economic crops, coccinellids help avert aphid pests, and earthworms are decomposers.

As may be clear from the above, many studies examining the effects of neonicotinoid pesticides on non-target species have been carried out since their application began in 1994. What has not yet been performed, however, is an assessment of the distribution and abundance of these species in the Netherlands, and a comparison with the doses of pesticides applied, as well as the residues in the surface waters. Such an assessment would give more information on the possible relationship between neonicotinoid pesticide application and species mortality.

## **Problem definition**

The distribution and abundance of the non-target species that may be affected by neonicotinoid pesticide use in the Netherlands had not yet been assessed, or compared to the doses of pesticides applied and the residues in the surface waters. Such an assessment would provide more information on the possible relationship between neonicotinoid pesticide application and species mortality, and thus, would give an indication as to whether the application should be allowed to continue, considering the importance of the species concerned.

To emphasise the importance of just the pollinating species included here: more than 20,000 different bee species pollinate 80% of the world's plant species. Without them, 80% of plants would become extinct (Vaissière et al., 2005).

'Neonicotinoid pesticides' comprises a group of several different pesticides, but the term is used here to indicate the four that are most widely used: imidacloprid, thiacloprid, clothianidin and thiamethoxam.

## **Objective**

The aim of this study was to find out whether the use of neonicotinoid pesticides and their residues in surface waters, affects the distribution of, and number of individuals per, non-target species in the Netherlands. And if significant differences in the distribution of, and number of individuals per species, were found, the aim was also to assess whether they are similar for all species studied, and whether this gives an indication as to the safety of neonicotinoid pesticides for non-target species in general.

## **Research question**

The research question focused on in this project, is:

Is there a correlation between the use of neonicotinoid pesticides, and their residues in soil and surface water, and the distribution of and number of individuals per non-target species, including bumblebees, earthworms and ladybugs, in the Netherlands?

To be able to answer this question, first, the following sub-questions had to be answered:

1. Where in the Netherlands are neonicotinoid pesticides applied, and in what amounts?
2. Where in the Netherlands can neonicotinoid pesticide residues be found in the soil or surface water, in significant amounts?
3. What are the effects of neonicotinoid pesticides and their residues on non-target species?
4. For which non-target species have the effects of neonicotinoid pesticides and their residues been reported in scientific literature?
5. Which non-target species appear to be most vulnerable to neonicotinoid pesticides, and which appear to be less vulnerable?
6. Has the distribution and number of different non-target species changed significantly since the application of neonicotinoid pesticides began?

## **Hypothesis**

There is a correlation between the use of neonicotinoid pesticides, and their residues in soil and surface water, and the distribution of and number of individuals per non-target species, in the Netherlands: where neonicotinoid pesticides are used, and/or there are significant amounts of residues in the soil and surface water, several non-target species are less abundant.

## Effects on susceptible species

In the mid-1990s, neonicotinoids were pesticides belonging to a new class of compounds, which had a new mode of action. After market introduction in the mid nineteen-nineties, neonics use has rapidly grown to become the most widely used class of insecticides world-wide with a 1.5 billion euro global market share (25% of the world insecticide market). The major application (765 MEuro/yr) is seed treatment. Large-scale world-wide use started around 2004. Neonics are nowadays registered in more than 120 countries. (Jeschke and Nauen, 2008; Jeschke et al., in press). Neonicotinoids seemed promising as pesticides which can be applied topically and in relatively small amounts, have high selectivity against non-target organisms (Iwaya & Kagabu, 1998) and may not threaten human health: because they work by intervening stimuli transmission in neuronal pathways that are specifically abundant in insects, they are much more poisonous to insects than to warm-blooded animals (Bonmatin et al., 2005).

Chloronicotinyl pesticides derive their toxicity by acting agonistically on, and blocking of, the post-synaptic nicotinic acetylcholine receptors (nAChRs), which means that normal nerve impulses become impaired. There may in fact be two different binding sites for imidacloprid in insects: one at the agonist binding site, which generates an electrical impulse, and one at a blocking site, which prevents ions from permeating. This also means the pesticides prevent species from feeding on plants by paralyzing sucking action, which causes starvation in the insects affected, although this is reversible at lower concentrations, contrary to the neuronal disorder symptoms occurring at higher concentrations (Iwaya & Kagabu, 1998).

In a study on rats and planthoppers, selectivity ratios of imidacloprid between the mammals and the insects were established as 10,000 to 33,000 times, which is exceptionally high. The reason for this is that the binding sites which are present in insects' nAChRs are absent in mammals (Iwaya & Kagabu, 1998).

Neonicotinoids are thus most toxic to insects, and this also goes for aquatic insects; more so than to other aquatic invertebrates (Overmyer et al., 2005), crustaceans and fish (Tišler et al., 2009). The toxicity to insects can be illustrated using the honeybee as an indicator species: the LD50 for honeybees of imidacloprid is 3.7 nanogram imidacloprid per honeybee. Table 1 shows the toxicity for honeybees of the systemic insecticides thiametoxam, fipronil, clotianidine and imidacloprid compared to classic insecticides.

In 2009 a scientific report in commission of Buglife<sup>1</sup> (UK) was published "The impact of neonicotinoid insecticides on bumblebees, Honey bees and other nontarget invertebrates" (Kindemba, 2009). It concluded that significant negative impacts of imidacloprid on bees and other non-target invertebrate occur at levels predicted to be present in the UK countryside and recommended precautionary suspension of all existing approvals for products containing neonicotinoids and fipronil for outdoor use. Italy has actually banned all seed treatment applications of neonicotinoids.<sup>2</sup>

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<sup>1</sup> A UK based nature conservation NGO devoted to the conservation of all invertebrates.

<sup>2</sup> <http://www.normativasanitaria.it/jsp/dettaglio.jsp?id=30174>

Pesticide	®	utilisation	LD50 (ng/honeybee)	Toxicity index relative to DDT
DDT	Dinocide	insecticide	27000	1
Amitraz	Apivar	insecticide / acaricide	12000	2
Coumaphos	Perizin	insecticide / acaricide	3000	9
Tau-fluvalinate	Apistan	insecticide / acaricide	2000	13.5
Methiocarb	Mesurool	insecticide	230	117
Carbofuran	Curater	insecticide	160	169
λ-cyhalothrin	Karate	insecticide	38	711
Deltamethrine	Decis	insecticide	10	2700
Thiamethoxam	Cruise	insecticide	5	5400
Fipronil	Regent	Insecticide	4.2	6475
Clothianidine	Poncho	Insecticide	4.0	6750
Imidacloprid	Gaucho	Insecticide	3.7	7297

Table 1. Toxicity of insecticides to honeybees compared to DDT. Median lethal dose (LD<sub>50</sub>) for honeybees are given in nanogram per honeybee. The final column expresses the toxicity relative to DDT. (Source: Bonmatin, 2009)

As was described above, starvation is a sub-lethal effect of neonicotinoids at lower concentrations. Other sub-lethal effects include regression in mating and oviposition (Iwaya & Kagabu, 1998), and greater susceptibility to parasites and infections such as *Nosema Ceranae* (Alaux et al., 2010). At concentrations that may be considered safe, sub-lethal effects in bumblebees have been reported to include negatively affected foraging behaviour, resulting in decreased pollination, lower reproduction and eventually mortality of the entire colony on account of a lack of food (Mommaerts et al., 2010). A sublethal dose as low as 0.1 nanogram imidacloprid per honeybee is enough to disturb the navigation of honeybees, which leads to weakening impacts on the colony, such as loss of foragers and thus less food supplied to the colony per unit of time (CST, 2003). This implies that concentrations of several parts per billion in the diet of social insects can already harm them on colony level. Desneux et al. (2007) have emphasised the importance of the investigation of sublethal effects on beneficial arthropods, in particular, as part of the analysis of pesticide impact. The sublethal effects mentioned by them include the impairment of olfactory memory, affected mobility, reduced learning, and a decrease in dancing, which, as it is a signal, leads to reduced foraging activity (Desneux et al., 2007).

## Behaviour of neonicotinoids in the environment

Over the last two decades, the worldwide production and utilisation of pesticides have greatly increased. An important thing to realise is that only a small part of the pesticide doses used reaches its intended target, while the major part of it ends up in the environment outside the field, where it can cause difficulties through its toxicity to non-target species, and accumulation can occur (Tišler 2009), especially if a pesticide is persistent.

Neonicotinoids are thought by some to be safer than other pesticides, since they are not particularly toxic to mammals yet are strong pesticides, which allows for their application at relatively low rates. But because neonicotinoids are used more and more, both in agriculture and for home use, the chance of their polluting water is still present despite the low application rates. This pollution can occur by means of accidental spilling, spray drift, and runoff, especially if the site of application is irrigated or natural rain occurs within two days of application (Overmyer et al., 2005).

### *Behaviour in water*

The solubility of imidacloprid in water is relatively high: 0.51 g/l, and its octanol-water partitioning coefficient is quite low:  $\text{Log } K_{ow} = 0.57$  (Gupta et al., 2002). Imidacloprid is generally persistent in water, and not easily biodegradable (Tišler et al., 2009). Indeed, Overmyer et al. (2005) found no significant differences in imidacloprid concentration in water over a 48-hour experiment, and Roberts & Hutson (1999) reported that it is likely to remain in the water column in aquatic systems, and has an aerobic sediment and water  $\text{DT}_{50}$  of 30 to 162 days (time for 50% decline of the initial pesticide concentration, or half-life time).

The influence of pH and formulation on the persistence of imidacloprid in water have also been studied, and it was found that a higher pH, meaning alkaline conditions, increases half-life time and thus persistence. Consistently, the lowest half-life time was found for the lowest pH value studied. The formulation of the pesticide also had a significant effect on persistence: the powder formulation Gaucho 70 WS was more persistent in water than the liquid formulation Confidor 200 SL (Sarkar et al., 1999). At pH values corresponding to environmental conditions, imidacloprid is stable to hydrolysis, but it can be rapidly degraded photolytically (Tišler et al., 2009).

### *Behaviour in soil*

Soil is a sink for the greater part of the pesticides applied in agriculture. Residues can be transported through the soil, and leaching is the main transportation process which leads to pollution of the ground water with pesticides. This is a global problem since ground water is used as drinking water and for irrigation in many countries. Pesticide leaching studies are important for determining a pesticide's capacity to pollute ground water, especially if the pesticide in question is highly soluble in water (Gupta et al., 2002), like imidacloprid. Data on the persistence of imidacloprid in soil are rather inconsistent, however. Some authors have reported that it is relatively immobile in soil and that leaching below the topmost layer and into the groundwater is not likely to occur, while other authors have claimed the exact opposite (Tišler et al., 2009).



Degradation of imidacloprid in soil is decreased if organic amendment, organic material used to improve soil quality, is added (Rouchaud et al., 1996). The lack of leaching that some authors have described, and this decrease in degradation upon addition of organic amendment, may well be due to the sorption-desorption characteristics of the chemicals. Sorption of imidacloprid, as well as its metabolites, increases when soil organic carbon content increases (Cox et al., 1997).

Peterson (2007) has reported that after an initial rapid decline in imidacloprid in the test plot soil, after two months a lagging phase occurs, with about 10% of initial imidacloprid still remaining in the soil after six months. The concentration was not influenced by the presence of vegetation, although mobility into lower soil layers was lower if vegetation was present, which was probably because of the effect of vegetation on soil moisture (Peterson, 2007).

### *Contamination of wild plants*

Imidacloprid can also be released into the environment when dressed seeds are drilled into the soil by pneumatic seed drills. 120 to 240 µg of imidacloprid was found per 1 g of filter paper used in a 240 second seed drilling test. Imidacloprid was also found on 'spontaneous vegetation', consisting of flowers and grass, which grew near the fields where the sowing test was carried out. This was the case on the day of sowing, but residues also remained present on the spontaneous vegetation until at least four days after sowing. The residue level on the flowers was higher than that on the grass (Greatti et al., 2006).

If no measures are taken to prevent it, seed drills can contaminate areas adjacent to arable land with imidacloprid, and they can remain polluted for a time period depending on the duration of the sowing time. In this way, pollinators can be exposed to the pesticide even if the crops they were applied on are not flowering plants. Testing also showed that despite careful cleaning of the pneumatic drills, it was not possible to remove all imidacloprid from them. This means that more areas could be polluted even if the seeds being drilled were not dressed. Additionally, pneumatic drills cause a lot of dust, which, when bound to pesticide molecules, could cause wind dispersion of the pesticides, leading to even less control of the pollution (Greatti et al., 2006).

## Metabolites

Imidacloprid chemistry is founded on nitromethylene derivatives, the activity or stability of which are improved by three processes, each of which increases activity approximately ten-fold: changing the heterocyclic ring to an imidazolidine ring; modifying the nitromethylene moiety to nitroimino; and introducing a pyridyl moiety which was part of the structure of nicotine (Iwaya & Kagabu, 1998).

The degradation processes of imidacloprid are related to the processes described above. The main metabolites of imidacloprid result from hydroxylation of the imidazolidine ring, in the case of 1-[(6-chloro-3-pyridinyl)methyl]-5-hydroxy-4,5-dihydro-N-nitro-1H-imidazol-2-amine, or dehydrogenation of the imidazolidine ring, in the case of 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-1H-imidazol-2-amine, or correspond to 6-chloronicotinic acid. 6-chloronicotinic acid has been found to be more toxic to honey bees than imidacloprid itself. In  $^{14}\text{C}$ -tests, only 0.1% of all recovered  $^{14}\text{C}$  was found as the nitroso derivative of imidacloprid, 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitroso-1H-imidazol-2-amine (Rouchaud et al., 1996).

Most of the degradation processes of imidacloprid are common, at least quantitatively, in plants, animals, soil and water (Iwaya & Kagabu, 1998).

### *Metabolites in plants*

In plants, the most important metabolic processes are: hydroxylation of the imidazolidine ring followed by removal or conjugation of water to form the olefin metabolite, which is also more toxic to honey bees than imidacloprid; reduction of the nitro group, resulting in the nitrosoimine compound, and loss of the nitro group, resulting in the guanidine metabolites; oxidative cleavage of the methylene bridge, resulting in 6-chloronicotinic acid and compounds related to it; and opening of the imidazolidine ring through elimination of the ethylene bridge, resulting in ring-opened guanidine metabolites which also degrade further into 6-chloronicotinic acid and related compounds (Iwaya & Kagabu, 1998).

### *Metabolites in soil*

The main metabolites of imidacloprid which have been identified in soil, include 1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidinone (imidacloprid-urea), 6-chloronicotinic acid, and 6-hydroxynicotinic acid (Rouchaud et al., 1996), which ultimately degrades to  $\text{CO}_2$  (Scholz & Spiteller, 1992). Metabolites which have been observed in plant cells, do not tend to accumulate in soil;  $^{14}\text{C}$  testing has shown that their total amount corresponded to less than 4% of total radioactivity recovered after soil biodegradation of imidacloprid (Rouchaud et al., 1996).

In paddy soil, when conditions were anaerobic, imidacloprid was found to be readily decomposed, resulting in guanidine as the main metabolite. Also, mineralisation to  $\text{CO}_2$  generally occurred – in a  $^{14}\text{C}$ -test using light sandy soil, approximately 40% of the total radioactivity of the parent compound was given off as  $\text{CO}_2$ , and 54% of it was fixed in the soil within 20 cm of the soil surface, while the lower layers contained hardly any. This seems logical, because the compounds resulting from degradation tend to be polar, and can be strongly absorbed by soil components (Iwaya & Kagabu, 1998).  $\text{CO}_2$  also formed the main product of soil biodegradation of imidacloprid (Rouchaud et al., 1996).

Guanidine metabolites have shown higher sorption in soil than the imidacloprid parent compound, while the urea metabolite showed lower sorption. This indicates that the functional groups of the imidazol ring of the molecule contribute to binding mechanisms. Desorption was found to be hysteretic in all cases, while it is possible that lower desorption in the more sorptive system means that hysteresis is caused by irreversible binding of the molecules to soil surfaces (Cox et al., 1997).

### **Neonicotinoid application in the Netherlands**

The total area used for growing arable crops in the Netherlands was 555,000 ha in 2009. Potatoes make out the largest part of the total production value, and 993,750 tons were exported in 2009. The arable land is used as follows:

Edible, starch and seed potatoes: 156,000 ha

Winter corn: 129,000 ha

Sugar beets: 73,000 ha

Summer barley: 40,000 ha

Onions for sowing: 20,000 ha

Other vegetables: 32,000 ha

The total area of greenhouses in the Netherlands is 9,640 ha, of which 4,830 ha are used for growing vegetables, 2,870 ha for cut flowers, and 1,940 ha for potted plants. The total amount of fresh greenhouse vegetables exported by the Netherlands in 2009 was 1,245 million kilograms, and the market share of the Netherlands in the European Union's flowers and potted plants export was 70%. Also, 23,560 ha are used for growing bulbs, and the market share in the EU bulbs export was even 93% (LTO Nederland, 2010).

The largest amounts of imidacloprid per hectare are allowed for the following horti- and agricultural products (CTGB, 2010):

- Flower bulbs and flowers grown from bulbs (plunging of bulb)  
Dosage: 210 g/ha
- Gladiola (treatment of plant or plunging of bulb)  
Dosage: 210 g/ha
- Lilies (flower bulbs and flowers grown from bulbs, open air)  
Dosage: 210 g/ha
- Floriculture crops, covered (not in the open air)  
Dosage: > 200 g/ha per season
- Chicory in the open air  
Dosage: 175 g/ha
- Seed potatoes  
Dosage: 175 g/ha

Figure 1, on the next page, shows how the different types of horti- and agriculture are distributed in the Netherlands. Note that the legends are not exactly the same for all maps.

Figure 2 shows locations where the MTR norm for maximum allowable risk level, 13 ng/l, was exceeded in the years 2003-2008 (a more recent map is not available).

The MTR norm is an ecotoxicological standard for general environmental quality and the minimum quality level that is desirable for all surface waters in the Netherlands. The MTR-value for a substance is the environmental concentration of that substance, at which the species in an ecosystem are safe from effects caused by the substance. The MTR should have been realised in 2000 (Bestrijdingsmiddelenatlas, 2010), but instead, as becomes clear from Figure 2, concentrations in the surface water have increased.

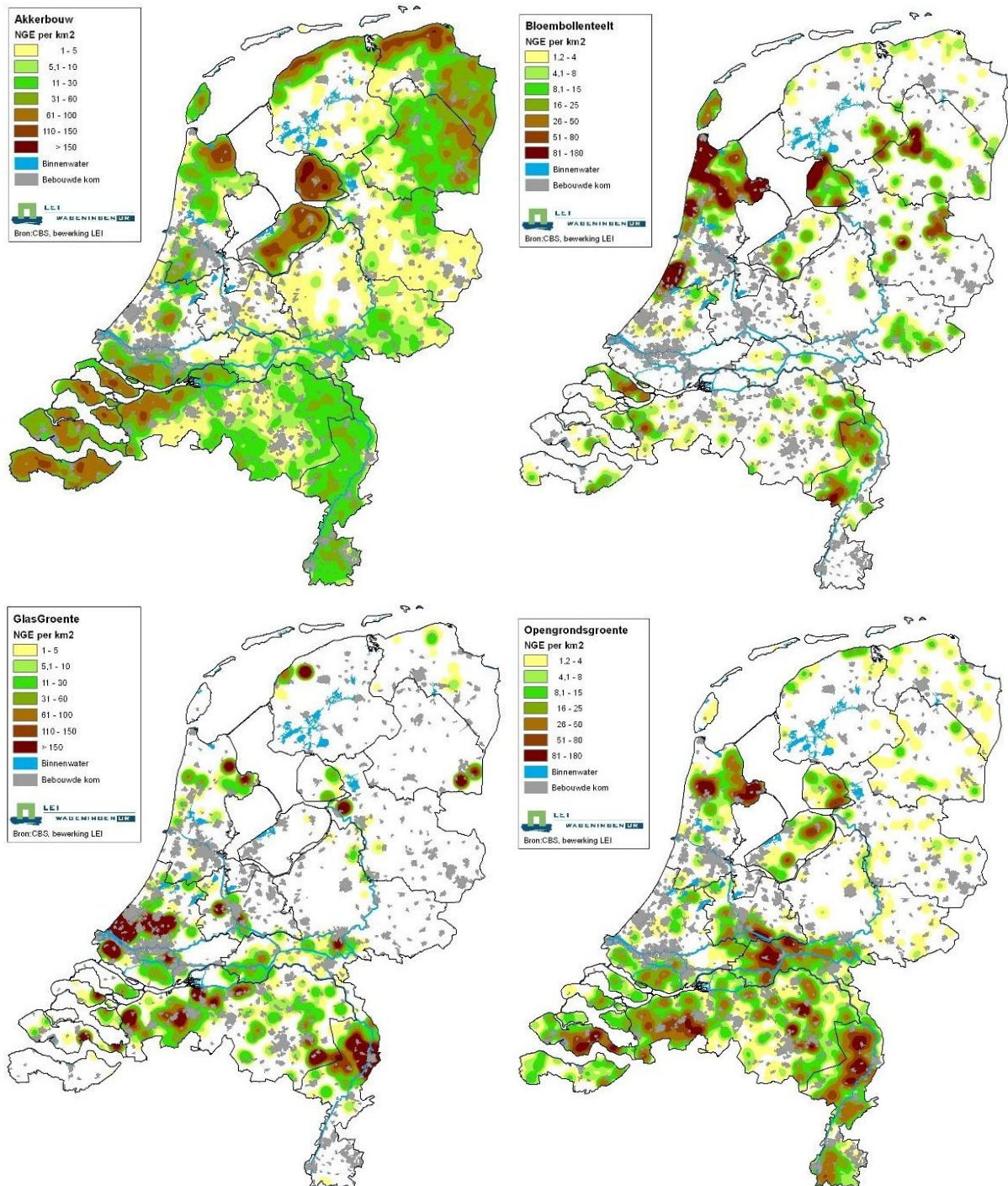


Figure 1. Maps showing where arable farming, bulb growing, cultivation of vegetables in greenhouses and open-air cultivation of vegetables are concentrated in the Netherlands. 100 NGE = € 140,000 in production value. Source: LEI Wageningen University

The locations in Figure 2 where the MTR norm was exceeded most often and most strongly, reflect the locations on the maps in Figure 1, where horti- and agriculture are concentrated. The norm was exceeded especially often in the provinces Zuid-Holland and Noord-Holland, where bulb growing and greenhouse cultivation of vegetables are common.

The norm was also exceeded in Flevoland, Groningen and, to a lesser extent, Zeeland, which are all areas with a lot of arable farming. Friesland is the only province where most concentrations measured are below the norm, which corresponds to the fact that there is not much arable farming or horticulture in this province, except in the north, where few concentration measurements were carried out.

It is difficult to draw any conclusions about most other areas of the Netherlands, since the concentration measurements are not distributed evenly over the country.

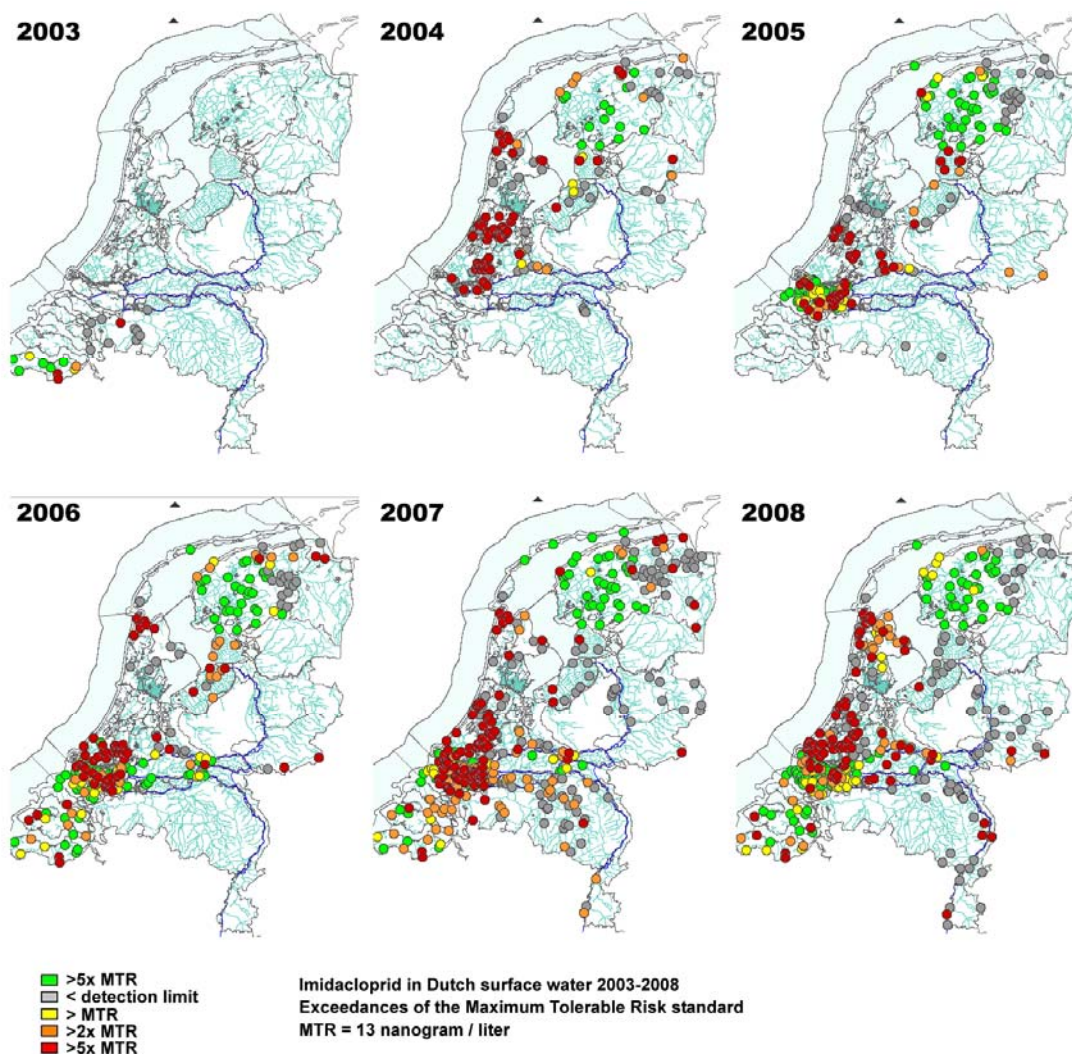


Figure 2. Map of the locations at which surface water concentrations of imidacloprid were measured, and where the MTR norm was exceeded. Measurements are expressed in violation of MTR (1x MTR=13ng/liter) (CML, 2010: [www.bestrijdingmiddelenatlas.nl](http://www.bestrijdingmiddelenatlas.nl)).

Until 2008, the MTR norm was not yet, or very rarely exceeded for the other neonicotinoid pesticides that are focused on here. The norm is different for each pesticide; in 2008, it was 25 ng/l for thiacloprid, 1 µg/l for thiamethoxam and 14 µg/l for clothianidin (CML, 2010).

Surface water concentrations of thiacloprid were measured in the provinces Zuid-Holland, Overijssel, Zeeland and Brabant. By far the greater part of these concentrations was below the norm for thiacloprid; only at seven locations did the concentration exceed the norm, and only at one location did it exceed the norm by more than five times. Concentrations of thiamethoxam were measured in Zuid-Holland and Zeeland; they were all below the norm in Zuid-Holland, except at one location, and all below the target value, which is lower than the norm, in Zeeland. Concentrations of clothianidin were only measured in the Zeeland and were all below the target value, as well.

*Where can high exposure to neonicotinoids be expected?*

The exact locations where high exposure is likely are clearly visible in Figure 2. More generally, high exposure of organisms to neonicotinoids can be expected:

- in areas with arable farming, especially of potatoes and chicory, and in areas where flowers and bulbs are commercially grown
- if the crops grown are flowering plants (for instance: bulbs); in this case, exposure is more likely than if they are not (for instance: sugar beets)
- if crops are grown on sandy soils; concentrations of neonicotinoids in surface waters are likely to be higher than if they are grown on clay soils, since clay particles bind the pesticide molecules better than do sand particles, and fewer pesticide molecules are transported to the ground water from clay soils than from sandy soils.

## Methods

### *Priority list*

As a first step to being able to assess the possible correlation between imidacloprid concentration and species abundance, an inventory was made of toxicity data from scientific literature. First, the available literature on the toxicity of neonicotinoid pesticides to non-target species was studied and the toxicity data were listed. This alphabetic list (see Appendix I) does not offer a complete overview of all published toxicity data, but does contain all those that could be found in the limited time span assigned to this activity, and in all the accessible papers.

Based on these data, the non-target species were listed in order of vulnerability in a 'priority list', so the species that were expected to be the most vulnerable to exposure to the pesticides could be identified: as it would not be possible to study the effect of neonicotinoids on every non-target species, the most vulnerable species were to take priority.

Initially the intention was to organise the listed species by product, unit and test duration, distinguishing between technical grade and commercial products. However, as there does not appear to be a standard unit for reporting toxicity, this resulted in such a large number of different categories that hardly any comparison could be made. Therefore, in re-ordering the list, it was assumed that:

- all reported values were in amount of active ingredient (a.i.) and not amount of the plant protection product used;
- synergy effects of the added substances in the commercial products were negligible, so reported values for technical and analytical grade, and all commercial products, are comparable;
- ppm indicates mass, not volume, and is approximately interchangeable with mg/l;
- "concentration expressed as percentage of solution (wt:vol) ( $\times 10^{-3}$ )" can be converted to mg/l by using the calculation:

$$\text{weight/volume percent (w/v)} = \frac{\text{weight solute, g}}{\text{volume solution, ml}} \times 100\%.$$

In many published toxicity studies information lacked to validate these assumptions, so the resulting list is not perfect. However, the assumptions seem reasonable, so the list can be used to assess relative vulnerability of non-target species to exposure to the four neonicotinoids studied. The ordered lists are presented in Appendix II.

### *Data collection*

The method of data collection chosen for this research, was consulting existing databases on the current and past (meaning: before the appliance of neonicotinoid pesticides began) distribution and abundance of selected species throughout the Netherlands. This method was chosen for two reasons. Collecting data through fieldwork would not have been efficient, since the aim of this research was to gain knowledge on the distribution and abundance of species in the entire Netherlands, and it is not possible for one person to sample an entire country, let alone during the relatively short time span of this research. Also, to be able to

analyse the effects of the pesticides on the species, measurements would have to have been taken, which would have brought about costs far exceeding the budget for this study.

Data on the distribution and abundance of four orders of species which topped the priority list as relatively vulnerable to neonicotinoids were available from Limnodata Neerlandica, an online database containing data provided mainly by the Dutch water boards (*waterschappen* or *hoogheemraadschappen*), but also the provinces and Rijkswaterstaat.

The water boards regularly sample a fixed set of locations in their areas by dragging close-meshed landing nets through the water over a distance of 5 or 10 m and through different habitats, including soil and vegetation. Afterwards, all macrofauna, diatoms, zooplankton, phytoplankton and macrophytes in the samples are determined. For this study, only the data on macrofauna were used. The database comprises about 36,000 measurements of, in total, more than 1,000 macrofauna taxa at 10,600 locations.

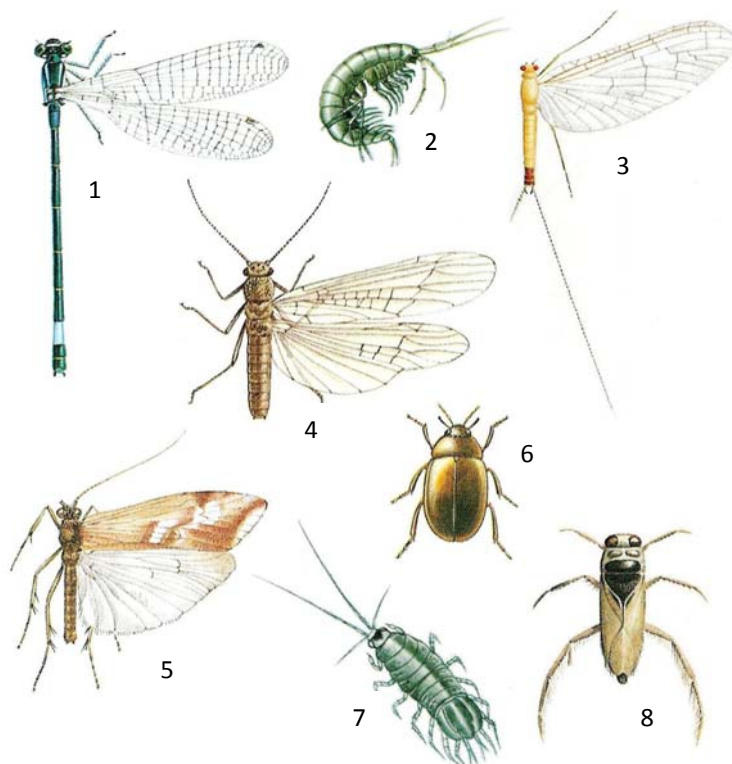


Figure 3. An example of an abundant species for eight of the orders (not to scale). 1 *Ischnura elegans* (Odonata), 2 *Gammarus pulex* (Amphipoda), 3 *Cloeon dipterum* (Ephemeroptera), 4 *Nemoura cinerea* (Plecoptera), 5 *Limnephilus lunatus* (Trichoptera), 6 *Enochrus testaceus* (Coleoptera), 7 *Asellus aquaticus* (Isopoda), 8 *Notonecta glauca* (Heteroptera). Sources: Elseviers Insectengids, De Grote Rebo Natuurgids

The four orders on which data were available from Limnodata Neerlandica and which were identified as vulnerable to neonicotinoids according to the priority list, were Diptera (true flies), Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). Data on thirteen other orders were available as well; based on the number of data available in the database, six of these were also selected for analysis: Hydracarina (water mites), Coleoptera (beetles), Heteroptera (bugs), Amphipoda (crustaceans), Isopoda (crustaceans), and Odonata (dragonflies and damselflies). Examples of species from these orders can be seen in Figure 3.

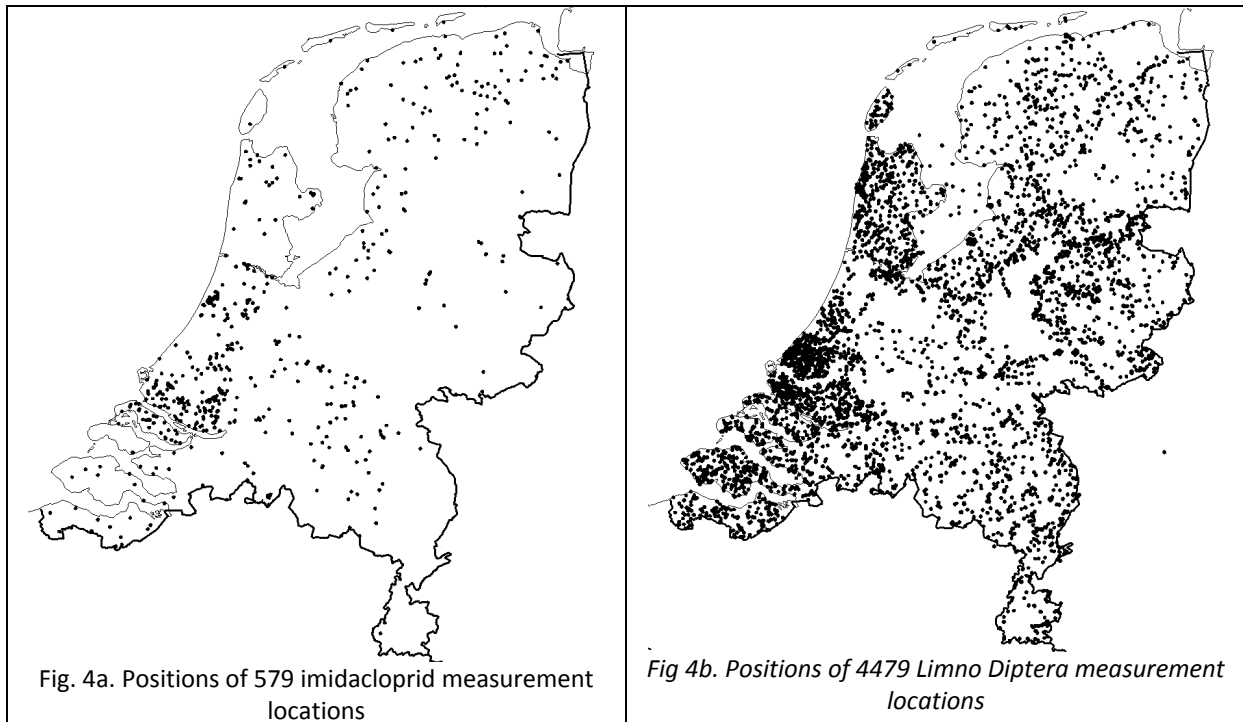
It should be noted that since these data are from water samples, all the sampled species from the orders described have at least one aquatic life stage. In the case of Diptera, Odonata, Ephemeroptera, Plecoptera and Trichoptera, this is the larval stage, which constitutes the main part of the lifespan of the latter three orders. In the case of Amphipoda and Isopoda, all life stages of the sampled species are aquatic, and the other orders are mainly aquatic but some species can fly between water bodies.



### *Combining species abundance and imidacloprid concentrations*

Measurements of imidacloprid concentrations in the Netherlands were available from [bestrijdingsmiddelenatlas.nl](http://bestrijdingsmiddelenatlas.nl), but only for the years 1998 and 2003-2007. Therefore, only the Limnodata sample data for those years could be analysed. Due to the nature of the data, comparing species abundance before the application of neonicotinoid pesticides began, and during application, was not possible.

The distribution of the locations at which imidacloprid concentrations were measured is shown in Figure 4.



The files with the amounts of organisms found per location per date, and those with imidacloprid concentration per location per date, were combined through a PHP script coupled to a MySQL database in which the imidacloprid measurements and the abundance data were stored. PHP stands for 'Hypertext Preprocessor' and is a scripting language originally meant for creating dynamic websites; MySQL is a system which acts as a server and can provide access to databases to many users (Wikipedia, 2010).

The PHP script did the following:

- For each row of species abundance data, search for data points in the imidacloprid concentrations file, which fall within a specified time frame and within a radius of 50 m from the sampling location;
- If more than one concentration measurement meets these requirements, take the unweighted average of these. This value is entered in the resulting file as *imi0*. If no values are found, *imi0* = 0;
- Was *imi0* > 0? If so, also enter its value as *imi1*, the value for a 1 km radius. If not, check again within a radius of 1 km and again take the unweighted average of all values found;

- Was  $imi1 > 0$ ? If so, enter its value as  $imi2$ , the value for a 2 km radius. If not, check again within a radius of 2 km and again take the unweighted average of all values found;
- And so forth, until radii of 3, 4 and 5 km have also been checked. If no concentration measurements are found which fall within the specified time frame and within a radius of 5 km, the species abundance data row is left out of the resulting file;
- The time frame, which could be varied, consisted of a number of days before the species sampling date (past) and a number of days after it (future);
- The detection limit could also be varied. This meant that only real values could be included, or also values which were below the detection limit used while measuring, and thus reported as the value that was the detection limit.

A second PHP script followed exactly the same steps but instead of the unweighted average imidacloprid concentration, it returned the median imidacloprid concentration.

The method of stepwise increase of the seek radius results in more data points being included in the resulting file than there would be if only a small radius (e.g. 50 m) was checked, but it does make sure that the most nearby concentration measurements available are always used. This is useful because the concentration at a closer location is a better measure of (c.q. proxy for) local bioavailability of imidacloprid than that measured at more distant locations.

#### *Preliminary data analysis: parameter selection*

The PHP script allowed for testing different scenarios by varying the time frame ( $d$ =number of days in the past-number of days in the future), as well as the detection limit ( $dl$ ). Three scenarios were tried. The default scenario, based on the maximum half-life time of imidacloprid in water (Roberts & Hutson, 1999), was  $d=160-0$  and  $dl=0$ . Scenarios for 0,5 and 2 times the half-life were also tested, which means  $d=80-0$  and  $d=320-0$ , respectively. Also, several values were tested for the detection limit.

Each output file of the script included the average of the imidacloprid concentrations found near each sampling point, for six distances: '0 km' (actually 50 m), 1 km, 2 km, 3 km, 4 km and 5 km. Since taking the average abundance for concentrations in a 3-5 km radius, resulted in relatively noisy output data, only the distances of 0-2 km were studied further.

The  $d=80-0$  scenario resulted in fewer usable data than the  $d=160-0$  scenario, whereas the  $d=320-0$  scenario, again, resulted in relatively noisy data. Therefore the  $d=160-0$  scenario was chosen for further analysis, as it was the most likely of the three to yield meaningful results. Including a number of days in the future did not greatly increase the number of usable data in the output files, while it did increase the chance of raised imidacloprid concentrations caused by imidacloprid application after the sampling data, which could thus not have had any influence, being included in the analysis. Therefore, no days in the future were included. Comparison of results for mean versus median imidacloprid concentration showed that the median caused less noise. This is consistent with what was expected on theoretical grounds: if multiple imidacloprid concentrations are found in the seek radius, the median is more representative for the local bioavailability of imidacloprid than the mean, because the concentrations show a log-normal distribution.

Many different detection limits were reported in the file with concentration measurements, the lowest being 5 ng/l and the highest 190 ng/l, and there were more low values: the average of the values reported as being not an actual value but the detection limit was 32

ng/l. The concentration data distribution is listed in Table 2. It shows many values for concentrations up to 25 times the MTR, while less than 7% of the measurements exceeds the MTR more than 125 times. The highest concentration in the sample is 25,000 times the MTR.

MTR	imi concentration (ng/l)	above detection limit	below detection limit		
		<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>
0-1x	0 - 13	120	1,204	2,022	161
1-5x	13 - 65	507			
5-25x	65 -325	501			
25-125x	325 -1625	252			
125-625x	1625-8125	55			
625-3125x	8125 -40625	20			
3125-15625x	40625-203125	6			
>15625x	>203125	4			
Total <i>n</i>		1,465	3,387		

Table 2. Distribution of all 4,852 available imidacloprid measurements (years 1998, 2003, 2004, 2005, 2006 and 2007 pooled). 1,465 data points are real measurements, 3,387 data points are measurements for which the actual concentration was below the detection limit of the measurement method used. Detection limits varied between 5 and 190 ng/l. (MTR = Maximum Allowable Risk level, here 13 ng/l)

Including measurements with concentrations below the detection limit would introduce unnecessary uncertainty in the analysis; therefore only the measurements with results above detection limit were used as default for further analysis of the data (coded as " *dl=0*" in the algorithm used).

#### Data analysis

After coupling the species abundance and concentration data, there were so few data points left for Plecoptera that the association between imidacloprid and species abundance of this order could not be analysed. The other nine orders were analysed using the default scenario of *d=160-0* and *dl=0*.

The data in each *d=160-0 dl=0* output file were first ordered in categories based on the MTR norm of 13 ng/l which was used in the Netherlands at the time the data were collected. Recently it has been proposed to relax the norm to 67 ng/l (Posthuma-Doodeman, 2008). MTR stands for maximum allowable risk level (Dutch: Maximaal Toelaatbaar Risiconiveau), and the norm is based on toxicity data of a pesticide for as many species as possible (Bestrijdingsmiddelenatlas, 2010).

The categories were:

0-1x the MTR norm	0-13 ng/l
1-5x	13-65 ng/l
5-25x	65-325 ng/l
25-125x	325-1625 ng/l
125-625x	1625-8125 ng/l
> 625x	> 8125 ng/l

As this classification resulted in very different sizes ( $n$ ) of the subsamples in each category, the data were also divided more evenly, into two groups for which  $n$  was as equal as possible. This provides a good base for testing significance of differences between categories where these differences indicate the existence of an association between species abundance and imidacloprid bioavailability.

Finally, the data were visualised in scatter plots. For each order, the abundance data were plotted against the average concentrations for imi2 (seek radius 2 km). Logarithmic axes were used to make up for the data distribution, in which there are many low values and few very high ones. Logarithmic trendlines were also added.

## Results

### *Priority list*

The ordered priority list (see Appendix II) contains 44 species, of which 18 are also found in the Netherlands (Nederlands Soortenregister, 2010). The list consistently shows that flying insects are the most vulnerable to neonicotinoid pesticides. They appear to be more vulnerable than mirid bugs, mites, spiders, aquatic crustaceans, fish and birds, the categories most other species in the list belong to.

Also, in 23 cases data about lethal doses or concentrations over different time spans are available, and in 21 of these cases, the lethal dose or concentration decreases with time. Generally this decrease amounts to about 50% over 24 hours, but in some cases, the factor is 10 or more. This indicates that as species are exposed to neonicotinoids longer, even very low concentrations will affect them. This phenomenon is toxicologically explained by Tennekes (2010) who showed that neonicotinoids in arthropods follow Haber's rule, which is characterised by a linear relationship (on logarithmic coordinates) between exposure concentration and median time to effect, i.e. mortality. Biochemically this can be understood from the mode of action of neonicotinoids, which derives from almost complete and virtually irreversible blockage of postsynaptic acetylcholine receptors in the central nervous system of insects (Tennekes, 2010).

The flying insect categories selected for further research, based on the priority list, were: flies, mayflies, caddisflies, parasitoid wasps, wild bees, mosquitoes and midges. However, data on parasitoid wasps and wild bees were not available.

### *Data analysis – MTR-based categories*

Table 3 shows the result of the classification by number of times the MTR, for two orders of species: Diptera and Amphipoda. The tables for the other orders can be found in Appendix III.

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
<b>Diptera</b>						
0-13 ng/l	32.3	31	31.4	59	25.4	182
13-65 ng/l	30.8	47	19.4	283	15.9	786
65-325 ng/l	25.1	74	18.0	132	14.3	359
325-1625 ng/l	11.2	36	8.4	52	9.8	158
1625-8125 ng/l	8.7	22	9.8	32	6.3	72
> 8125 ng/l					1.8	10
<b>Amphipoda</b>						
0-13 ng/l	841.3	10	620.8	14	396.3	29
13-65 ng/l	29.8	12	46.5	35	41.7	85
65-325 ng/l	144.0	25	110.7	34	77.5	55
325-1625 ng/l	17.4	7	15.6	8	28	22
1625-8125 ng/l			50	1	58	6

Table 3. Average abundance and *n* classified by number of times the MTR, for Diptera (top) and Amphipoda (bottom).  $d=160-0$   $dl=0$

As can be seen in Table 3, the species abundance for Diptera tends to decrease with increasing imidacloprid concentration. This is the case for all radii and all categories, apart from 325-1625 ng/l and 1625-8125 ng/l for a radius of 1 km.

The results for Amphipoda are more ambiguous. For all radii, the average abundance is by far the largest at the lowest imidacloprid concentration, 0-13 ng/l. The average abundance for this concentration is more than five times as high as the average abundance for any other category. However, abundance does not decrease with every higher concentration category, as the abundance at 65-325 is actually higher than that at 13-65, and the abundance at 1625-8125 ng/l is higher than that at 325-1625 ng/l. As mentioned though, they are much smaller than the abundance at the lowest concentration, and also, *n* is much smaller for the higher concentrations, which makes the average abundance for those categories less reliable.

The results for the other orders, which can be found in Appendix III, are also ambiguous. For Ephemeroptera, the highest abundances are found in the 325-1625 and 1625-8125 ng/l categories, although *n* is rather small for both. For Trichoptera there is no big difference between the abundances so it is difficult to observe a clear trend, but there does not appear to be any. For Hydracarina, on the other hand, a reverse trend is observable: for a 2 km radius, species abundance consistently increases with imidacloprid concentration for this order. For Coleoptera, the difference in abundance is extremely small, even smaller than that for Trichoptera; therefore, no trend is observable there, either. Heteroptera and Odonata both seem to show a trend of decreasing abundance with increasing imidacloprid concentration, except for a relatively high value in the 325-1625 ng/l category in the case of Heteroptera, and 1625-8125 ng/l in the case of Odonata - they are even the highest abundances found for these orders. Finally, for Isopoda it may be possible that abundance decreases with increasing imidacloprid concentration once 325 times the norm has been reached, but *n* is too small to be able to assess this with certainty.

*Data analysis – Two groups of equal size*

As the classification on the basis of the MTR resulted in very different subsample sizes *n*, the data were divided again, but more roughly, into two groups for which *n* was as equal as possible. This provides a good base for testing significance. The division was made for the data for a 2 km radius. Since the total *n* was different for each order, the concentration at which the groups were divided is also different for each order.

Table 4 shows the results for Diptera and Amphipoda. The PAST software package for statistical analysis (<http://folk.uio.no/ohammer/past>) was used to compute a Permutation *t* test with *N*=100,000 permutations, to test the null hypothesis that the means in both subsamples for the 2 km radius case are equal. The results of the analyses of the other orders can be found in Appendix IV.

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
<b>Diptera</b>						
0-40 ng/l	34.3	70	22.8	292	19.6	788
> 40 ng/l	17.9	140	14.4	266	11.4	779
Significance						<i>p</i> = 0.00131
<b>Amphipoda</b>						
0-54 ng/l	503.6	17	240.6	42	146.8	98
> 54 ng/l	106.3	37	83.0	50	59.4	99
Significance						<i>p</i> = 0.20218

Table 4. Average abundance for two groups with equal *n*, and the significance of the difference for a 2 km radius. *d*=160-0 *dl*=0

The extremely skewed abundance distribution, with many low values and a couple of high extremes, resulted in very high variances (up to several hundreds or thousands). This is because the species abundance data appear to exhibit a distribution with a very high coefficient of variation. Therefore, the data were assessed once more after a square root transformation had been carried out. This means that the square root of all the abundance data was taken, which results in a transformed data set with much lower variance. Then the two groups were again subjected to a Permutation *t* test. The results can be found in Table 5.

<b>Order</b>	<b>Diptera</b>	<b>Ephemeroptera</b>	<b>Trichoptera</b>	<b>Hydracarina</b>	<b>Coleoptera</b>
Significance	$p < 1 \cdot 10^{-5}$	$p = 0.92837$	$p = 0.51723$	$p < 1 \cdot 10^{-5}$	$p = 0.06782$
<b>Order</b>	<b>Heteroptera</b>	<b>Amphipoda</b>	<b>Isopoda</b>	<b>Odonata</b>	
Significance	$p = 0.40059$	$p = 0.1090$	$p = 0.73996$	$p = 0.12976$	

Table 5. The significance of the difference between the two groups for a 2 km radius, after root transformation.  $d=160-0$   $dl=0$

According to these results, the only orders for which there is a significant difference between the abundance at lower imidacloprid concentrations, and those at higher concentrations, at a 95% confidence level, are Diptera and Hydracarina. In the case of Diptera, this is a strong indication that species abundance negatively correlates with imidacloprid bioavailability. But in the case of Hydracarina, the opposite is true, since an inverse trend could be observed from the results of the MTR classification.

For these two orders, the difference was already significant before the square root transformation was applied, but their *p* values have become even smaller because of it.

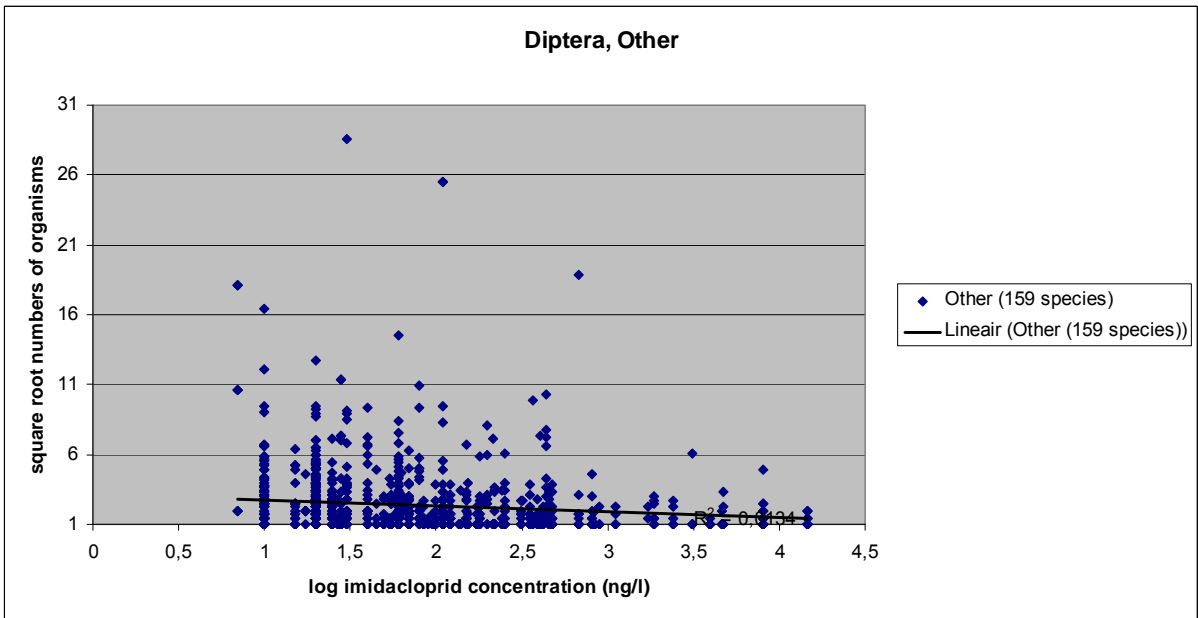
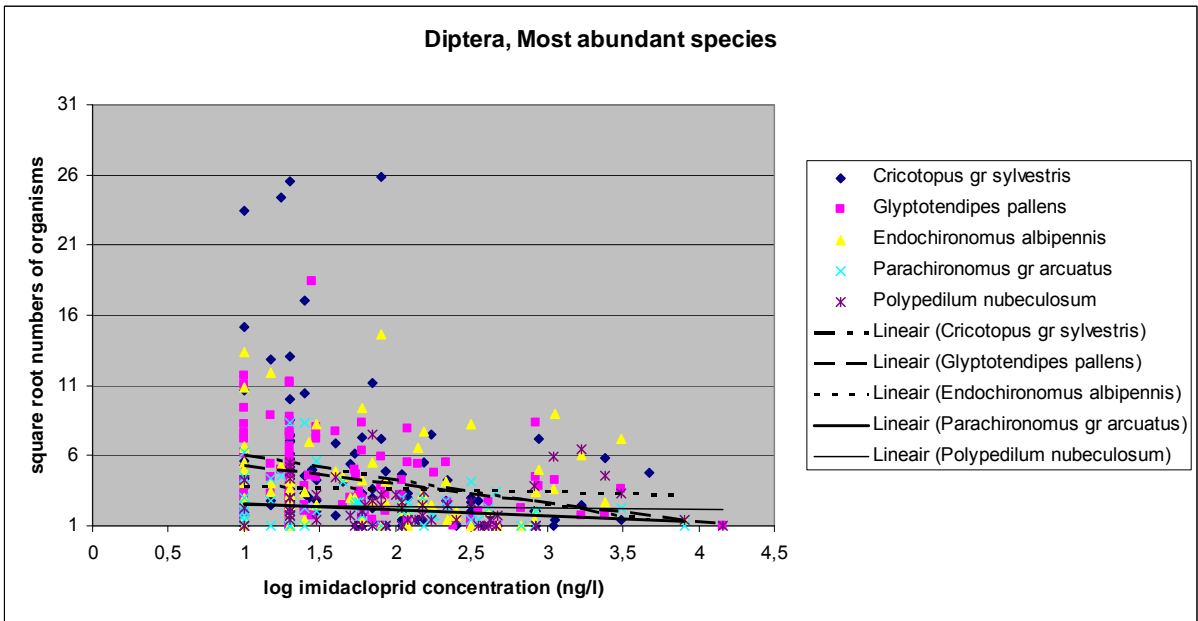
After the transformation, the *p* value for Coleoptera is 0.067, and those for Amphipoda and Odonata are 0.108 and 0.129 respectively. Although these values are not within the 95% confidence interval, they are relatively high values for this kind of research, where only a proxy of the bioavailability could be constructed because simultaneous imidacloprid measurements at the same locations where species abundance was measured are not available. They indicate 93, 89 and 87% chances, respectively, that the species abundance is negatively affected by the presence of imidacloprid in the water. It is well possible that if all species samples and imidacloprid concentration measurements had been taken at the same locations and the same dates, *p* values would have been much smaller.

#### *Data analysis – Scatter plots*

Finally, the data were visualised in scatter plots. For each order, the data for the most abundant species, the other species, and the data for the entire order, were plotted in separate graphs against the average concentrations for a 2 km radius. Again, this was done after a transformation had been applied to the data, in order to more closely approximate a normal distribution: the square root of the abundances was taken, and the logarithm of the imidacloprid concentrations. Linear trendlines were also added.

Only species which were present in at least 20 samples in the output file were plotted as ‘most abundant species’, with a maximum of five species per order.

Figure 4 shows the scatter plots for Diptera. Graphs for the other orders can be found in Appendix V.





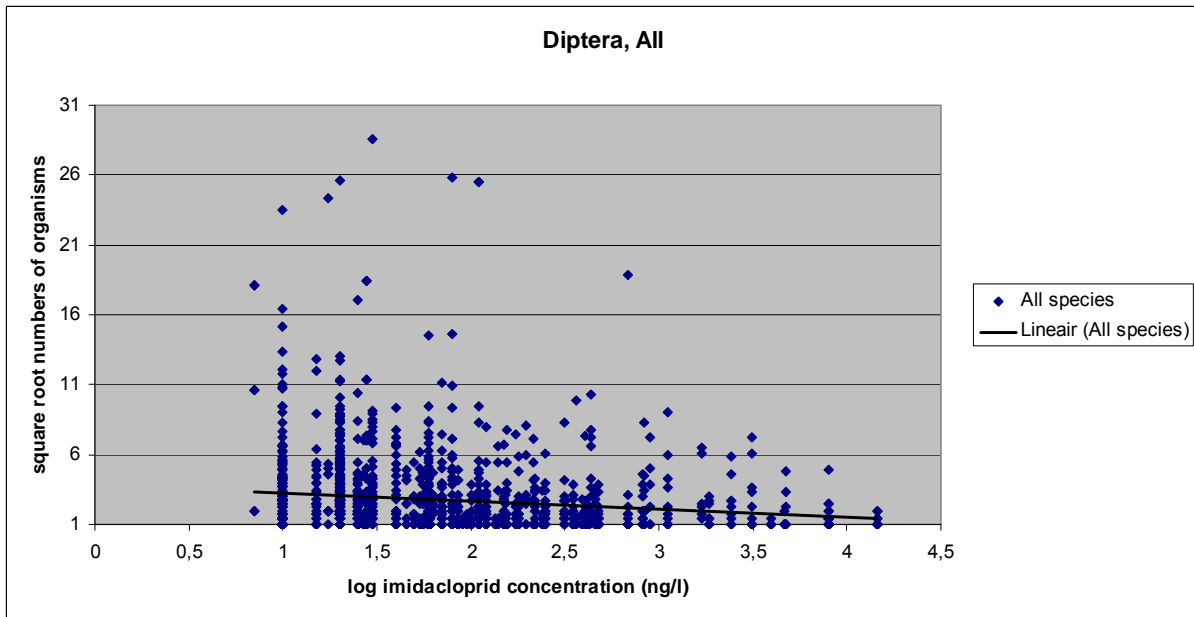


Figure 4. Scatter plots for Diptera, with linear trendlines. Top: the most abundant species, middle: the other species, bottom: all species.  $d=160-0$   $dl=0$

Again, the results for Diptera consistently show a trend of decreasing abundance with increasing imidacloprid concentration. This is the case for all species together, for the ‘other species’ category, and for all of the most abundant species, although there is some variety in trendline slope.

Regression analysis was also carried out to test the significance of the correlation shown by the trendlines in these graphs, again using PAST. The graphs were not created using this software, as it does not allow for several series to be plotted in one graph. Table 5 shows the results for Diptera; results for the other orders can be found in Appendix V.

Diptera				
Species	<i>C. gr sylvestris</i>	<i>G. pallens</i>	<i>E. albipennis</i>	<i>P. gr arcuatus</i>
Regression	$r = -0.22225$	$r = -0.25408$	$r = -0.051467$	$r = -0.19518$
Significance	$p$ (uncorr) = 0.01649	$p$ (uncorr) = 0.00740	$p$ (uncorr) = 0.63792	$p$ (uncorr) = 0.09334
Species	<i>P. nubeculosum</i>	Other (159 species)	All	
Regression	$r = -0.044888$	$r = -0.11587$	$r = -0.14243$	
Significance	$p$ (uncorr) = 0.72684	$p$ (uncorr) = 0.00010	$p$ (uncorr) = $1.49 \cdot 10^{-8}$	

Table 5. Results of significance analyses of the trendlines in the scatter plots.  $d=160-0$   $dl=0$

The  $r$  value indicates correlation. If  $r=0$  there is no correlation. If  $r=1$  there is a perfect positive correlation, and if  $r=-1$  there is a perfect negative correlation.  $p$  (uncorrelated) expresses the chance that the found correlation is based on coincidence, rather than a real correlation.

$r$  is negative for all species of Diptera, both when analysed separately and together, but the strength of the correlation varies. However, the correlation is significant at a 95% confidence level for the species *C. gr sylvestris* and *G. pallens*, for the other species, and especially for all species together. There is also a 91% chance that the correlation for *P. gr arcuatus* is not coincidental.

There is also at least one declining trendline for each of the other orders, though again with variation in steepness, and though not all these correlations are significant.

A very significant negative correlation is present for all species together for Amphipoda. Again, there are also several correlations which, although not within the 95% confidence interval, have a relatively high level of significance if the nature of this research is considered. This is the case for *M. longicornis*, a species of Trichoptera (92%), *P. minutissima minutissima*, a species of Heteroptera (85%), the other species and all species of Heteroptera (91 and 90%, respectively), *G. tigrinus*, a species of Amphipoda (93%), as well as the other species of Amphipoda (92%).

There were also some significant positive correlations. Consistent with the results of the previous analyses, this was the case for many species of Hydracarina, but also for *C. dipterum*, a species of Ephemeroptera, all species of Ephemeroptera together, and *T. bicolor*, a species of Trichoptera.

Although these results are ambiguous, they do indicate that even among the orders for which no significant differences were found in the previous analyses, there may be species which are adversely affected by increasing imidacloprid concentration.

This also means it is possible that if the previous analyses had been tried for separate species instead of entire orders, more significant differences might have been found. Unfortunately this was not possible due to lack of data.

## Conclusions

Neonicotinoid pesticides are applied in the largest amounts where potatoes, horticultural products and chicory are grown. In these areas, which are mainly found in the Dutch provinces of Zuid-Holland, Noord-Holland, Zeeland and Groningen, imidacloprid can be found in the surface water in concentrations often far exceeding the MTR norm of formerly 13 ng/l and presently 67 ng/l.

Neonicotinoid pesticides work by inhibiting nerve impulses in susceptible species, particularly insects, which leads to their demise and, at lower concentrations, several sublethal effects including reduced learning, signalling and starvation.

The effects of neonicotinoid pesticides on many non-target species have been reported in scientific literature – in this study, toxicity data from previous research were listed for 44 different species. Flying insects quite consistently appeared to be the most vulnerable to neonicotinoids, and therefore species abundance of three orders of flying insects was combined with imidacloprid concentrations in the Dutch surface water, to see whether any correlation between these properties existed. Nine other orders, of aquatic insects and crustaceans, were also included in this analysis.

Species abundance for the flying insect order Diptera tends to decrease with increasing imidacloprid concentration. This was shown by all three different methods of analysis used: classification by number of times the MTR norm, classification by groups with equal  $n$ , and visualisation of data in scatter plots. The difference between the average abundances of the groups with equal  $n$  was significant for Diptera.

The order Hydracarina showed an opposite effect, as abundances were higher at high imidacloprid concentrations. Results for the other orders were often more ambiguous, However, after a square root transformation had been applied to the data, the significance of the finding that Coleoptera, Amphipoda and Odonata are negatively influenced by the presence of imidacloprid was shown to be at a 93, 89 and 87% confidence level, respectively. Also, the scatter plots of transformed data for all orders showed declining trendlines for separate species and sometimes for all species together, and these were significant for Diptera and all species of Amphipoda together, and had a relatively high level of significance for a species of Trichoptera and most species of Heteroptera.

This indicates that if the amount of data available had permitted analysing separate species, more significant differences might well have been found, as the presence of insensitive species may have distorted results for the entire order. If analysis of separate species had been possible, the hypothesis drafted for this study might have been confirmed unequivocally. As is, the hypothesis has been confirmed for Diptera, and, less strongly, for Coleoptera, Amphipoda, Heteroptera and Odonata. It was invalidated for Hydracarina, and for the other orders results were ambiguous.

## Discussion

During the inventory of the available scientific literature on the effects of neonicotinoid pesticides on non-target species, two things became clear. First, there is still a considerable gap in the knowledge on these effects on many species, including, for instance, butterflies. Perhaps it would be advisable for organisations specialising in the conservation of such species to conduct research on this topic. Second, there does not appear to be a standard method of research, or standard unit for the publication of research results, used in toxicity studies. This made comparing the results found for different species difficult in many cases. Standardising toxicity research, would facilitate comparison of research results. This would also be helpful for authors conducting new research, as it would enable them to compare their results to previously published results, and to assess whether they correspond.

Forming an objective image on the effects of pesticides on different species, and on their general safety, is complicated by the fact that many studies are financed at least in part by large chemical concerns such as Bayer, which produce the pesticides in question. This was the case with Sarkar et al. (1999). Also, some studies involve researchers employed by such concerns; this was the case with Cox et al. (1997). Finally, some studies on toxicity to non-target species are published in publications issued by the aforementioned concerns, instead of in peer-reviewed independent journals; this applies to Schmuck (2001).

A factor that has not been dealt with in the methods and results sections but may have influenced results slightly, is that besides a detection limit, there is also a quantification limit applicable when measuring concentrations. In some cases, this quantification limit may have been entered in the original imidacloprid concentrations data set, but classified as a real value. Alternatively, when they were entered in the database, the data may not have been flagged as being below detection limit. In any case, a 'snap to detection limit' mechanism seems to have been present in the composition of the dataset: this is indicated by the fact that many of the scatter plots contain vertical 'lines' of data points, caused by many similar concentrations.

During data analysis it became clear that one of the sub-questions drafted for this study, "Has the distribution and number of different non-target species changed significantly since the application of neonicotinoid pesticides began?", could not be answered, since continuous species abundance data over a time span of several decades did not appear to be available for any species or order of species.

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## Appendix I – Alphabetic list of toxicity data

### Imidacloprid (alphabetical list)

Species (names in Latin, English and Dutch)	Acute toxicity	Chronic toxicity	NOEC	Synergy effects	Source
<i>Allolobophora icterica</i> , earth worm, Gevlekte worm (?)		LC <sub>50</sub> : 2.81 mg/kg dry soil; 14 d exposure  (Confidor, 200 g/l in 100% DMSO)			Capowiez et al., 2005
<i>Amblyseius cucumeris</i> , predatory mite, roofmijt	LC <sub>50</sub> : 10,000 to >10,000 ppm; determined 24 h after 5 seconds' dipping in solution, and 72 h direct contact with dipped leaf; females only, obtained from three companies  (Admire 240 F)				Lee et al., 2002
<i>Anagrus nilaparvatae</i> Pang et Wang, parasitoid wasp, parasitaire wesp	LC <sub>50</sub> : 0.021 mg a.i./l; determined 8 h after 1 hour's exposure  (Technical grade, 95.3% a.i., 50 mg a.i./l)				Wang et al., 2008
<i>Anaphes iole</i> Girault, fairyfly, parasitaire wesp		ST <sub>50</sub> : 2.64 d; contact with leaves with field-weathered residues; females only (Provado 1.6 F, 0.053 kg)			Williams et al., 2003

		a.i./ha, applied at field rate)			
<i>Aphelinus mali</i> , wasp, sluipwesp	LC <sub>50</sub> : 0.16 ppm; 24 h exposure to leaf dipped in solution for 10 s, not systemic  (Confidor 35 wettable powder)				Cohen et al., 1996
<i>Aporrectodea nocturna</i> , earth worm, regenworm		LC <sub>50</sub> : 3.74 mg/kg dry soil; 14 d exposure  (Confidor, 200 g/l in 100% DMSO)			Capowiez et al., 2005
<i>Artemia</i> sp., Brine shrimps, Pekelkreeftjes (?)	LC <sub>50</sub> : 361.23 mg/l; 48 h exposure under hyperosmotic conditions: 100% artificial salt water			Mortality increased dramatically, exceeding 75%, when salinity increased from 10 to 200‰ over 48 h; decreasing salinity did not cause a change in mortality	Song et al., 1997 and Song & Brown, 2006
<i>Asellus aquaticus</i> L., Water louse, Waterpissebed	LC <sub>50</sub> : 8.5 mg/l; 48 h  (Confidor SL 200)				Lukančič et al., 2010
<i>Baetis rhodani</i> , mayfly, haft of eendagsvlieg	LC <sub>50</sub> : 8.49 µg/l; 48 h exposure; larvae only  (Analytical grade powder)				Beketov and Liess, 2008
<i>Bombus impatiens</i> , Common eastern bumblebee, hommelmel	LC <sub>50</sub> : 3.22 (concentration expressed as percentage of solution (wt:vol) (x10 <sup>-3</sup> )); 48 h direct contact toxicity; females only		For survival: 10 ppb (with and without foraging)  For reproduction: 20 ppb (without foraging), <2.5 ppb (with foraging)		Scott-Dupree et al., 2009



	(Technical grade, >95% purity)				
<i>Bombus terrestris</i> L., Large earth bumblebee or Buff-tailed bumblebee, Aardhommel	LD <sub>50</sub> : 0.04 µg/bumblebee; 24 h after oral contact LD <sub>50</sub> : 0.02 µg/bumblebee; 72 h after oral contact LD <sub>50</sub> : 0.02 µg/bumblebee; 72 h after topical contact (Commercial product fed dispersed in water and syrup)				Marletto et al., 2003
<i>Bombus terrestris</i> , Large earth bumblebee or Buff-tailed bumblebee, Aardhommel		LC <sub>50</sub> without foraging: 59 ppb; EC <sub>50</sub> : 37 ppb LC <sub>50</sub> with foraging: 20 ppb; EC <sub>50</sub> : 3.7 ppb Experiment duration 11 wk, monitored weekly (Confidor® 20% SC)	10 ppb		Mommaerts et al., 2010
<i>Ceriodaphnia dubia</i> Richard, crustacean, watervlo	LC <sub>50</sub> : 2.07 µg/l; 48 h (Admire Pro; 42.8% active ingredient)			8 days' exposure to a mixture of the nonylphenol polyethoxylate, R-11 and imidacloprid resulted in a population size 3 times smaller than with R-11 alone, and 13 times smaller than with imidacloprid alone	Chen et al., 2009
<i>Chironomus tentans</i> , Midge, Watermug	LC <sub>50</sub> : 5.75 µg/l; 96 h constant exposure, larvae	LC <sub>50</sub> : 0.91 µg/l; 28 d constant exposure	1.03 µg/l for technical imidacloprid, 5.11 µg/l for Admire®, 96 h exposure	The difference between the effects of 99.2% pure imidacloprid and Admire®	Stoughton et al., 2008

	(99.2% pure imidacloprid) LC <sub>50</sub> : 5.40 µg/l; 96 h constant exposure, larvae (Admire®)	(Admire®)	(difference is due to high variability in survival among Admire® replicates at NOEC concentration)  1.14 µg/l for Admire®, 28 d exposure; 3.47 µg/l for Admire®, 96 h pulse exposure, observed after 10 d (28 d: same)	was not significant	
<i>Chydorus sphaericus</i> , planktonic cladoceran, watervlo	LC <sub>50</sub> : 161950 µg/l; 24 h LC <sub>50</sub> : 132673 µg/l; 48 h (Technical grade imidacloprid, 99.5% pure)			EC <sub>50</sub> was also determined, and was 2-13 times lower under dark than under normal (16 h light : 8 h dark) laboratory conditions	Sánchez-Bayo & Goka, 2006
<i>Coleomegilla maculata lengi</i> Timberlake, Pink spotted ladybird, lieveheersbeestje	LD <sub>50</sub> adults: 0.074 µg a.i./insect; 48 hours after ventral application  LD <sub>50</sub> 3 <sup>rd</sup> instar larvae: 0.034 µg a.i./insect; 48 h after ventral application  LD <sub>50</sub> adults: 60.8 mg/l; 48 hours exposure to foliage and <i>Leptinotarsa decemlineata</i> eggs dipped in solution  LD <sub>50</sub> 3 <sup>rd</sup> instar larvae: 12.8 mg/l; 48 h, same treatment (Admire® 240 water flowable solution)	LD <sub>50</sub> adults: 0.013 µg a.i./insect; 6 days after ventral application  LD <sub>50</sub> 3 <sup>rd</sup> instar larvae: 0.008 µg a.i./insect; same treatment  LD <sub>50</sub> adults: 14.4 mg/l; 6 days exposure to foliage and <i>Leptinotarsa decemlineata</i> eggs dipped in solution  LD <sub>50</sub> 3 <sup>rd</sup> instar larvae: 3.2 mg/l; 6 days, same treatment (Admire® 240 water flowable solution)			Lucas et al., 2004
<i>Cyprretta seurati</i> ,	LC <sub>50</sub> : 732 µg/l; 24 h			EC <sub>50</sub> was also determined,	Sánchez-Bayo &

planktonic crustacean, planktonisch schaaldier	LC <sub>50</sub> : 301 µg/l; 48 h (Technical grade imidacloprid, 99.5% pure)			and was 4-16 times lower under dark than under normal (16 h light : 8 h dark) laboratory conditions	Goka, 2006
<i>Cypridopsis vidua</i> , planktonic crustacean, planktonisch schaaldier	LC <sub>50</sub> : >4000 µg/l; 24 h LC <sub>50</sub> : 715 µg/l; 48 h (Technical grade imidacloprid, 99.5% pure)			LC <sub>50</sub> was lower under dark than under normal (16 h light : 8 h dark) laboratory conditions: 24 h: 542 µg/l 48 h: 273 µg/l	Sánchez-Bayo & Goka, 2006
<i>Cypridopsis vidua</i> , planktonic crustacean, planktonisch schaaldier	LC <sub>50</sub> : 3,951 µg/l; 24 h LC <sub>50</sub> : 391 µg/l; 48 h LC <sub>50</sub> : 7.1 µg/l; 96 h				Sánchez-Bayo, 2009
<i>Cyrtorhinus lividipennis</i> , mirid bug, wants	LC <sub>50</sub> : 0.36 ppm; 24 h after dipping in solution; females (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000
<i>Danio rerio</i> , Zebrafish, Zebrafish	LC <sub>50</sub> : 241 mg/l; 96 h (Analytical grade) LC <sub>50</sub> : 214 mg/l (Concentration of imi. in Confidor SL 200)				Tišler et al., 2009
<i>Daphnia magna</i> , water flea, watervlo	EC <sub>50</sub> : 97.9 mg/l; 24 h EC <sub>50</sub> : 56.5 mg/l; 48 h (Analytical grade imidacloprid)		21 d NOEC = 1.25 mg/l	The toxicity of commercial formulation Confidor SL 200 was intensified in comparison to the analytical grade	Tišler et al., 2009

	EC <sub>50</sub> : 38 mg/l; 24 h EC <sub>50</sub> : 30 mg/l; 48 h (Concentration of imi. in Confidor SL 200)			imidacloprid	
<i>Daphnia magna</i> , water flea, watervlo	LC <sub>50</sub> : 64,873 µg/l; 48 h	LC <sub>50</sub> : 9,500; 10 d			Sánchez-Bayo, 2009
<i>Dendrobaena octaedra</i> , earth worm, Koperworm (?)		LC <sub>50</sub> : 5.7 mg/kg LC <sub>10</sub> : about 2 mg/kg and significant weight losses among survivors at 3 mg/kg; all for 35 days?	No effects on cocoon production among survivors at 3 mg/kg; 35 days?		Kreutzweiser et al., 2008a
<i>Diadegma insulare</i> , parasitoid wasp, parasitaire wesp	LC <sub>50</sub> : 0.081 mg a.i./ml (!); 30 min exposure to dipped leaves LC <sub>50</sub> : 0.002 mg a.i./ml (!); 24 h exp. to dipped leaves (Provado 1.6 F)				Hill & Foster, 2000
<i>Diaeretiella rapae</i> , wasp, Bladluiswesp (?)	LC <sub>50</sub> : 3,390 mg/l; 1 h exposure; F <sub>0</sub> : adults collected from regularly sprayed field, thus considered resistant LC <sub>50</sub> : 2,051 mg/l; 1 h; F <sub>11</sub> : 11 <sup>th</sup> generation progeny reared from resistant adults LC <sub>50</sub> : 132 mg/l; 1 h; F <sub>21</sub> : 21 <sup>st</sup> generation progeny, considered susceptible LC <sub>50</sub> : 7,839 mg/l; 9 h; 8 h			All tested synergists had a significant effect, that of piperonyl butoxide (PB) being the greatest. Synergism was greater in F <sub>0</sub> parents than in F <sub>11</sub> and F <sub>21</sub> progeny, but was still significant in F <sub>11</sub> progeny. Only PB synergism was significant in F <sub>21</sub> progeny. LC <sub>50</sub> : imi + 50 mg/l PB: 879 mg/l; 9 h; F <sub>0</sub>	Wu et al., 2004

	<p>after 1 h exposure; F<sub>0</sub></p> <p>LC<sub>50</sub>: 2,558 mg/l; 9 h; F<sub>11</sub></p> <p>LC<sub>50</sub>: 105 mg/l; 9 h; F<sub>21</sub></p> <p>LC<sub>50</sub>: 5.11 mg/l; 24 h: 23 h after 1 h exposure; other adult population collected from regularly sprayed field</p> <p>LC<sub>50</sub>: 0.17 mg/l; 24 h; F<sub>21</sub></p>			<p>LC<sub>50</sub>: imi + 50 mg/l triphenyl phosphate (TPP): 1,245 mg/l; 9h, F<sub>0</sub></p> <p>LC<sub>50</sub>: imi + 50 mg/l diethyl maleate (DEM): 1,026 mg/l; 9h; F<sub>0</sub></p> <p>LC<sub>50</sub>: imi + 100 mg/l PB: 420 mg/l</p> <p>LC<sub>50</sub>: imi + 100 mg/l TPP: 815 mg/l; 9h; F<sub>0</sub></p> <p>LC<sub>50</sub>: imi + 100 mg/l DEM: 653 mg/l; 9h; F<sub>0</sub></p> <p>Insecticide: synergist = 1:1 (v:v), and the terminal concentrations of PB, TPP, and DEM were 50 and 100 mg/liter, respectively.</p>	
<p><i>Eisenia fetida</i>, Common brandling worm or Common dung-worm, Tijgerworm of Mestpier (?)</p>	<p>LC<sub>50</sub>: 1.23 mg/l; 24 h, in solution</p> <p>LC<sub>50</sub>: 0.77 mg/l; 48 h, in solution</p> <p>LC<sub>50</sub>: 0.100 µg/cm<sup>2</sup>; 24 h, direct contact with filter paper</p> <p>LC<sub>50</sub>: 0.034 µg/cm<sup>2</sup>; 48 h, direct contact with filter paper (Imidacloprid &gt;95% pure)</p>	<p>LC<sub>50</sub>: 3.48 mg/kg dry soil; 7 d, in artificial soil</p> <p>LC<sub>50</sub>: 2.30 mg/kg dry soil; 14 d, in artificial soil</p>			<p>Luo et al., 1999 and Zang et al., 2000</p>
<p><i>Eisenia fetida</i>, Common brandling worm or Common dung-worm, Tijgerworm of Mestpier (?)</p>		<p>LC<sub>50</sub>: 25 mg/kg, with significant weight losses at 14 mg/kg (14 days?). No significant effects on microbial decomposition of</p>			<p>Kreutzweiser et al., 2008a</p>

		leaf material at the maximum test concentration of 1400 mg/kg (35 days?)			
<i>Forficula auricularia</i> L., Common earwig or European earwig, Gewone oorwurm	LD <sub>50</sub> : 2.47 µg a.i./cm <sup>2</sup> ; 24 h (Confidor® 350 SC)				Nicholas, 2000
<i>Gammarus fossarum</i> Koch, Stream scud, vlokreeft	LC <sub>50</sub> : 0.8 mg/l (?); 48 h (Confidor SL 200)				Lukančič et al., 2010
<i>Gammarus pulex</i> , crustacean, Brakwater vlokreeft	LC <sub>50</sub> : 270 µg/l; 96 h exposure (Analytical grade powder)				Beketov and Liess, 2008
<i>Gnathonarium exsiccatum</i> , spider, spin	LC <sub>50</sub> : 801 ppm; 48 h, individuals dipped in solution, 1 <sup>st</sup> instars only (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000
<i>Gonatocerus ashmeadi</i> , parasitoid fairyfly wasp, parasitaire wesp	LC <sub>50</sub> : 65.68 ng imidacloprid per cm <sup>2</sup> leaf; 48 h (Study focused on residues; Admire 2F)				Byrne & Toscano, 2007
<i>Haplogonatopus apicalis</i> , Dryinid wasp, parasitaire wesp	LC <sub>50</sub> : 0.12 ppm; 24 h; females dipped in solution (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000

<p><i>Harmonia axyridis</i>, Multicoloured Asian lady beetle, Veelkleurig Aziatisch lieveheersbeestje</p>	<p>LC<sub>50</sub> 1<sup>st</sup> inst.: &lt;8.79 mg a.i./l; 48 h</p> <p>LC<sub>50</sub> 2<sup>nd</sup> inst.: &lt;8.79 mg a.i./l; 48 h</p> <p>LC<sub>50</sub> 3<sup>rd</sup> inst.: 30.3 mg a.i./l; 48 h</p> <p>LC<sub>50</sub> 4<sup>th</sup> inst.: 190.2 mg a.i./l; 48 h</p> <p>LC<sub>50</sub> adults: 364.07 mg a.i./l; 48 h</p> <p>Instars and adults were exposed via topical application, eggs and pupae were dipped in solution for 10 s</p> <p>(Confidor wettable powder)</p>	<p>LC<sub>50</sub> eggs: &lt;8.79 mg a.i./l; 7 d</p> <p>LC<sub>50</sub> pupae: &gt;1000.0 mg a.i./l; 7 d</p>			<p>Youn et al., 2003</p>
<p><i>Hyalella azteca</i>, crustacean, vlokreeft</p>	<p>LC<sub>50</sub> juveniles: 65.43 µg/l; 96 h (99.2% pure imidacloprid)</p> <p>LC<sub>50</sub> juveniles: 17.44µg/l; 96 h (Admire®)</p>	<p>LC<sub>50</sub>: 7.08 µg/l; 28 d, constant exposure (7.01 µg/l after 10 d)</p>	<p>NOEC and values were similar for the two products; 54.24 µg/l for imi, 48.75 µg/l for Admire (96 h). 3.44 µg/l for 28 d constant exposure (Admire); 3.53 µg/l after 10 days. 11.93 µg/l for 96 h pulse exposure (Admire) observed after 10 d, 3.53 µg/l observed after 28 d</p>		<p>Stoughton et al., 2008</p>
<p><i>Ilyocypris dentifera</i>, planktonic crustacean, planktonisch schaaldier</p>	<p>LC<sub>50</sub>: 1122 µg/l; 24 h</p> <p>LC<sub>50</sub>: 517 µg/l; 48 h (Technical grade)</p>			<p>LC<sub>50</sub> was lower under dark than under normal (16 h light : 8 h dark) laboratory conditions:</p>	<p>Sánchez-Bayo &amp; Goka, 2006</p>

	imidacloprid, 99.5% pure)			24 h: 759 µg/l 48 h: 214 µg/l	
<i>Megachile rotundata</i> , Alfalfa leafcutting bee, bij	LC <sub>50</sub> : 0.17 (concentration expressed as percentage of solution (wt:vol) (x10 <sup>-3</sup> )); 48 h, direct contact toxicity, females and males  (Technical grade, >95% purity)				Scott-Dupree et al., 2009
<i>Nannotrigona perilampoides</i> , stingless bee, angelloze bij	LD <sub>50</sub> : 0.0011 µg per bee for foragers (average weight of workers 0.0082 g); topical application, mortality observed for 24 h  (Technical grade)				Valdovinos-Núñez et al., 2009
<i>Orius laevigatus</i> Fieber, predatory pirate bug, roofwants	LC <sub>50</sub> residual contact, 5 <sup>th</sup> instar nymphs: 0.04 mg a.i./l, adults: 0.3 mg a.i./l.  LC <sub>50</sub> ingestion, 5 <sup>th</sup> instar nymphs: 1.1 mg a.i./l, adults: 2.1 mg a.i./l  (Confidor 200 SL)				Delbeke et al., 1997
<i>Oryzias latipes</i> , Medaka fish or Japanese ricefish, Japanese rijstvis	Exposure to Admire GR (1% imidacloprid) at 1.5 times the recommended rate of application on commercial rice fields, had no significant effect on survival/mortality rate over the 4 month test period. However, 40-100% of the fish were infested with the parasite <i>Trichodina</i> , against 40% in the control group				Sánchez-Bayo & Goka, 2005
<i>Osmia lignaria</i> Cresson, Orchard mason bee or Blue orchard bee, bij	LC <sub>50</sub> : 0.07 (concentration expressed as percentage of solution (wt:vol) (x10 <sup>-3</sup> )); 48 h, direct contact toxicity,				Scott-Dupree et al., 2009



	females and males (Technical grade, >95% purity)				
<i>Palaemonetes pugio</i> , Daggerblade grass shrimp, garnaal	LC <sub>50</sub> larvae: 308.8 µg/l; 96 h exposure LC <sub>50</sub> adults: 563.5 µg/l; 96 h exposure (Technical grade, 99.5% pure)		NOEC larvae: 100 µg/l; 96 h exposure	96 h LC <sub>50</sub> for imidacloprid in the presence of atrazine was significantly lower compared to imidacloprid alone. A mixture of fipronil and imidacloprid resulted in significantly lower toxicity compared to each insecticide alone	Key et al., 2007
<i>Pardosa pseudoannulata</i> , thin-legged wolf spider, wolfspin	LC <sub>50</sub> : 440 ppm; 48 h, individuals dipped in solution, 1 <sup>st</sup> instars only (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000
<i>Pheretima</i> spp., Earth worms, regenwormen	LC <sub>50</sub> : 155 mg/kg; 24 h exposure in artificial soil LC <sub>50</sub> : 5 mg/kg; 48 h exposure in artificial soil	LC <sub>50</sub> : 3 mg/kg; 7 d exposure in artificial soil	NOEL 21 d: 350.0 g a.i./l		Mostert et al., 2002 NOEL from Ecotox Database
<i>Phytoseiulus persimilis</i> , predatory mite, roofmijt	LC <sub>50</sub> : 1500, 1800 and 8500 ppm; 24 h after 5 seconds' dipping in solution LC <sub>50</sub> : >10,000 ppm; 72 h direct contact with dipped leaf, females only, obtained from three companies (Admire 240 F)				Lee et al., 2002

<i>Picromerus bidens</i> , predatory bug, schildwants	LC <sub>50</sub> 4 <sup>th</sup> instar: 7.12 mg a.i./l; residual contact, mortality checked every day for four days  (17.7% suspension concentrate)	LC <sub>50</sub> adult female: 9.87 mg a.i./l; residual contact, mortality checked every day for six days  (17.7% suspension concentrate)			Mahdian et al., 2007
<i>Podisus nigrispinus</i> Dallas nymphs, predatory stinkbug, roofkever		LC <sub>50</sub> 2 <sup>nd</sup> instar: 34.40 mg; residual  LC <sub>50</sub> 5 <sup>th</sup> instar: 147.66 mg; residual  LC <sub>50</sub> 2 <sup>nd</sup> instar: 0.13 mg; ingestion  LC <sub>50</sub> 5 <sup>th</sup> instar: 0.44 mg; ingestion  All mortalities summarized after 5 days exposure  (Imidacloprid 700 g/kg as Confidor WDGr)			Torres & Ruberson, 2004
<i>Porcellio scaber</i> , Common rough woodlouse or Common slater, Kelderpissebed			NOEC mortality, juvenile: >50 µg/g dry food NOEL mortality, juv: >1.95 µg/g body weight/day  NOEC mortality, adult: >25 µg/g dry food  NOEL mortality, adult: >0.32 µg/g body weight/day (Imidacloprid 99.8% pure)		Drobne et al., 2008
<i>Pteronarcys dorsata</i> , stonefly, vlieg		LC <sub>50</sub> (14 d, nymphs): 70.1 µg/l (Confidor™ 200SL, 200 g a.i./l)	NOEL 14 d: A 24 µg/l (		Kreutzweiser et al., 2008b  NOEL from Ecotox

					Database
<i>Rana limnocharis</i> , Boie's wart frog or Cricket frog, kikker	<p>LC<sub>50</sub> tadpoles: 235 mg/l; 24 h exposure</p> <p>LC<sub>50</sub> tadpoles: 165 mg/l; 48 h exposure</p> <p>LC<sub>50</sub> tadpoles: 116 mg/l; 72 h exposure</p> <p>LC<sub>50</sub> tadpoles: 82 mg/l; 96 h exposure</p> <p>(Imidacloprid &gt;95% pure)</p>				Feng et al., 2004
<i>Rana nigromaculata</i> Hallowell, Japanese pond frog or Dark-spotted frog, kikker	<p>LC<sub>50</sub> tadpoles: 268 mg/l; 24 h exposure</p> <p>LC<sub>50</sub> tadpoles: 219 mg/l; 48 h exposure</p> <p>LC<sub>50</sub> tadpoles: 177 mg/l; 72 h exposure</p> <p>LC<sub>50</sub> tadpoles: 129 mg/l; 96 h exposure</p> <p>(Imidacloprid &gt;95% pure)</p>				Feng et al., 2004
<i>Simulium latigonium</i> , blackfly, vlieg	<p>LC<sub>50</sub>: 3.73 µg/l; 96 h exposure, larvae</p> <p>(Analytical grade powder)</p>				Beketov and Liess, 2008
<i>Simulium vittatum</i> Zetterstedt cytospecies IS-7, blackfly, vlieg	<p>LC<sub>50</sub>: between 9.54 and 6.75 µg/l; 48 h, larvae</p> <p>(Analytical grade, ≥98% pure)</p>		NOEC: likely 2 to 5 µg/l		Overmyer et al., 2005

<i>Tetragnatha maxillosa</i> , spider, spin	LC <sub>50</sub> : 136 ppm; 48 h, individuals dipped in solution, 1 <sup>st</sup> instars only  (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000
<i>Tipula</i> spp., Craneflies or Daddy long legs, Langpootmuggen		LC <sub>50</sub> : 139.0 µg/l, 14 d, larvae (Confidor™ 200SL, 200 g a.i./l)	NOEL 14 d: A 93 µg/l		Kreutzweiser et al., 2008b  NOEL from Ecotox Database
<i>Ummeliata insecticeps</i> , spider, spin	LC <sub>50</sub> : 995 ppm; 48 h, individuals dipped in solution, 1 <sup>st</sup> instars only  (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000

### Thiacloprid (alphabetical list)

Species	Acute toxicity	Chronic toxicity	NOEC	Synergy effects	Source
<i>Aleochara bilineata</i> , rove beetle, kortschildkever		ER <sub>50</sub> : < 187.5 g a.i./ha; 28 d contact toxicity, quartz sand (SC 480)  LR <sub>50</sub> : > 375.0 g a.i./ha; 28 d contact toxicity, natural soil (SC 480)	NOER: 375 g a.i./ha; 28 d contact toxicity, natural soil (SC 480)		Schmuck, 2001
<i>Anas platyrhynchos</i> , Mallard duck, Wilde			NOED: 4.8 mg a.i./kg b.w. NOEC: 55.2 mg a.i./kg food		Schmuck, 2001

eend			(Technical grade)		
<i>Aphidius rhopalosiphi</i> , parasitoid wasp, parasitaire wesp	LR <sub>50</sub> : 6.5 g a.i./ha; 48 h contact toxicity (SC 480) LR <sub>50</sub> : 6.8 g a.i./ha; 48 h contact toxicity (WG 36)		NOER: 1.0 g a.i./ha  NOER: 1.0 g a.i./ha		Schmuck, 2001
<i>Asellus aquaticus</i> , Water louse, Waterpissebed	LC <sub>50</sub> : >698.5 µg/l; 24 h LC <sub>50</sub> : 299 µg/l; 96 h (Analytical grade powder?)	LC <sub>50</sub> : 153.4 µg/l; 19 d (Analytical grade powder?)			Beketov and Liess, 2007
<i>Baetis rhodani</i> , mayfly, haft of eendagsvlieg	LC <sub>50</sub> : 4.60 µg/l; 96 h exposure, larvae (Analytical grade powder)				Beketov and Liess, 2008
<i>Bombus terrestris</i> , Large earth bumblebee or Buff-tailed bumblebee, Aardhommel		LC <sub>50</sub> (without foraging; time?): 18 ppm EC <sub>50</sub> (without foraging; time?): 12 ppm Experiment duration 11 wk, monitored weekly (Calypso® 48% SC)	1.2 ppm		Mommaerts et al., 2010
<i>Coccinella septempunctata</i> , Seven-spotted ladybird, Zevenstippelig lieveheersbeestje		LR <sub>50</sub> : c 19.2 g a.i./ha; 15 d, contact with apple seedlings, larvae (SC 480) LR <sub>50</sub> : c 24.8 g a.i./ha; 18 d, larvae	NOER: 9.6 g a.i./ha; 15 d contact with apple seedlings, larvae (SC 480) NOER: 9.6 g a.i./ha; 14 d		Schmuck, 2001

		(WG 36) LR <sub>50</sub> : c 14.4-57.6 g a.i./ha; 12 d, adults (SC 480) Contact toxicity (and possibly oral for larvae)	(WG 36) NOER: <4.8 g a.i./ha; 14 d (SC 480)		
<i>Colinus virginianus</i> , Northern bobwhite quail, Noordelijke boomkwartel of Virginische boomkwartel	LD <sub>50</sub> : 2716 mg a.i./kg body weight; acute oral (Technical grade)		NOED: 70.74 mg a.i./kg b.w. NOEC: 467 mg a.i./kg food (Technical grade)		Schmuck, 2001
<i>Coturnix japonica</i> , Japanese quail, Japanse kwartel	LD <sub>50</sub> : 49 mg a.i./kg body weight; acute oral (Technical grade)		NOED: 20.74 mg a.i./kg b.w. NOEC: 157 mg a.i./kg food (Technical grade)		Schmuck, 2001
<i>Culex pipiens</i> , House mosquito, Gewone steekmug	LC <sub>50</sub> : 7.35 µg/l; 24 h, larvae LC <sub>50</sub> : 7.10; 96 h, larvae	LC <sub>50</sub> : 5.76; 7 d, larvae LC <sub>50</sub> : 6.04; 14 d, larvae			Beketov and Liess, 2007
<i>Cyprinodon variegates</i> , Sheepshead minnow, Edelsteentandkarper	LC <sub>50</sub> : 19.7 mg a.i./l; 96 h static (Technical grade)				Schmuck, 2001
<i>Daphnia magna</i> , water flea, watervlo	LC <sub>50</sub> : 7,200 µg/l; 24 h LC <sub>50</sub> : 4,400; 96 h	LC <sub>50</sub> : 4,400; 14 d LC <sub>50</sub> : 4,100; 30 d			Beketov and Liess, 2007
<i>Eisenia fetida</i> , Common brandling worm or Common dung-worm, Tijgerworm of Mestpier		LC <sub>50</sub> : 105 mg a.i./kg dry wt soil; 14 d (Technical grade) LC <sub>50</sub> : 51 mg a.i./kg dry wt	Technical grade: 1 mg ai./kg dry wt soil; 14 d SC 480: 0.32 mg ai./kg dry wt soil; 14 d	The effect of formulated thiacloprid is stronger than that of technical grade imidacloprid; WG 36 > SC	Schmuck, 2001

(?)		soil; 14 d (SC 480) LC <sub>50</sub> : 87 mg a.i./kg dry wt soil; 14 d (WG 36)	WG 36: 0.12 mg ai./kg dry wt soil; 14 d SC 480: <62.5 g ai./ha; 56 d	480	
<i>Gammarus pulex</i> , crustacean, Brakwater vlokreeft	LC <sub>50</sub> : 350 µg/l; 96 h exposure (Analytical grade powder)				Beketov and Liess, 2008
<i>Gammarus pulex</i> , crustacean, Brakwater vlokreeft	LC <sub>50</sub> : >9,520.0 µg/l; 24 h LC <sub>50</sub> : 580 µg/l; 96 h	LC <sub>50</sub> : 190 µg/l; 17 d			Beketov and Liess, 2007
<i>Hyaliodes vitripennis</i> Say, predaceous mirid, roofwants	LC <sub>50</sub> , adults: 0.0003 g a.i./l; 24 h LC <sub>50</sub> , nymphs: 0.0015 g a.i./l; 24 h (Insects and apple leaf sprayed with 480 g/l SC Calypso®)				Bostanian et al., 2005
<i>Lepomis macrochirus</i> , Bluegill, baars	LC <sub>50</sub> : 25.2 mg a.i./l; 96 h static (Technical grade) LC <sub>50</sub> : 38.7 mg a.i./l; 96 h static (SC 480)				Schmuck, 2001
<i>Mysidopsis bahia</i> , mysid shrimp, garnaal	LC <sub>50</sub> : 0.031 mg a.i./l; 96 h flow-through		NOEC: 0.0011 mg a.i./l; 32 d (Technical grade)		Schmuck, 2001

	(Technical grade) LC <sub>50</sub> : 0.050 mg a.i./l; 96 h flow-through (SC 480)				
<i>Nannotrigona perilampoides</i> , stingless bee, angelloze bij	LD <sub>50</sub> : 0.007 µg per bee for foragers (average weight of workers 0.0082 g); topical application, mortality observed for 24 h (Technical grade)				Valdovinos-Núñez et al., 2009
<i>Notidobia ciliaris</i> , caddisfly, schietmot	LC <sub>50</sub> : 7.7 µg/l; 24 h, larvae only  LC <sub>50</sub> : 7 µg/l; 96 h, larvae only	LC <sub>50</sub> : 6.8 µg/l; 15 d, larvae only			Beketov and Liess, 2007
<i>Oncorhynchus mykiss</i> , Rainbow trout, Regenboogforel	LC <sub>50</sub> : 30.5 mg a.i./l; 96 h static (Technical grade)		NOEC: 0.244 mg a.i./l (body length/weight); flow- through, 97 d (Technical grade)		Schmuck, 2001
<i>Pardosa</i> spp., Thin- legged wolf spiders, wolfspinnen		ER <sub>50</sub> : c 375.0 g a.i./ha; 14 d contact toxicity (SC 480)	NOER: < 187.5 g a.i./ha; 14 d contact toxicity (SC 480)		Schmuck, 2001
<i>Pimephales promelas</i> , Fathead minnow, Amerikaanse dikkop- elrits	LC <sub>50</sub> : > 104 mg a.i./l; 96 h static (Technical grade)		NOEC: 0.170 mg a.i./l (max. test concentration); flow- through, 33 d  NOEC: 0.780 mg a.i./l (F <sub>0</sub> male body length/weight); flow-through, 290 d		Schmuck, 2001



			(Technical grade)		
<i>Poecilus cupreus</i> , Ground beetle, loopkever		LR <sub>50</sub> : > 211.2 g a.i./ha; 14 d contact toxicity, quartz sand (SC 480) LR <sub>50</sub> : > 150 g a.i./ha; 21 d contact toxicity, natural soil (SC 480)	NOER: < 98.0 g a.i./ha; 14 d, quartz sand (SC 480) NOER: 150 g a.i./ha; 21 d, natural soil (SC 480)		Schmuck, 2001
<i>Simulium latigonium</i> , blackfly, vlieg	LC <sub>50</sub> : 10.1 µg/l; 24 h, larvae only LC <sub>50</sub> : 7.8 µg/l; 96 h, larvae only	LC <sub>50</sub> : 5.5 µg/l; 11 d, larvae only			Beketov and Liess, 2007
<i>Sympetrum striolatum</i> , Common darter dragonfly, Bruinrode heidelibel	LC <sub>50</sub> : >113.3, µg/l; 24 h, larvae only LC <sub>50</sub> : 47.6 µg/l; 96 h, larvae only	LC <sub>50</sub> : 31.2 µg/l; 11 d, larvae only			Beketov and Liess, 2007
<i>Typhlodromus pyri</i> , predatory mite, Appelroofmijt		LR <sub>50</sub> : c 400.0 g a.i./ha; 14 d contact toxicity (SC 480)	NOER: < 60.0 g a.i./ha; 14 d (SC 480)		Schmuck, 2001

### Clothianidin (alphabetical list)

Species	Acute toxicity	Chronic toxicity	NOEC	Synergy effects	Source
<i>Bombus impatiens</i> , Common eastern bumblebee, hommelt	LC <sub>50</sub> : 0.39 (concentration expressed as percentage of solution (wt:vol) (x10 <sup>-3</sup> )); 48 h direct contact toxicity,				Scott-Dupree et al., 2009

	females only (Technical grade, >95% purity)				
<i>Megachile rotundata</i> , Alfalfa leafcutting bee, bij	LC <sub>50</sub> : 0.08 (concentration expressed as percentage of solution (wt:vol) (x10 <sup>-3</sup> )); 48 h direct contact toxicity, females and males  (Technical grade, >95% purity)				Scott-Dupree et al., 2009
<i>Osmia lignaria</i> Cresson, Orchard mason bee or Blue orchard bee, bij	LC <sub>50</sub> : 0.10 (concentration expressed as percentage of solution (wt:vol) (x10 <sup>-3</sup> )); 48 h, direct contact toxicity, females and males  (Technical grade, >95% purity)				Scott-Dupree et al., 2009

### Thiamethoxam (alphabetical list)

Species	Acute toxicity	Chronic toxicity	NOEC	Synergy effects	Source
<i>Amblyseius cucumeris</i> , predatory mite, roofmijt	LC <sub>50</sub> : 1000 to >1000 ppm; 24 h after 5 seconds' dipping in solution; females only, obtained from three companies  (Actara 240 F)				Lee et al., 2002
<i>Anagrus nilaparvatae</i> Pang et Wang, parasitoid	LC <sub>50</sub> : 0.520 mg a.i./l; determined 8 h after 1				Wang et al., 2008

wasp, parasitaire wesp	hour's exposure (Technical grade, 95.87% a.i., 10 mg a.i./l)				
<i>Anaphes iole</i> Girault, fairyfly, parasitaire wesp		ST <sub>50</sub> : 8.94 d; contact with leaves with field-weathered residues; females only;  (Actara 25 WG, 0.1 kg a.i./ha, applied at field rate)			Williams et al., 2003
<i>Anaphes iole</i> Girault, fairyfly, parasitaire wesp	LC <sub>50</sub> : 1.70 µg/ml; after 48 h direct contact; females only  (Actara 25 WG 0.1 kg a.i./ha)				Williams & Price, 2004
<i>Bombus terrestris</i> , Large earth bumblebee or Buff-tailed bumblebee, Aardhommel		LC <sub>50</sub> (without foraging; time?): 0.12 ppm;  EC <sub>50</sub> (without foraging; time?): 35 ppb  Experiment duration 11 wk, monitored weekly  (Actara® 25% WG)	10 ppb		Mommaerts et al., 2010
<i>Harmonia axyridis</i> , Multicoloured Asian lady beetle, Veelkleurig Aziatisch lieveheersbeestje	LC <sub>50</sub> eggs: 382.31 mg a.i./l LC <sub>50</sub> 1 <sup>st</sup> inst.: 37.01 mg a.i./l LC <sub>50</sub> 2 <sup>nd</sup> inst.: 81.09 mg a.i./l LC <sub>50</sub> 3 <sup>rd</sup> inst.: 124.03 mg a.i./l LC <sub>50</sub> 4 <sup>th</sup> inst.: 249.23 mg a.i./l LC <sub>50</sub> pupae: >2500.0 mg				Youn et al., 2003

	<p>a.i./l</p> <p>LC<sub>50</sub> adults: 150.46 mg a.i./l</p> <p>Instars and adults were exposed via topical application, eggs and pupae were dipped in solution for 10 s</p> <p>(Actara WG)</p>				
<p><i>Hyaliodes vitripennis</i> Say, predaceous mirid, roofwants</p>	<p>LC<sub>50</sub> adults: 0.0005 g a.i./l; 24 h</p> <p>LC<sub>50</sub> nymphs: 0.0143 g a.i./l; 24 h</p> <p>(Insects and apple leaf sprayed with Actara® WG, 250 g/kg)</p>				<p>Bostanian et al., 2005</p>
<p><i>Nannotrigona perilampoides</i>, stingless bee, angelloze bij</p>	<p>LD<sub>50</sub>: 0.004 µg per bee for foragers (average weight of workers 0.0082 g); topical application, mortality observed for 24 h</p> <p>(Technical grade)</p>				<p>Valdovinos-Núñez et al., 2009</p>
<p><i>Phytoseiulus persimilis</i>, predatory mite, roofmijt</p>	<p>LC<sub>50</sub>: &gt;1000 ppm; 24 h after 5 seconds' dipping in solution, and 72 h direct contact with dipped leaf; females only, obtained from three companies</p> <p>(Actara 240 F)</p>				<p>Lee et al., 2002</p>
<p><i>Podisus nigrispinus</i></p>		<p>LC<sub>50</sub> 2<sup>nd</sup> instar: 18.39 mg</p>			<p>Torres &amp; Ruberson,</p>

Dallas nymphs, predatory stink bug, roofkever		(a.i.); residual LC <sub>50</sub> 5 <sup>th</sup> instar: 98.84 mg; residual LC <sub>50</sub> 2 <sup>nd</sup> instar: 0.05 mg; ingestion LC <sub>50</sub> 5 <sup>th</sup> instar: 0.06 mg; ingestion Mortalities summarized after 5 days exposure (Actara WG, 250 g/kg)			2004
<i>Trissolcus nigripedius</i> , parasitoid wasp, parasitaire wesp	LT <sub>50</sub> : 15.5 h; topical application, 0.05% a.i. LT <sub>50</sub> : 20.6 h; residue, 0.8 ml LT <sub>50</sub> : 12.9 h; oral ingestion, 0.0033% a.i.				Lim & Mahmoud, 2008

LC<sub>50</sub> = reported median lethal concentration

EC<sub>50</sub> = effective concentration

ST<sub>50</sub> = LT<sub>50</sub>

NOEC = No Observable Effect Concentration

NOEL = No Observable Effect Level

NOER = No Observable Effect Rate

a.i. = active ingredient

WG = wettable granules

F = flowable

SC = suspension concentrate

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\* N.B. Schmuck's article was not published in a peer-reviewed journal, but in a publication by Bayer, producer of imidacloprid products



## Appendix II – Ordered priority list

### Imidacloprid

#### LC<sub>50</sub> – 96 h direct contact – species in order of vulnerability

**1** *Simulium latigonium*, blackfly, vlieg

LC<sub>50</sub>: 0.00373 mg/l; 96 h exposure, larvae  
(Analytical grade powder)

**2** *Chironomus tentans*, Midge, Watermug

LC<sub>50</sub>: 0.00575 mg/l; 96 h constant exposure, larvae  
(99.2% pure imidacloprid)  
LC<sub>50</sub>: 0.00540 mg/l; 96 h constant exposure, larvae  
(Admire®)

**3** *Hyalella azteca*, crustacean, vlokreeft

LC<sub>50</sub> juveniles: 0.06543 mg/l; 96 h  
(99.2% pure imidacloprid)  
LC<sub>50</sub> juveniles: 0.01744 mg/l; 96 h  
(Admire®)

**4** *Gammarus pulex*, crustacean, Brakwater vlokreeft

LC<sub>50</sub>: 0.270 mg/l; 96 h exposure  
(Analytical grade powder)

**5** *Palaemonetes pugio*, Daggerblade grass shrimp, garnaal

LC<sub>50</sub> larvae: 0.3088 mg/l; 96 h exposure  
LC<sub>50</sub> adults: 0.5635 mg/l; 96 h exposure  
(Technical grade, 99.5% pure)

**6** *Rana limnocharis*, Boie's wart frog or Cricket frog, kikker

LC<sub>50</sub> tadpoles: 82 mg/l; 96 h exposure  
(Imidacloprid >95% pure)

**7** *Rana nigromaculata* Hallowell, Japanese pond frog or Dark-spotted frog, kikker

LC<sub>50</sub> tadpoles: 129 mg/l; 96 h exposure  
(Imidacloprid >95% pure)

**8** *Danio rerio*, Zebrafish, Zebravis

LC<sub>50</sub>: 241 mg/l; 96 h  
(Analytical grade)  
LC<sub>50</sub>: 214 mg/l  
(Concentration of imi. in Confidor SL 200)

#### LC<sub>50</sub> – 48 h direct contact – species in order of vulnerability

**1** *Osmia lignaria* Cresson, Orchard mason bee or Blue orchard bee, bij

LC<sub>50</sub>: 0.07 (concentration expressed as percentage of solution (wt:vol) (x10<sup>-3</sup>)) = 0.0007 mg/l; 48 h, direct contact toxicity, females and males  
(Technical grade, >95% purity)

- 2** *Megachile rotundata*, Alfalfa leafcutting bee, bij  
LC<sub>50</sub>: 0.17 (concentration expressed as percentage of solution (wt:vol) (x10<sup>-3</sup>)) = 0.0017 mg/l; 48 h, direct contact toxicity, females and males  
(Technical grade, >95% purity)
- 3** *Ceriodaphnia dubia* Richard, crustacean, watervlo  
LC<sub>50</sub>: 0.00207 mg/l; 48 h  
(Admire Pro; 42.8% active ingredient)
- 4** *Simulium vittatum* Zetterstedt cytospecies IS-7, blackfly, vlieg  
LC<sub>50</sub>: between 0.00954 and 0.00675 mg/l; 48 h, larvae  
(Analytical grade, ≥98% pure)
- 5** *Baetis rhodani*, mayfly, haft of eendagsvlieg  
LC<sub>50</sub>: 0.00849 mg/l; 48 h exposure; larvae only  
(Analytical grade powder)
- 6** *Bombus impatiens*, Common eastern bumblebee, hommelmel  
LC<sub>50</sub>: 3.22 (concentration expressed as percentage of solution (wt:vol) (x10<sup>-3</sup>)) = 0.0322 mg/l; 48 h direct contact toxicity; females only  
(Technical grade, >95% purity)
- 7** *Cyprretta seurati*, planktonic crustacean, planktonisch schaaldier  
LC<sub>50</sub>: 0.301 mg/l; 48 h  
(Technical grade imidacloprid, 99.5% pure)
- 8** *Cypridopsis vidua*, planktonic crustacean, planktonisch schaaldier  
LC<sub>50</sub>: 0.391 mg/l; 48 h
- 9** *Ilyocypris dentifera*, planktonic crustacean, planktonisch schaaldier  
LC<sub>50</sub>: 0.517 mg/l; 48 h  
(Technical grade imidacloprid, 99.5% pure)
- 10** *Cypridopsis vidua*, planktonic crustacean, planktonisch schaaldier  
LC<sub>50</sub>: 0.715 mg/l; 48 h  
(Technical grade imidacloprid, 99.5% pure)
- 11** *Eisenia fetida*, Common brandling worm or Common dung-worm, Tijgerworm of Mestpier (?)  
LC<sub>50</sub>: 0.77 mg/l; 48 h, in solution  
(Imidacloprid >95% pure)
- 12** *Gammarus fossarum* Koch, Stream scud, vlokreeft  
LC<sub>50</sub>: 0.8 mg/l (?); 48 h  
(Confidor SL 200)
- 13** *Asellus aquaticus* L., Water louse, Waterpissebed  
LC<sub>50</sub>: 8.5 mg/l; 48 h  
(Confidor SL 200)
- 14** *Coleomegilla maculata lengi* Timberlake, Pink spotted ladybird, lieveheersbeestje  
LD<sub>50</sub> 3<sup>rd</sup> instar larvae: 12.8 mg/l; 48 hours exposure to foliage and *Leptinotarsa decemlineata* eggs dipped in solution  
(Admire® 240 water flowable solution)
- 15** *Coleomegilla maculata lengi* Timberlake, Pink spotted ladybird, lieveheersbeestje  
LD<sub>50</sub> adults: 60.8 mg/l; 48 hours exposure to foliage and *Leptinotarsa decemlineata* eggs dipped in solution  
(Admire® 240 water flowable solution)

**16** *Daphnia magna*, water flea, watervlo

LC<sub>50</sub>: 64.873 mg/l; 48 h

**17** *Chydorus sphaericus*, planktonic cladoceran, watervlo

LC<sub>50</sub>: 132.673 mg/l; 48 h

(Technical grade imidacloprid, 99.5% pure)

**18** *Rana limnocharis*, Boie's wart frog or Cricket frog, kikker

LC<sub>50</sub> tadpoles: 165 mg/l; 48 h exposure

(Imidacloprid >95% pure)

**19** *Rana nigromaculata* Hallowell, Japanese pond frog or Dark-spotted frog, kikker

LC<sub>50</sub> tadpoles: 219 mg/l; 48 h exposure

(Imidacloprid >95% pure)

**20** *Artemia* sp., Brine shrimps, Pekelkreeftjes (?)

LC<sub>50</sub>: 361.23 mg/l; 48 h exposure under hyperosmotic conditions: 100% artificial salt water

### **LC<sub>50</sub> – 24 h direct contact – species in order of vulnerability**

**1** *Aphelinus mali*, wasp, sluipwesp

LC<sub>50</sub>: 0.16 ppm; 24 h exposure to leaf dipped in solution for 10 s, not systemic

(Confidor 35 wettable powder)

**2** *Cyprretta seurati*, planktonic crustacean, planktonisch schaaldier

LC<sub>50</sub>: 0.732 mg/l; 24 h

(Technical grade imidacloprid, 99.5% pure)

**3** *Ilyocypris dentifera*, planktonic crustacean, planktonisch schaaldier

LC<sub>50</sub>: 1.122 mg/l; 24 h

(Technical grade imidacloprid, 99.5% pure)

**4** *Eisenia fetida*, Common brandling worm or Common dung-worm, Tijgerworm of Mestpier (?)

LC<sub>50</sub>: 1.23 mg/l; 24 h, in solution

(Imidacloprid >95% pure)

**5** *Diadegma insulare*, parasitoid wasp, sluipwesp

LC<sub>50</sub>: 2 mg a.i./l; 24 h exp. to dipped leaves

(Provado 1.6 F)

**6** *Cypridopsis vidua*, planktonic crustacean, planktonisch schaaldier

LC<sub>50</sub>: 3.951 mg/l; 24 h

**7** *Cypridopsis vidua*, planktonic crustacean, planktonisch schaaldier

LC<sub>50</sub>: >4.0 mg/l; 24 h

(Technical grade imidacloprid, 99.5% pure)

**8** *Chydorus sphaericus*, planktonic cladoceran, watervlo

LC<sub>50</sub>: 161.950 mg/l; 24 h

(Technical grade imidacloprid, 99.5% pure)

**9** *Rana limnocharis*, Boie's wart frog or Cricket frog, kikker

LC<sub>50</sub> tadpoles: 235 mg/l; 24 h exposure

(Imidacloprid >95% pure)

**10** *Rana nigromaculata* Hallowell, Japanese pond frog or Dark-spotted frog, kikker  
LC<sub>50</sub> tadpoles: 268 mg/l; 24 h exposure  
(Imidacloprid >95% pure)

### **LC<sub>50</sub> – 48 h after topical contact – species in order of vulnerability**

**1** *Harmonia axyridis*, Multicoloured Asian lady beetle, Veelkleurig Aziatisch lieveheersbeestje

LC<sub>50</sub> 1<sup>st</sup> inst.: <8.79 mg a.i./l; 48 h

LC<sub>50</sub> 2<sup>nd</sup> inst.: <8.79 mg a.i./l; 48 h

LC<sub>50</sub> 3<sup>rd</sup> inst.: 30.3 mg a.i./l; 48 h

Instars and adults were exposed via topical application, eggs and pupae were dipped in solution for 10 s  
(Confidor wettable powder)

**2** *Tetragnatha maxillosa*, spider, spin

LC<sub>50</sub>: 136 ppm; 48 h after topical application (dipping in solution); 1<sup>st</sup> instars  
(Imidacloprid 10% wettable powder, 1% granules)

**3** *Harmonia axyridis*, Multicoloured Asian lady beetle, Veelkleurig Aziatisch lieveheersbeestje

LC<sub>50</sub> 4<sup>th</sup> inst.: 190.2 mg a.i./l; 48 h

LC<sub>50</sub> adults: 364.07 mg a.i./l; 48 h

Instars and adults were exposed via topical application, eggs and pupae were dipped in solution for 10 s  
(Confidor wettable powder)

**4** *Pardosa pseudoannulata*, thin-legged wolf spider, wolfspin

LC<sub>50</sub>: 440 ppm; 48 h after topical application (dipping in solution); 1<sup>st</sup> instars  
(Imidacloprid 10% wettable powder, 1% granules)

**5** *Gnathonarium exsiccatum*, spider, spin

LC<sub>50</sub>: 801 ppm; 48 h after topical application (dipping in solution); 1<sup>st</sup> instars  
(Imidacloprid 10% wettable powder, 1% granules)

**6** *Ummeliata insecticeps*, spider, spin

LC<sub>50</sub>: 995 ppm; 48 h after topical application (dipping in solution); 1<sup>st</sup> instars  
(Imidacloprid 10% wettable powder, 1% granules)

### **LC<sub>50</sub> – 24 h after topical contact – species in order of vulnerability**

**1** *Haplogonatopus apicalis*, Dryinid wasp, sluipwesp

LC<sub>50</sub>: 0.12 ppm; 24 h after topical application (dipping in solution); females  
(Imidacloprid 10% wettable powder, 1% granules)

**2** *Cyrtorhinus lividipennis*, mirid bug, wants

LC<sub>50</sub>: 0.36 ppm; 24 h after topical application (dipping in solution); females  
(Imidacloprid 10% wettable powder, 1% granules)

**3** *Phytoseiulus persimilis*, predatory mite, roofmijt

LC<sub>50</sub>: 1500, 1800 and 8500 ppm; 24 h after 5 seconds' dipping in solution  
(Admire 240 F)

**4** *Amblyseius cucumeris*, predatory mite, roofmijt

LC<sub>50</sub>: 10,000 to >10,000 ppm; determined 24 h after 5 seconds' dipping in solution; females only, obtained from three companies  
(Admire 240 F)

## Thiacloprid

### LC<sub>50</sub> – 96 h exposure – species in order of vulnerability

- 1** *Baetis rhodani*, mayfly, haft of eendagsvlieg  
LC<sub>50</sub>: 0.00460 mg/l; 96 h exposure, larvae  
(Analytical grade powder)
- 2** *Notidobia ciliaris*, caddisfly, schietmot  
LC<sub>50</sub>: 0.007 mg/l; 96 h, larvae only
- 3** *Culex pipiens*, House mosquito, Gewone steekmug  
LC<sub>50</sub>: 0.00710 mg/l; 96 h, larvae
- 4** *Simulium latigonium*, blackfly, vlieg  
LC<sub>50</sub>: 0.0078 mg/l; 96 h, larvae only
- 5** *Mysidopsis bahia*, mysid shrimp, garnaal  
LC<sub>50</sub>: 0.031 mg a.i./l; 96 h flow-through  
(Technical grade)
- 6** *Sympetrum striolatum*, Common darter dragonfly, Bruinrode heidelibel  
LC<sub>50</sub>: 0.0476 mg/l; 96 h, larvae only
- 7** *Mysidopsis bahia*, mysid shrimp, garnaal  
LC<sub>50</sub>: 0.050 mg a.i./l; 96 h flow-through  
(SC 480)
- 8** *Asellus aquaticus*, Water louse, Waterpissebed  
LC<sub>50</sub>: 0.299 mg/l; 96 h  
(Analytical grade powder?)
- 9** *Gammarus pulex*, crustacean, Brakwater vlokreeft  
LC<sub>50</sub>: 0.350 mg/l; 96 h exposure  
(Analytical grade powder)
- 10** *Gammarus pulex*, crustacean, Brakwater vlokreeft  
LC<sub>50</sub>: 0.580 mg/l; 96 h
- 11** *Cyprinodon variegates*, Sheepshead minnow, Edelsteentandkarper  
LC<sub>50</sub>: 19.7 mg a.i./l; 96 h static  
(Technical grade)
- 12** *Oncorhynchus mykiss*, Rainbow trout, Regenboogforel  
LC<sub>50</sub>: 30.5 mg a.i./l; 96 h static  
(Technical grade)
- 13** *Lepomis macrochirus*, Bluegill, baars  
LC<sub>50</sub>: 38.7 mg a.i./l; 96 h static  
(SC 480)  
LC<sub>50</sub>: 25.2 mg a.i./l; 96 h static  
(Technical grade)
- 14** *Pimephales promelas*, Fathead minnow, Amerikaanse dikkop-elrits  
LC<sub>50</sub>: > 104 mg a.i./l; 96 h static  
(Technical grade)

**15** *Daphnia magna*, water flea, watervlo  
LC<sub>50</sub>: 4.400 mg/l; 96 h

### **LC<sub>50</sub> – 24 h exposure – species in order of vulnerability**

**1** *Culex pipiens*, House mosquito, Gewone steekmug  
LC<sub>50</sub>: 0.00735 mg/l; 24 h, larvae

**2** *Notidobia ciliaris*, caddisfly, schietmot  
LC<sub>50</sub>: 0.0077 mg/l; 24 h, larvae only

**3** *Simulium latigonium*, blackfly, vlieg  
LC<sub>50</sub>: 0.0101 mg/l; 24 h, larvae only

**4** *Sympetrum striolatum*, Common darter dragonfly, Bruinrode heidelibel  
LC<sub>50</sub>: >0.1133, mg/l; 24 h, larvae only

**5** *Hyaliodes vitripennis* Say, predaceous mirid, roofwants  
LC<sub>50</sub>, adults: 0.3 mg a.i./l; 24 h  
(Insects and apple leaf sprayed with 480 g/l SC Calypso®)

**6** *Asellus aquaticus*, Water louse, Waterpissebed  
LC<sub>50</sub>: >0.6985 mg/l; 24 h  
(Analytical grade powder?)

**7** *Hyaliodes vitripennis* Say, predaceous mirid, roofwants  
LC<sub>50</sub>, nymphs: 1.5 mg a.i./l; 24 h  
(Insects and apple leaf sprayed with 480 g/l SC Calypso®)

**8** *Daphnia magna*, water flea, watervlo  
LC<sub>50</sub>: 7.200 mg/l; 24 h

**9** *Gammarus pulex*, crustacean, Brakwater vlokreeft  
LC<sub>50</sub>: >9.520 mg/l; 24 h

### **Clothianidin**

#### **LC<sub>50</sub> - 48 h direct contact – species in order of vulnerability**

**1** *Megachile rotundata*, Alfalfa leafcutting bee, bij  
LC<sub>50</sub>: 0.08 (concentration expressed as percentage of solution (wt:vol) (x10<sup>-3</sup>)) = 0.0008 mg/l; females and males

**2** *Osmia lignaria* Cresson, Orchard mason bee or Blue orchard bee, bij  
LC<sub>50</sub>: 0.10 (concentration expressed as percentage of solution (wt:vol) (x10<sup>-3</sup>)) = 0.001 mg/l; females and males

**3** *Bombus impatiens*, Common eastern bumblebee, hommelm  
LC<sub>50</sub>: 0.39 (concentration expressed as percentage of solution (wt:vol) (x10<sup>-3</sup>)) = 0.0039 mg/l; females only

## Appendix III – Classification by number of times the MTR

### Diptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-13 ng/l	32.3	31	31.4	59	25.4	182
13-65 ng/l	30.8	47	19.4	283	15.9	786
65-325 ng/l	25.1	74	18.0	132	14.3	359
325-1625 ng/l	11.2	36	8.4	52	9.8	158
1625-8125 ng/l	8.7	22	9.8	32	6.3	72
> 8125 ng/l					1.8	10

### Ephemeroptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-13 ng/l	19.3	3	12.9	7	14.3	17
13-65 ng/l	3.4	5	45.3	34	30.4	100
65-325 ng/l	3.4	8	3.4	18	7.8	51
325-1625 ng/l			112.3	3	159.3	16
1625-8125 ng/l	166	2	124.7	3	182.6	10
> 8125 ng/l					15.5	2

### Trichoptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-13 ng/l			6	4	3.1	15
13-65 ng/l	6	5	10.9	41	7.0	118
65-325 ng/l	15	4	13.2	15	5.4	47
325-1625 ng/l	2	1	2	1	6.1	16
1625-8125 ng/l	24	1	8.8	4	10.8	16

### Hydracarina, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-13 ng/l	7.1	15	6.6	19	4.8	56
13-65 ng/l	10.6	38	6.8	127	7.0	326
65-325 ng/l	6.9	72	8.1	115	8.9	210
325-1625 ng/l	11.8	38	17.0	57	20.1	133
1625-8125 ng/l	15.1	36	14.2	44	23.4	105
> 8125 ng/l	80.3	3	80.3	3	30.1	12

### Coleoptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-13 ng/l	1.9	9	1.9	22	2.6	46
13-65 ng/l	1.7	18	3.3	74	3.6	214
65-325 ng/l	1.5	28	1.8	43	2.2	107
325-1625 ng/l	2.9	11	3.4	37	3.3	97
1625-8125 ng/l	2.5	4	2.3	6	2.5	33
> 8125 ng/l					1	4

### Heteroptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-13 ng/l	14.5	11	13.3	25	14.5	55
13-65 ng/l	11.2	16	17.4	88	15.8	262
65-325 ng/l	3.8	16	16.6	25	10.9	109
325-1625 ng/l	1	6	7.4	17	19.0	53
1625-8125 ng/l			16.3	4	6.1	28

### Amphipoda, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-13 ng/l	841.3	10	620.8	14	396.3	29
13-65 ng/l	29.8	12	46.5	35	41.7	85
65-325 ng/l	144.0	25	110.7	34	77.5	55
325-1625 ng/l	17.4	7	15.6	8	28	22
1625-8125 ng/l			50	1	58	6

### Isopoda, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-13 ng/l	64.3	9	48	13	115.6	23
13-65 ng/l	21.2	9	19.8	32	25.2	93
65-325 ng/l	692.8	19	507.1	26	243.3	56
325-1625 ng/l	21.5	4	16	6	15.4	13
1625-8125 ng/l					17.3	4
> 8125 ng/l					1	1

### Odonata, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-13 ng/l	9.3	3	13.6	9	9.8	21
13-65 ng/l	5.6	7	11.9	26	11.5	72
65-325 ng/l	3	7	5.2	10	8.4	34
325-1625 ng/l	3	2	3.3	3	7.5	15
1625-8125 ng/l	1	1	15.5	2	21.5	6
> 8125 ng/l					1	1



## Appendix IV – Two groups of equal size

### Diptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>	
0-40 ng/l	34.3	70	22.8	292	19.6	788	
> 40 ng/l	17.9	140	14.4	266	11.4	779	
Significance						$p = 0.00131$	

### Ephemeroptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>	
0-55 ng/l	9.4	8	45.9	34	32.4	93	
> 55 ng/l	35.9	10	27.1	31	49.2	103	
Significance						$p = 0.44332$	

### Trichoptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>	
0-40 ng/l	6.8	4	11.2	40	6.2	105	
> 40 ng/l	12.7	7	10.2	25	7.0	107	
Significance						$p = 0.81263$	

### Hydracarina, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>	
0-80 ng/l	10.0	61	6.9	161	7.1	427	
> 80 ng/l	11.5	141	12.9	204	16.6	415	
Significance						$p = 1 \cdot 10^{-5}$	

### Coleoptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>	
0-60 ng/l	1.7	27	3.0	96	3.4	260	
> 60 ng/l	2.0	43	2.5	86	2.7	241	
Significance						$p = 0.1026$	

### Heteroptera, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>	
0-30 ng/l	14.2	21	18.0	101	16.4	255	
> 30 ng/l	3.9	28	11.2	58	12.3	252	
Significance						$p = 0.41198$	

**Amphipoda, d=160-0 dl=0**

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-54 ng/l	503.6	17	240.6	42	146.8	98
> 54 ng/l	106.3	37	83.0	50	59.4	99
Significance	$p = 0.20218$					

**Isopoda, d=160-0 dl=0**

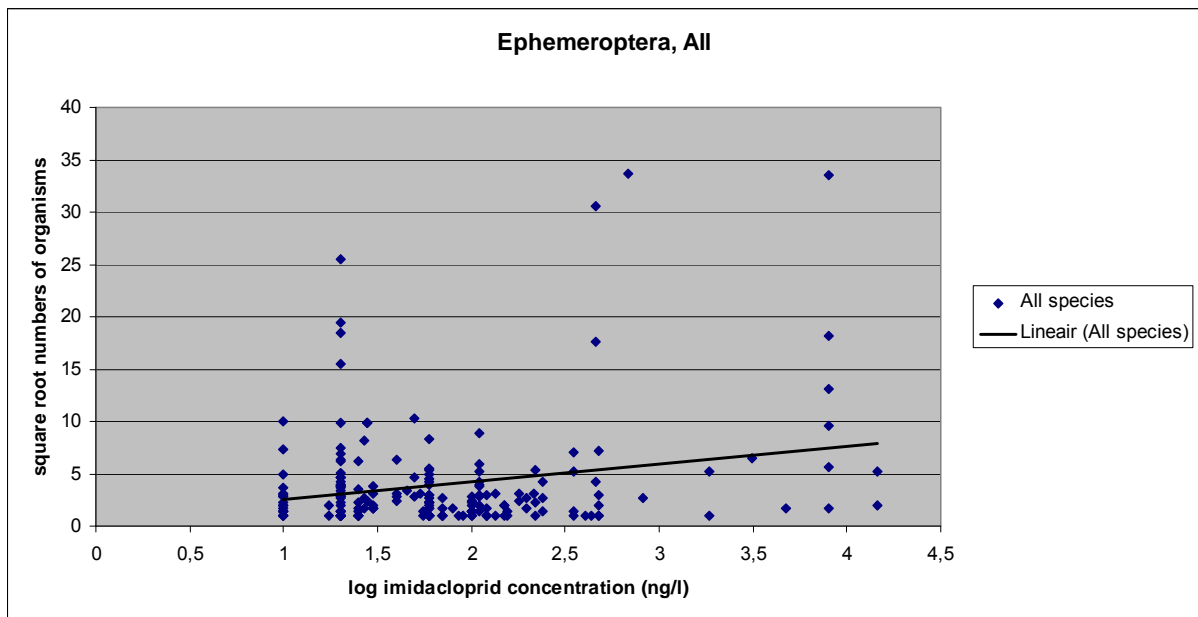
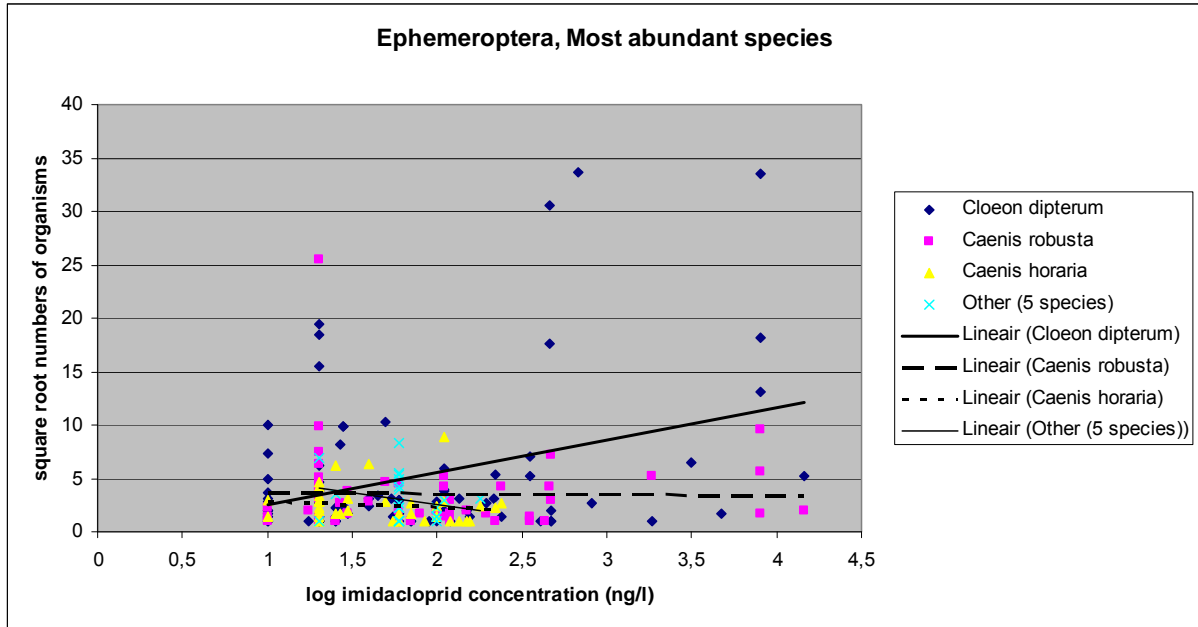
Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-55 ng/l	46.4	15	31.1	36	48.4	96
> 55 ng/l	512.5	26	327.2	41	151.6	94
Significance	$p = 0.49540$					

**Odonata, d=160-0 dl=0**

Category	0 km radius av. abundance	0 km radius <i>n</i>	1 km radius av. abundance	1 km radius <i>n</i>	2 km radius av. abundance	2 km radius <i>n</i>
0-30 ng/l	6.7	10	13.0	33	12.1	77
> 30 ng/l	2.8	10	5.6	17	8.8	72
Significance	$p = 0.2279$					

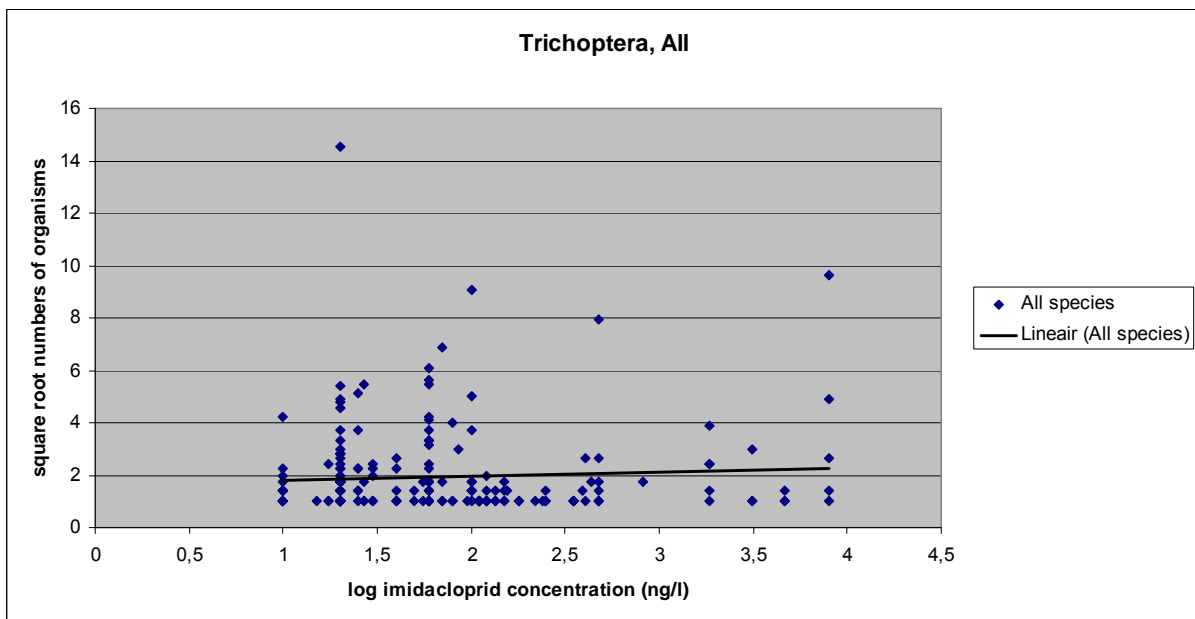
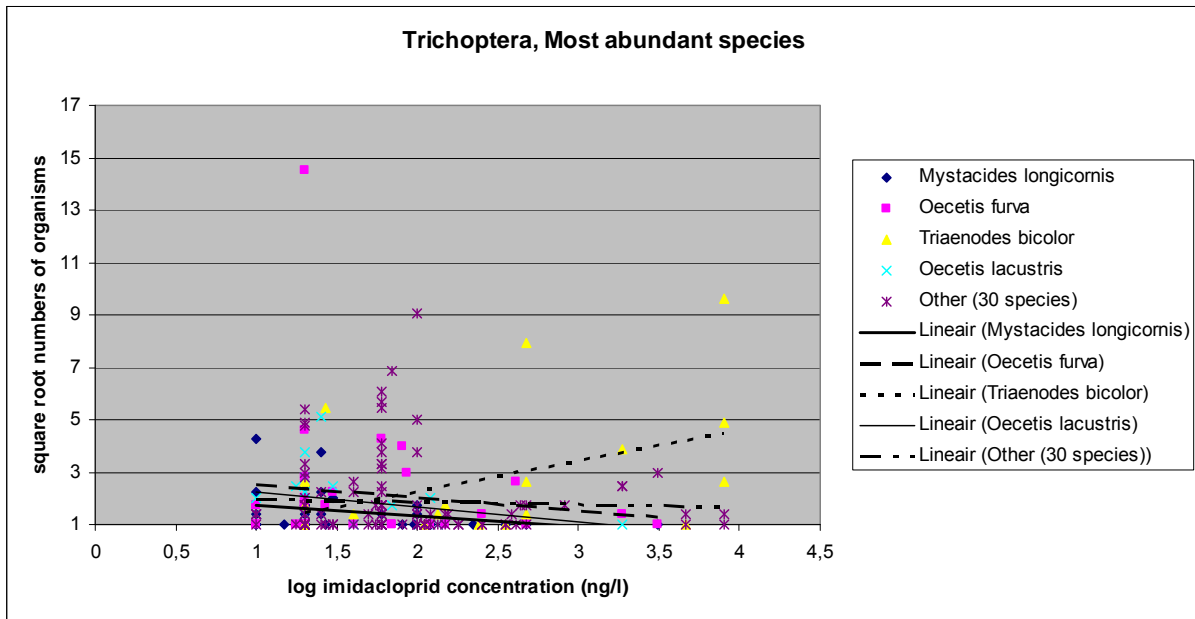
## Appendix V – Scatter plots

### Ephemeroptera



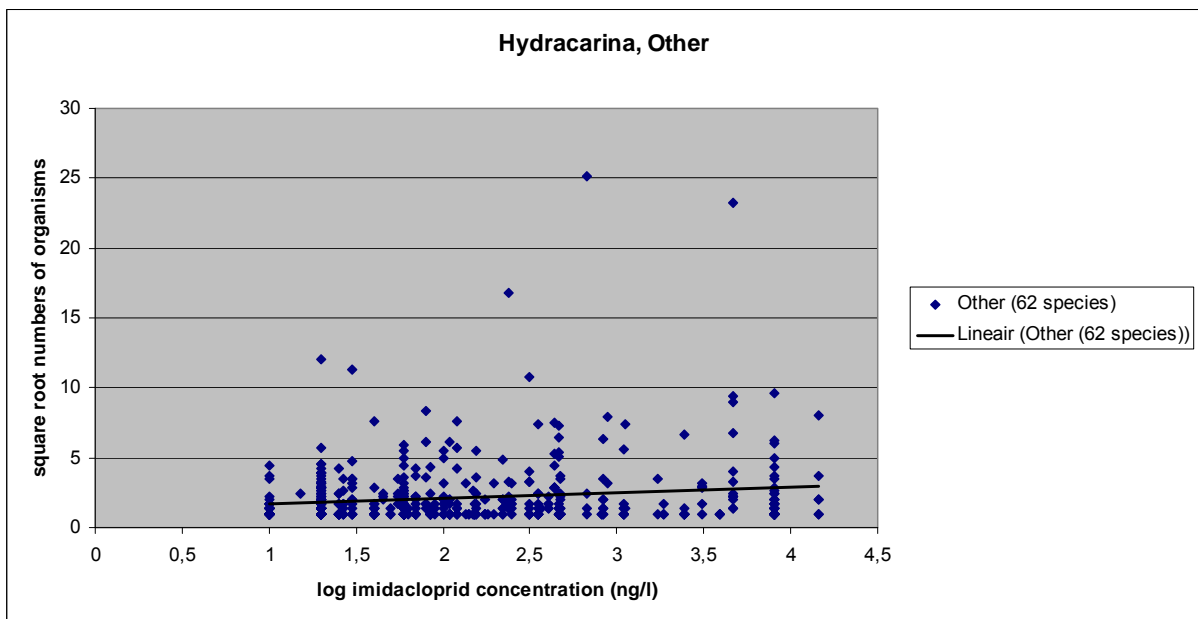
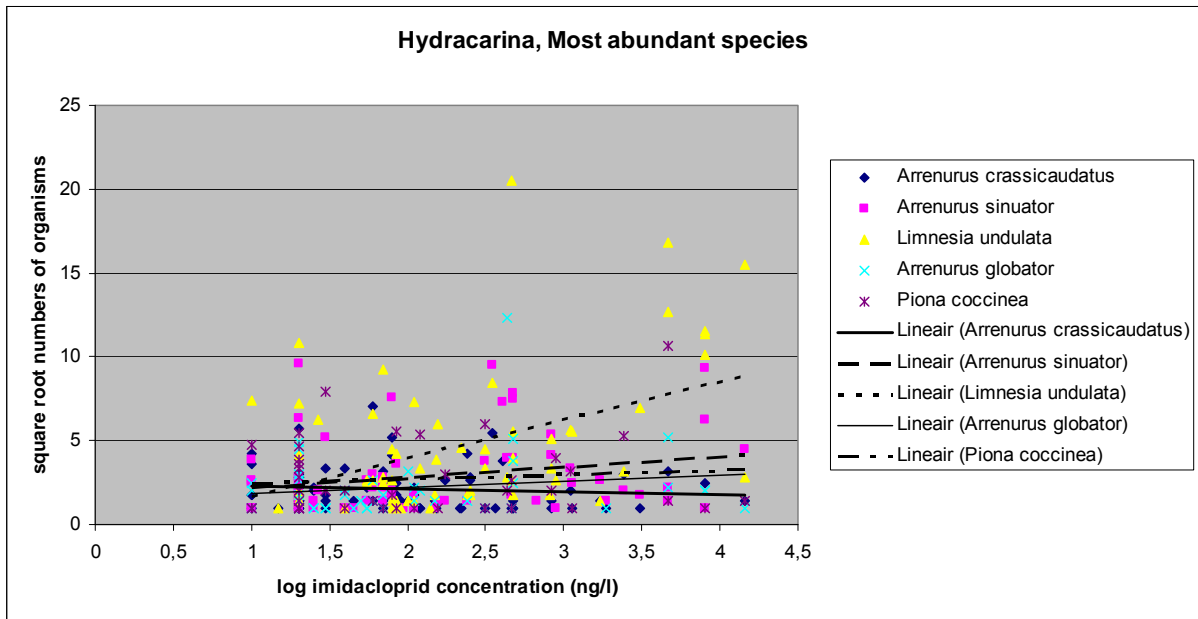
Ephemeroptera			
Species	<i>C. dipterum</i>	<i>C. robusta</i>	<i>C. horaria</i>
Regression	$r = 0.34176$	$r = -0.01519$	$r = -0.12311$
Significance	$p$ (uncorr) = 0.00168	$p$ (uncorr) = 0.91491	$p$ (uncorr) = 0.45526
Species	Other (5 species)	All	
Regression	$r = -0.23093$	$r = 0.22903$	
Significance	$p$ (uncorr) = 0.28907	$p$ (uncorr) = 0.00124	

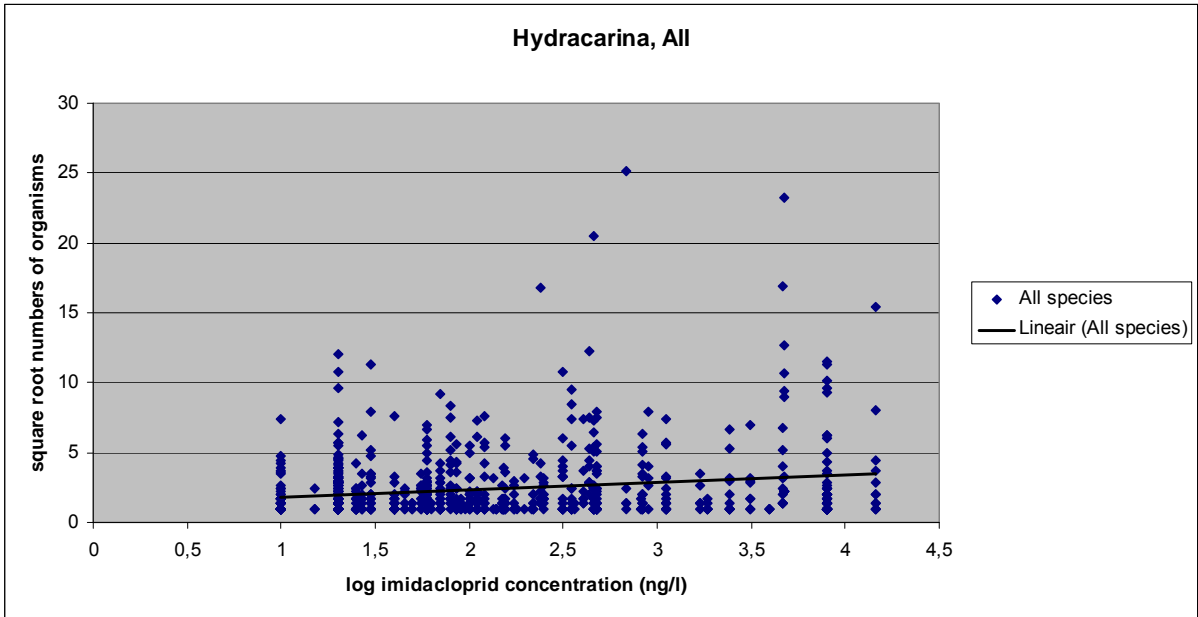
## Trichoptera



Trichoptera			
Species	<i>M. longicornis</i>	<i>O. furva</i>	<i>T. bicolor</i>
Regression	$r = -0.3126$	$r = -0.12221$	$r = 0.45666$
Significance	$p \text{ (uncorr)} = 0.08152$	$p \text{ (uncorr)} = 0.52001$	$p \text{ (uncorr)} = 0.03744$
Species	<i>O. lacustris</i>	Other (30 species)	All
Regression	$r = -0.27616$	$r = -0.039507$	$r = 0.059028$
Significance	$p \text{ (uncorr)} = 0.23856$	$p \text{ (uncorr)} = 0.68337$	$p \text{ (uncorr)} = 0.39248$

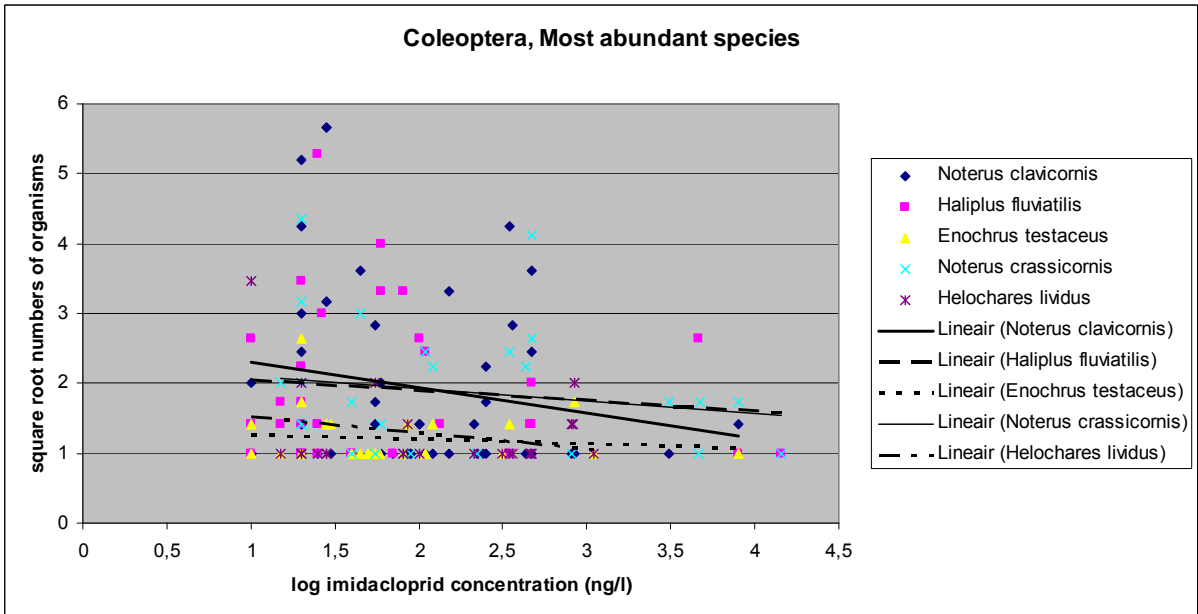
## Hydracarina

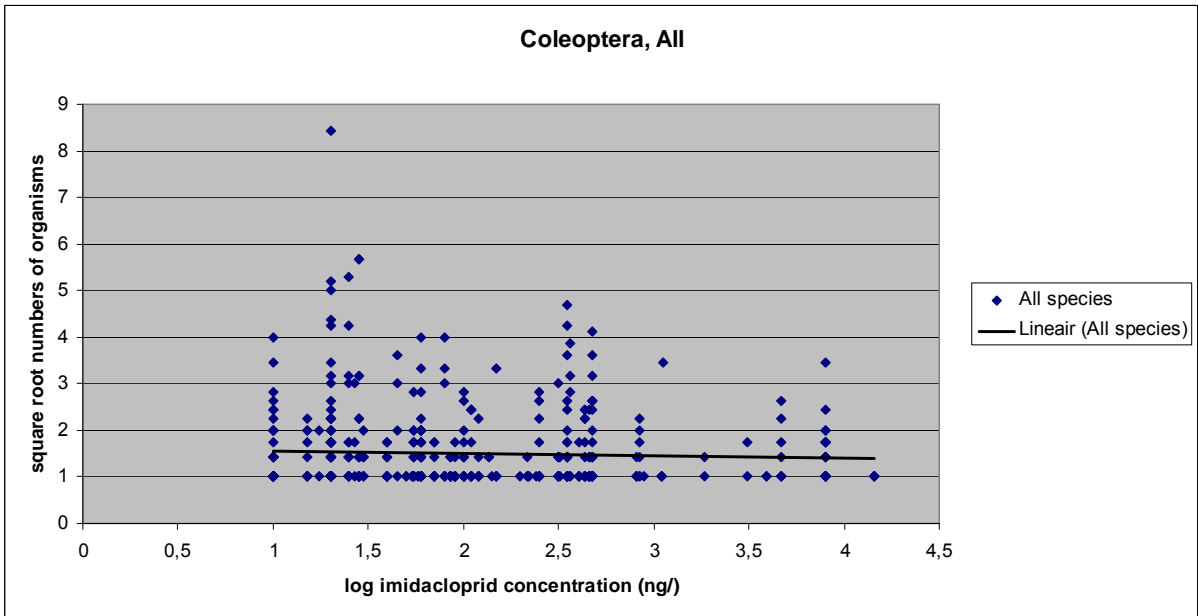
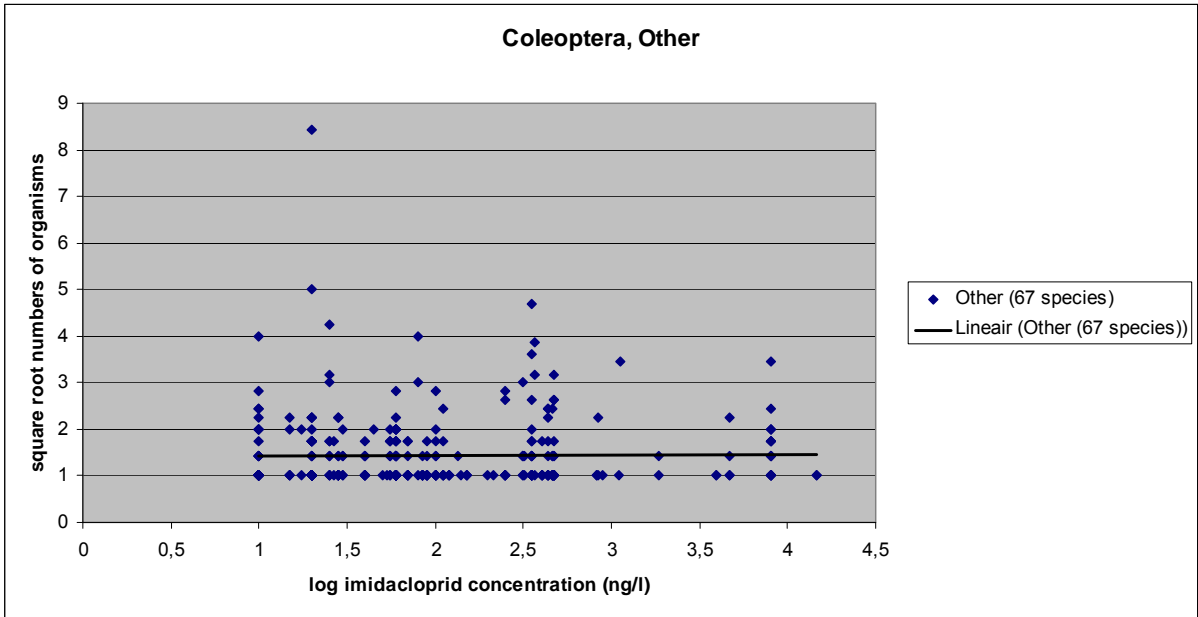




Hydracarina				
Species	<i>A. crassicaudatus</i>	<i>A. sinuator</i>	<i>L. undulata</i>	<i>A. globator</i>
Regression	$r = -0.09980$	$r = 0.23492$	$r = 0.43865$	$r = 0.15638$
Significance	$p$ (uncorr) = 0.39105	$p$ (uncorr) = 0.05028	$p$ (uncorr) = 0.00032	$p$ (uncorr) = 0.30498
Species	<i>P. coccinea</i>	Other (62 species)	All	
Regression	$r = 0.11101$	$r = 0.1515$	$r = 0.1891$	
Significance	$p$ (uncorr) = 0.47852	$p$ (uncorr) = 0.00039	$p$ (uncorr) = $3.22 \cdot 10^{-8}$	

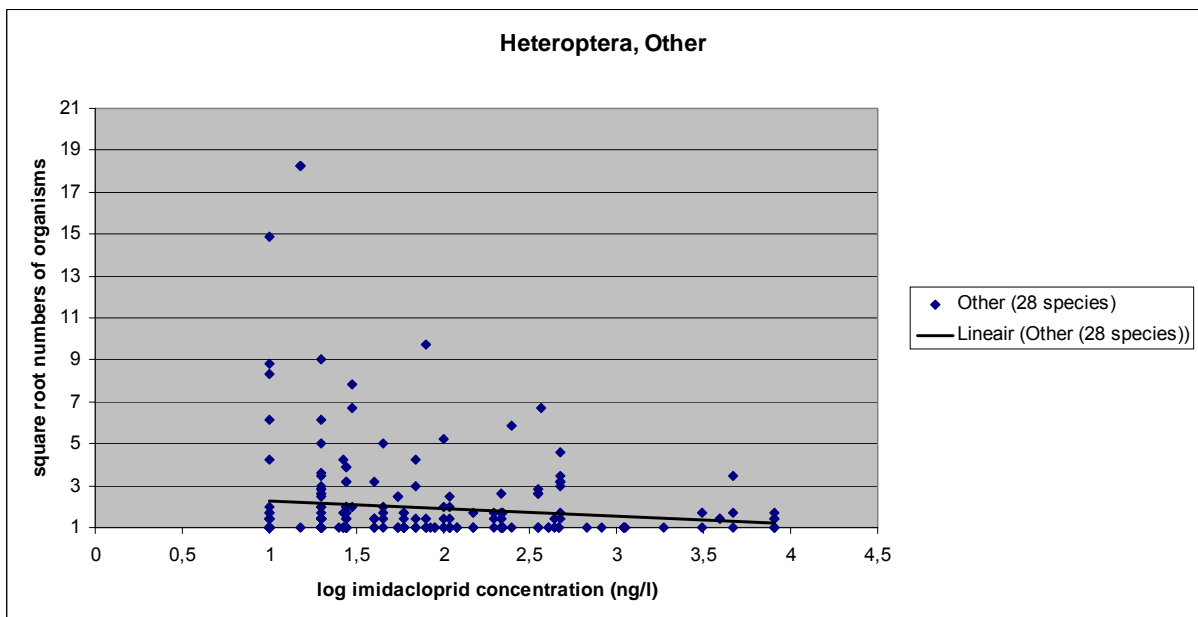
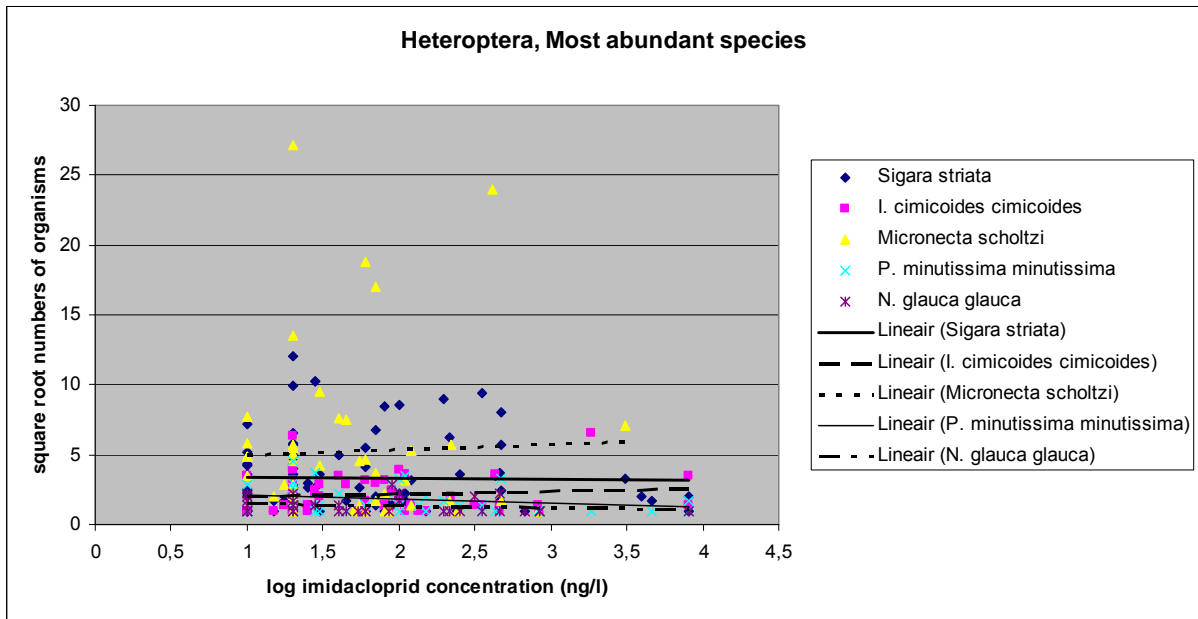
## Coleoptera



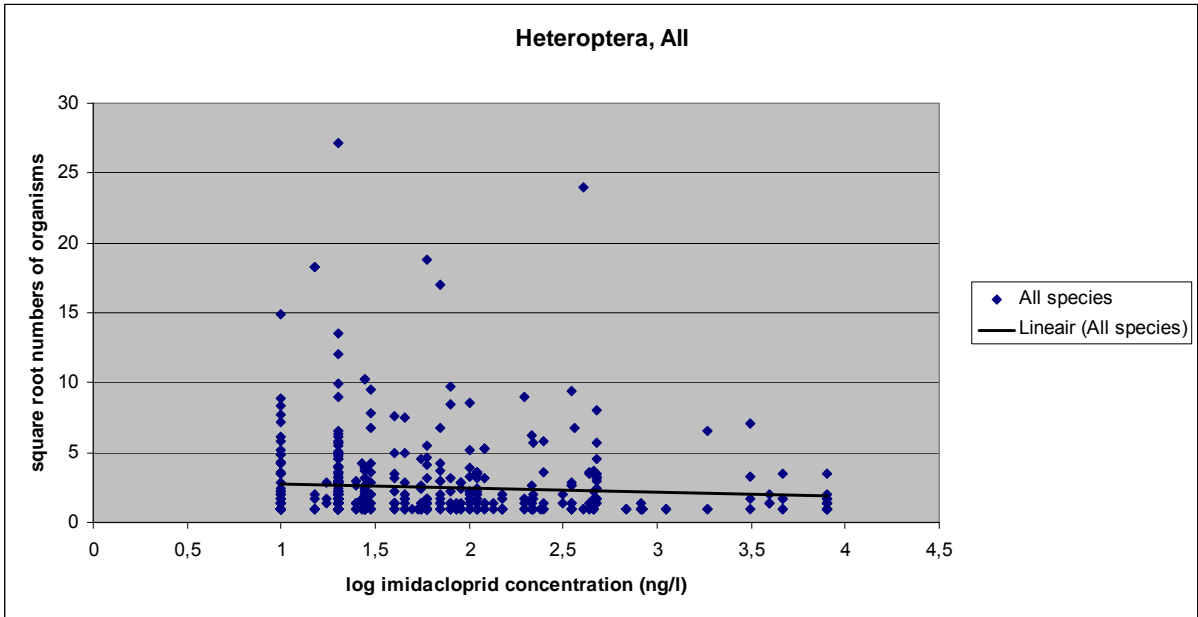


Coleoptera				
<b>Species</b>	<i>N. clavicornis</i>	<i>H. fluviatilis</i>	<i>E. testaceus</i>	<i>N. crassicornis</i>
Regression	$r = -0.18148$	$r = -0.11729$	$r = -0.11127$	$r = -0.16467$
Significance	$p$ (uncorr) = 0.20719	$p$ (uncorr) = 0.51566	$p$ (uncorr) = 0.55124	$p$ (uncorr) = 0.41178
<b>Species</b>	<i>H. lividus</i>	Other (67 species)	All	
Regression	$r = -0.25136$	$r = 0.014434$	$r = -0.042746$	
Significance	$p$ (uncorr) = 0.2361	$p$ (uncorr) = 0.79208	$p$ (uncorr) = 0.33966	

## Heteroptera

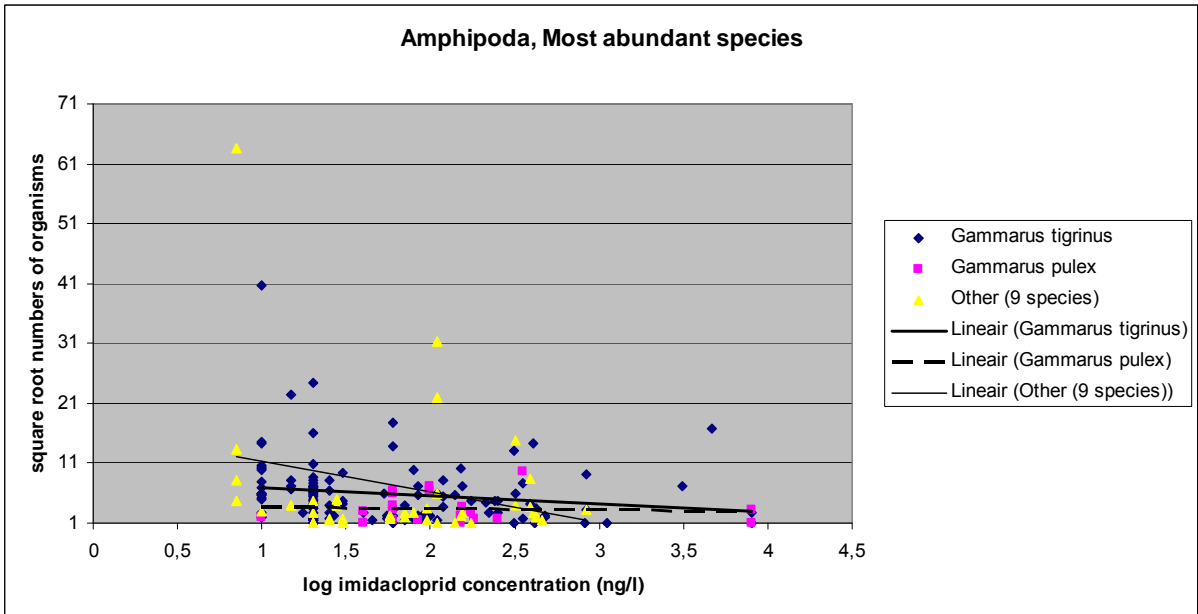


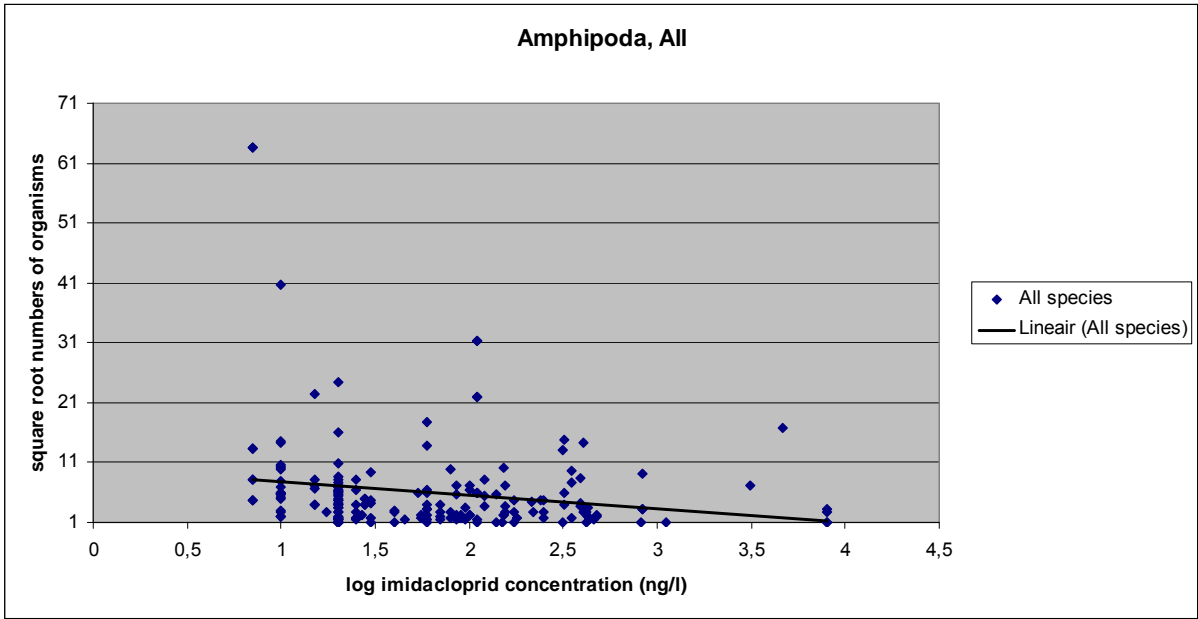




Heteroptera				
<b>Species</b>	<i>S. striata</i>	<i>I. cimicoides cim.</i>	<i>M. scholtzi</i>	<i>P. minutissima min.</i>
Regression	$r = -0.018234$	$r = 0.10923$	$r = 0.035921$	$r = -0.23284$
Significance	$p$ (uncorr) = 0.86397	$p$ (uncorr) = 0.39806	$p$ (uncorr) = 0.80643	$p$ (uncorr) = 0.15373
<b>Species</b>	<i>N. glauca glauca</i>	Other (28 species)	All	
Regression	$r = -0.21728$	$r = -0.11092$	$r = -0.071829$	
Significance	$p$ (uncorr) = 0.20992	$p$ (uncorr) = 0.09259	$p$ (uncorr) = 0.10622	

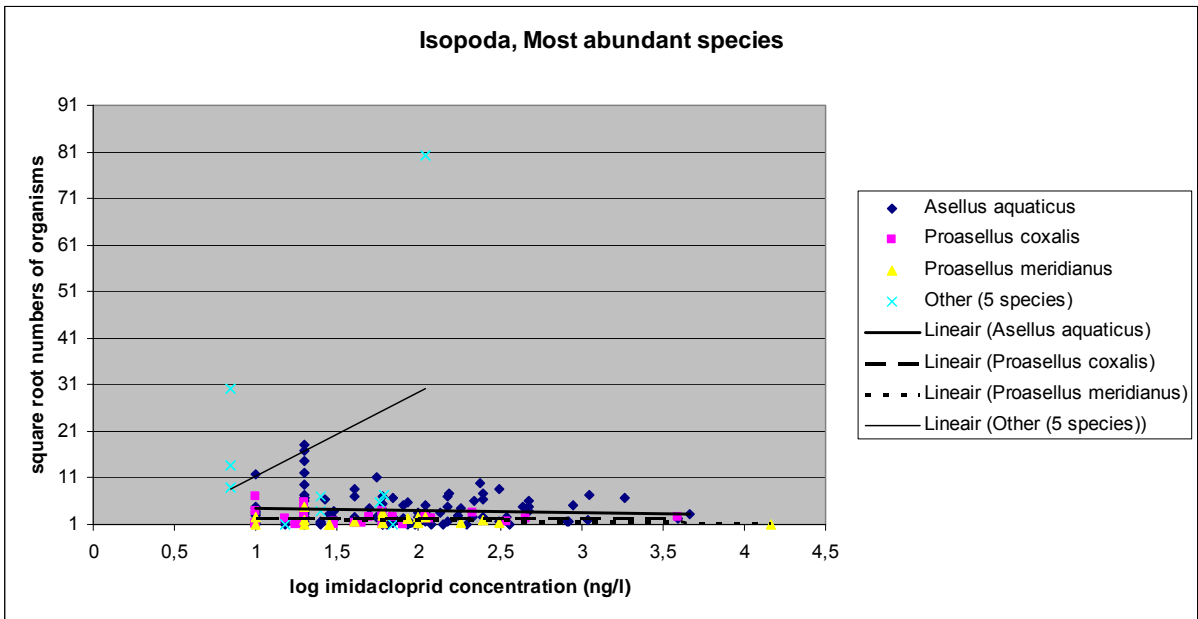
### Amphipoda

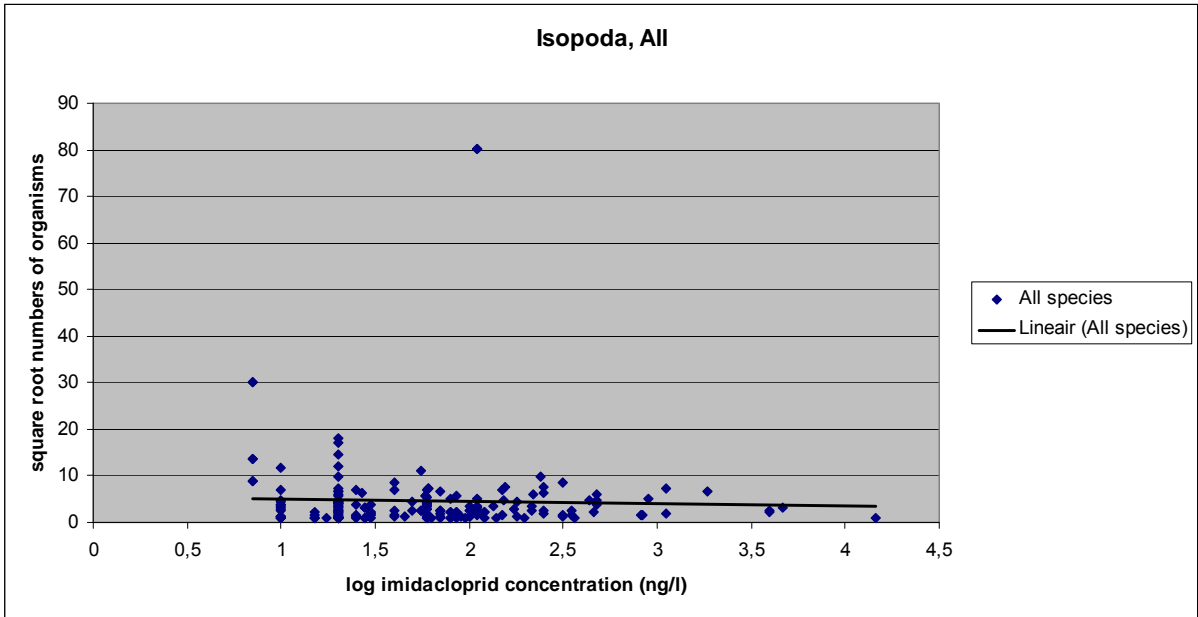




Amphipoda				
Species	<i>G. tigrinus</i>	<i>G. pulex</i>	Other (9 species)	All
Regression	$r = -0.16554$	$r = -0.053635$	$r = -0.23453$	$r = -0.18314$
Significance	$p \text{ (uncorr)} = 0.06842$	$p \text{ (uncorr)} = 0.8223$	$p \text{ (uncorr)} = 0.08480$	$p \text{ (uncorr)} = 0.00100$

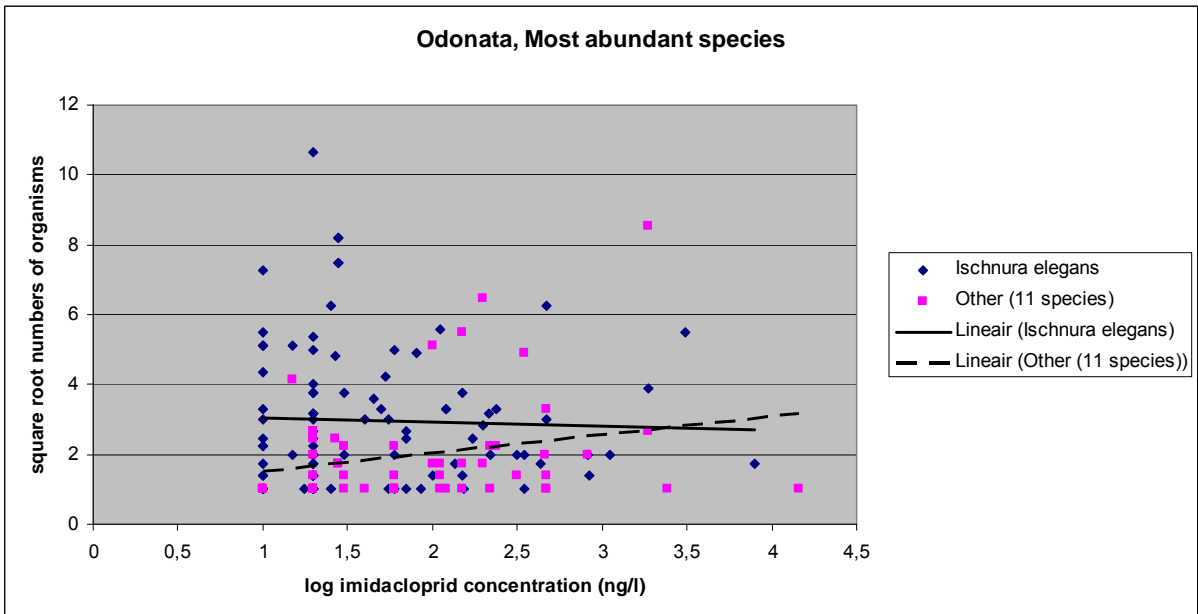
### Isopoda

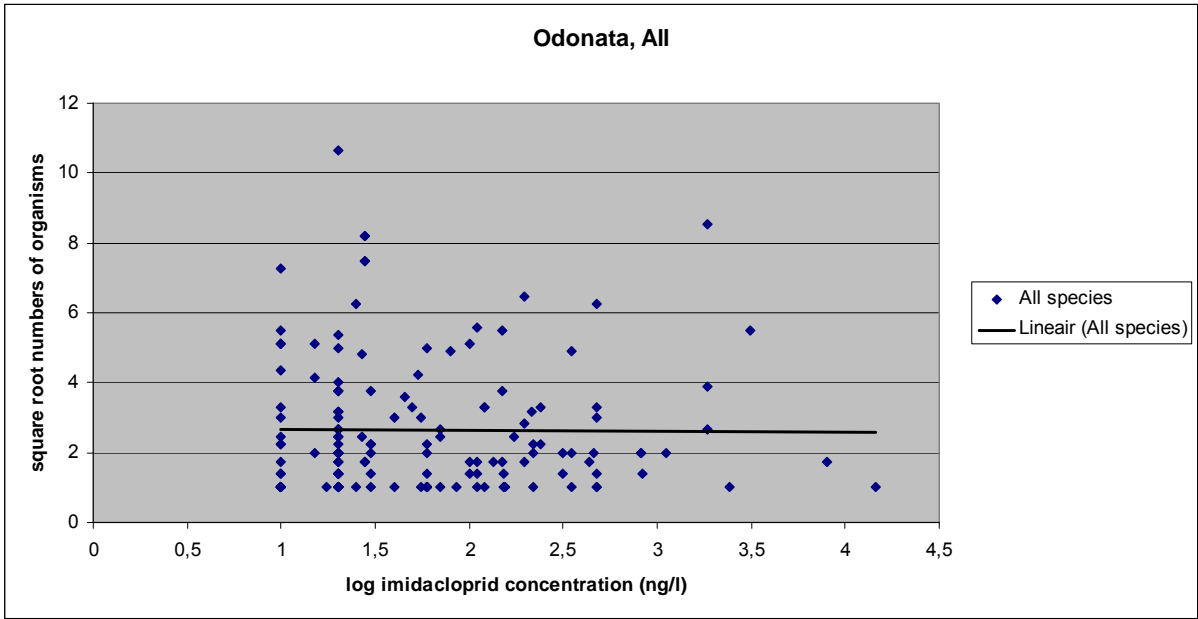




Isopoda			
Species	<i>A. aquaticus</i>	<i>P. coxalis</i>	<i>P. meridianus</i>
Regression	$r = -0.078004$	$r = -0.031175$	$r = -0.21133$
Significance	$p$ (uncorr) = 0.41578	$p$ (uncorr) = 0.84657	$p$ (uncorr) = 0.35779
Species	Other (5 species)	All	
Regression	$r = 0.34309$	$r = -0.035866$	
Significance	$p$ (uncorr) = 0.17759	$p$ (uncorr) = 0.62323	

### Odonata





Odonata			
Species	<i>I. elegans</i>	Other (11 species)	All
Regression	$r = -0.037403$	$r = 0.22731$	$r = -0.010467$
Significance	$p$ (uncorr) = 0.7175	$p$ (uncorr) = 0.10166	$p$ (uncorr) = 0.89918