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®, the active substance of which is imidacloprid, was toxic for honeybees (Bonmatin et al., 2005; Maxim & Van der Sluijs, 2009), and several studies have provided supporting evidence for this link (e.g. CST, 2003; Yang et al., 2008).
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 had 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. 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, 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 paralysing 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).

As was described above, starvation is a sublethal effect of neonicotinoids at lower concentrations. Other sublethal 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, sublethal 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 navigation in 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 it that only a small part of the pesticide doses used reaches its intended target, while the major part 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: 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 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) 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 were 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 hydroxylization 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}C-tests, only 0.1% of all recovered ^{14}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 CO_2 (Scholz & Spiteller, 1992). Metabolites which have been observed in plant cells do not tend to accumulate in soil; ^{14}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 CO_2 generally occurred – in a ^{14}C-test using light sandy soil, approximately 40% of the total radioactivity of the parent compound was given off as 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). 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 tonnes 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 1,245,000 tonnes, 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 as much as 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 2007 (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 it. 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.

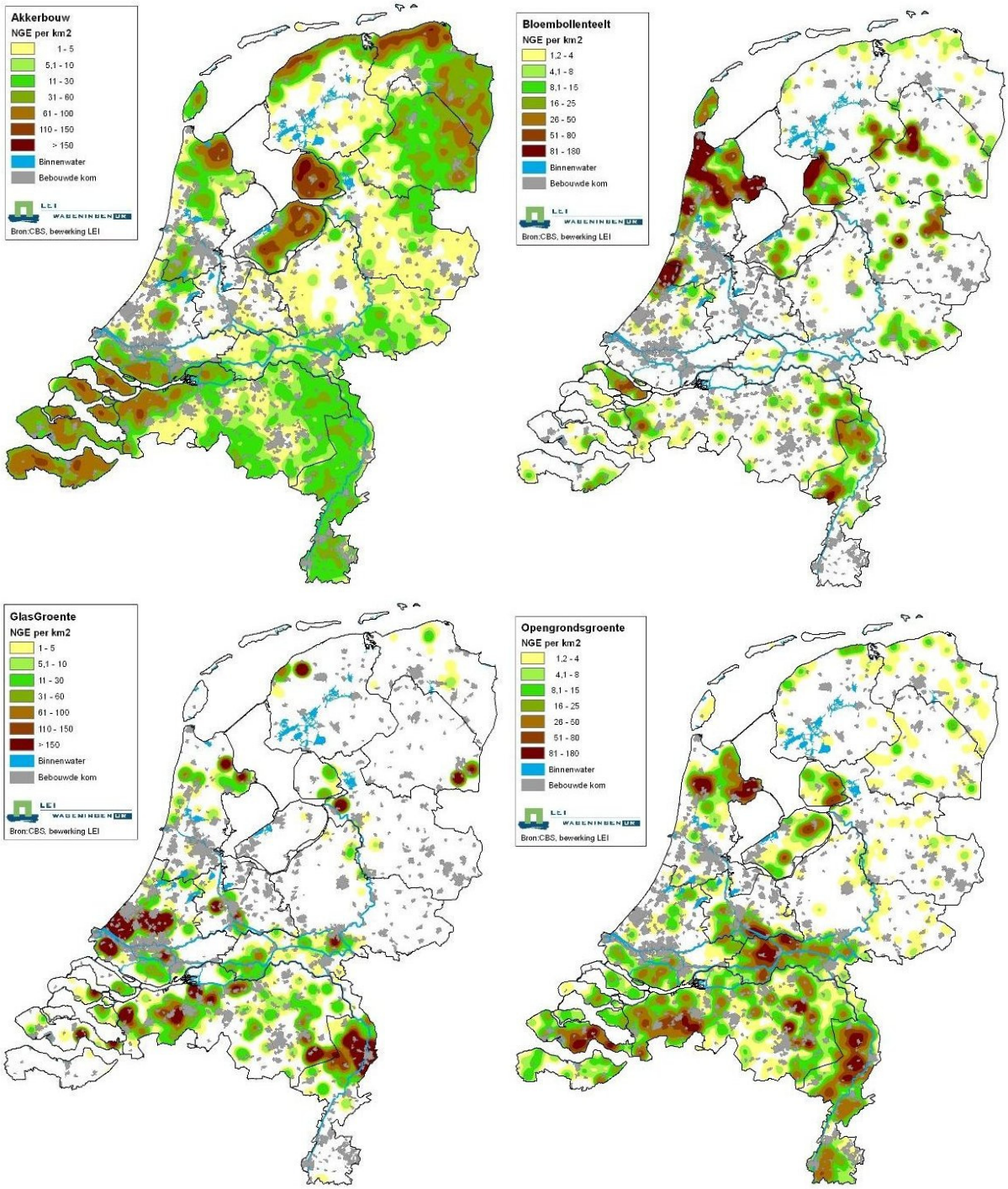


Figure 1. Maps showing where arable farming, bulb growing, cultivation of vegetables in greenhouses and open-air cultivation of vegetables are concentrated in the Netherlands. 100 NGE = € 140,000 in production value. Source: LEI Wageningen University

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 provinces with a lot of arable farming. Friesland is the only province where most concentrations measured were 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.

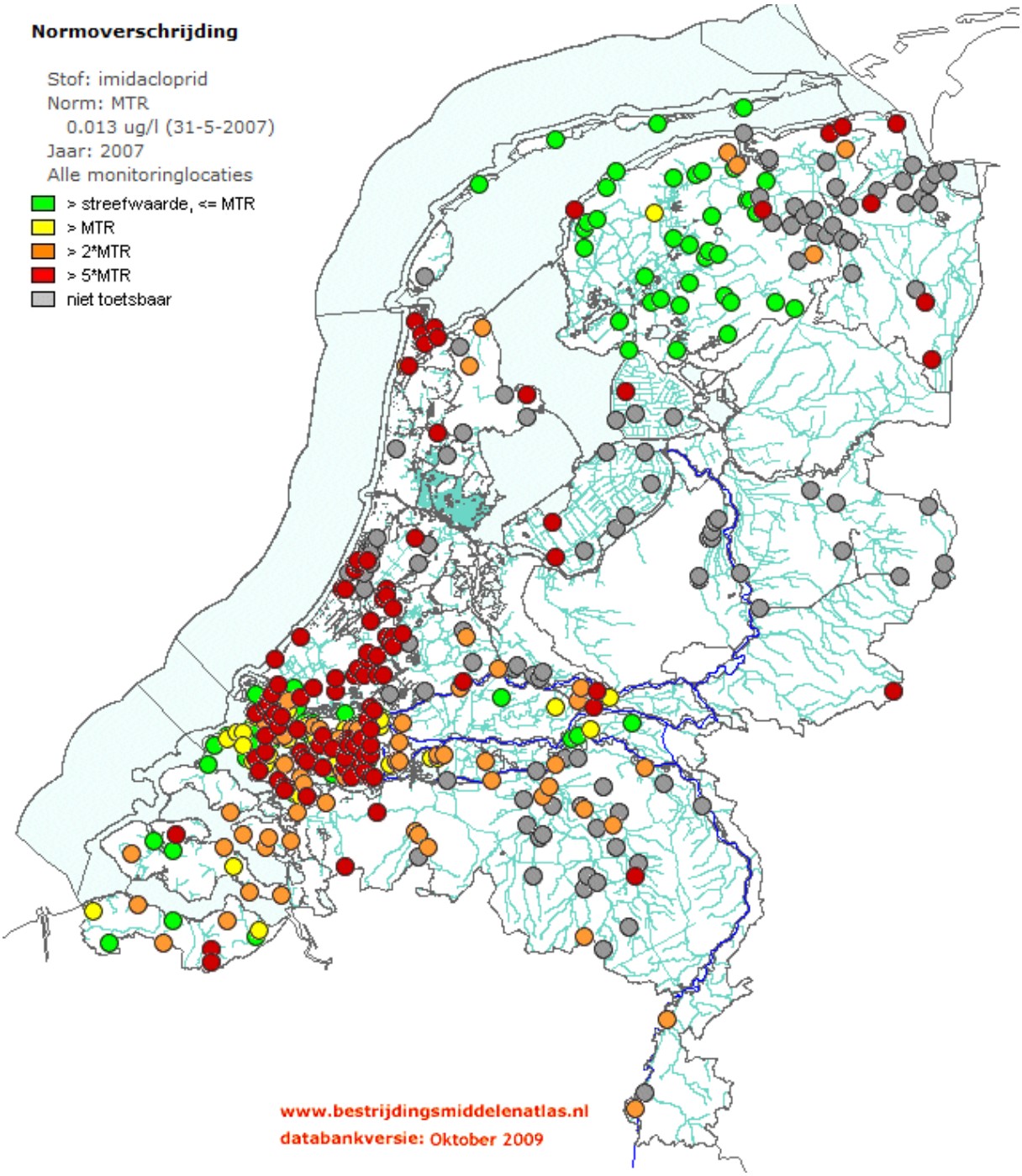


Figure 2. Map of the locations at which surface water concentrations of imidacloprid were measured, and where the MTR norm was exceeded.

In 2007, 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 2007, it was 25 ng/l for thiacloprid, 1 µg/l for thiamethoxam and 14 µg/l for clothianidin (Bestrijdingsmiddelenatlas, 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 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; in this case, concentrations of neonicotinoids in surface waters are likely to be higher than if crops 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 neonicotinoid 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) ($x10^{-3}$)" 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, and thus 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.

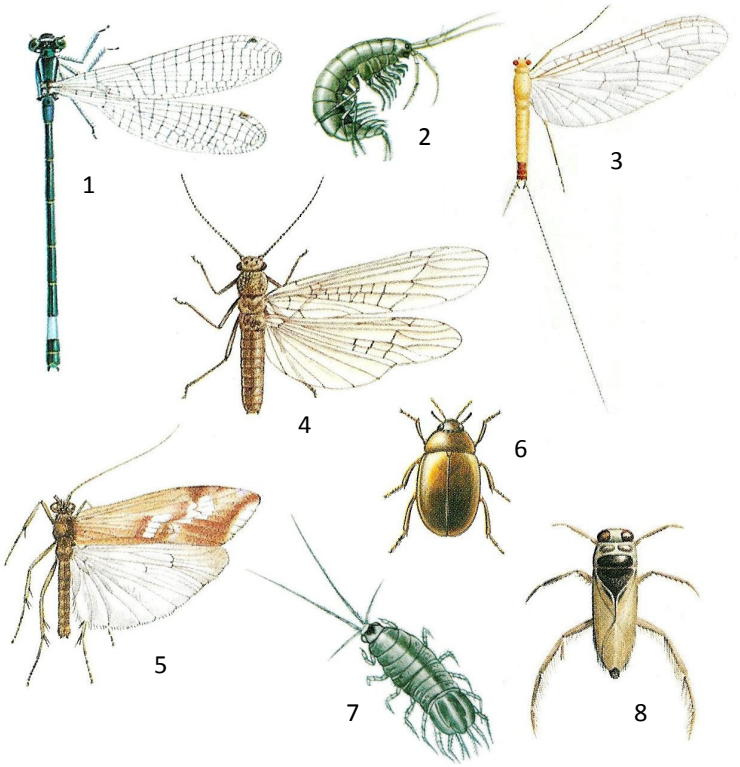


Figure 3. An example of an abundant species for eight of the orders (not to scale). 1 *Ischnura elegans* (Odonata), 2 *Gammarus pulex* (Amphipoda), 3 *Cloeon dipterum* (Ephemeroptera), 4 *Nemoura cinerea* (Plecoptera), 5 *Limnephilus lunatus* (Trichoptera), 6 *Enochrus testaceus* (Coleoptera), 7 *Asellus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

The three 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) and Trichoptera (caddisflies). Data on thirteen other orders were available as well; based on the number of data available in the database, seven of these were also selected for analysis: Plecoptera (stoneflies) 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 or mainly 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, 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 to that during application, was not possible.
The distribution of the locations at which imidacloprid concentrations were measured is shown in Figure 2.

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, while it does make sure that the most nearby concentration measurements available are always used. This is useful because a concentration at a closer location is a better measure of (or proxy for) local bioavailability of imidacloprid than one measured at a more distant location.

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 1, on the next page. 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. Setting the detection limit to a higher value than 0 would introduce unnecessary uncertainty in the analysis; therefore *dl*=0 was used as default for further analysis of the data.

	imi concentration	*dl*=0	*dl*>0		
MTR	(ng/l)	*n*	*n*	*n*	*n*
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 *n*	1,465	3,387		

Table 1. 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)

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 for 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; the norm has since been raised 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 (Bestrijdings-middelenatlas, 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 sub samples 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 square roots of the abundance data were plotted against the logarithm of the average concentrations for imi2 (seek radius 2 km). These transformations were applied to make up for the data distribution, in which there are many low values and a few very high ones. Linear trend lines 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 2 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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
Diptera						
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
Amphipoda						
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 2. 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 2, 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 sub sample sizes *n*, the data were divided again, but more evenly, 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 3 shows the results for Diptera and Amphipoda. The PAST software package for statistical analysis (http://folk.uio.no/ohammer/past) was used to perform a Permutation *t* test with *N*=100,000 permutations, to test the null hypothesis that the means in both sub samples 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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
Diptera						
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					$p = 0.00131$	
Amphipoda						
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					$p = 0.20218$	

Table 3. 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 poisson 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 4.

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

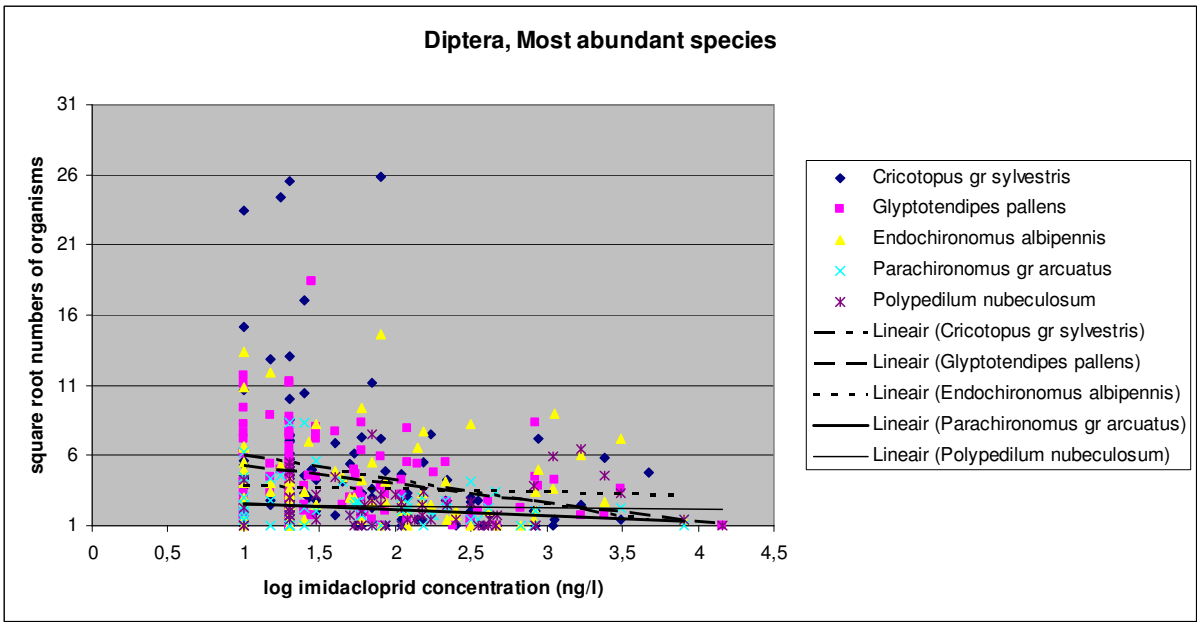
Table 4. The significance of the difference between the two groups for a 2 km radius, after root transformation. *d*=160-0 *dl*=0

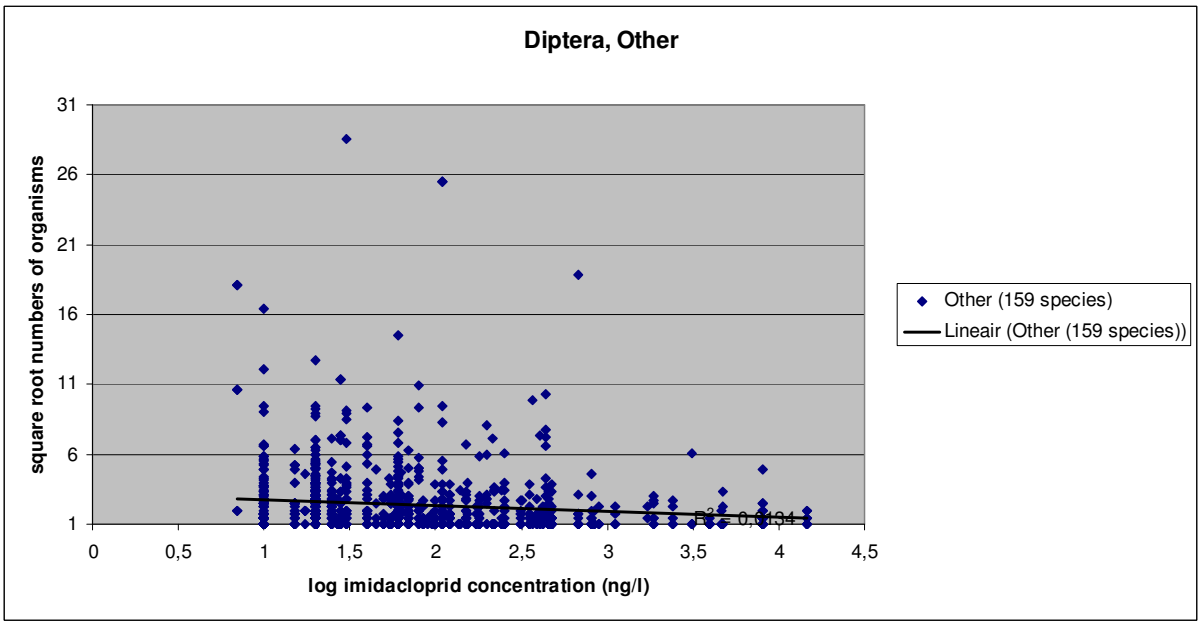
According to these results, the only orders for which there is a significant difference between the abundance at lower imidacloprid concentrations, and those at higher concentrations, at a 95% confidence level, are Diptera and Hydracarina. In the case of Diptera, this is a strong indication that species 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 trend lines 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.


Diptera, Most abundant species
square root numbers of organisms
31
26
21
16
11
6
1
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
log imidacloprid concentration (ng/l)
Cricotopus gr sylvestris
Glyptotendipes pallens
Endochironomus albipennis
Parachironomus gr arcuatus
Polypedilum nubeculosum
Lineair (Cricotopus gr sylvestris)
Lineair (Glyptotendipes pallens)
Lineair (Endochironomus albipennis)
Lineair (Parachironomus gr arcuatus)
Lineair (Polypedilum nubeculosum)


Diptera, Other
square root numbers of organisms
31
26
21
16
11
6
1
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
log imidacloprid concentration (ng/l)
Other (159 species)
Lineair (Other (159 species))

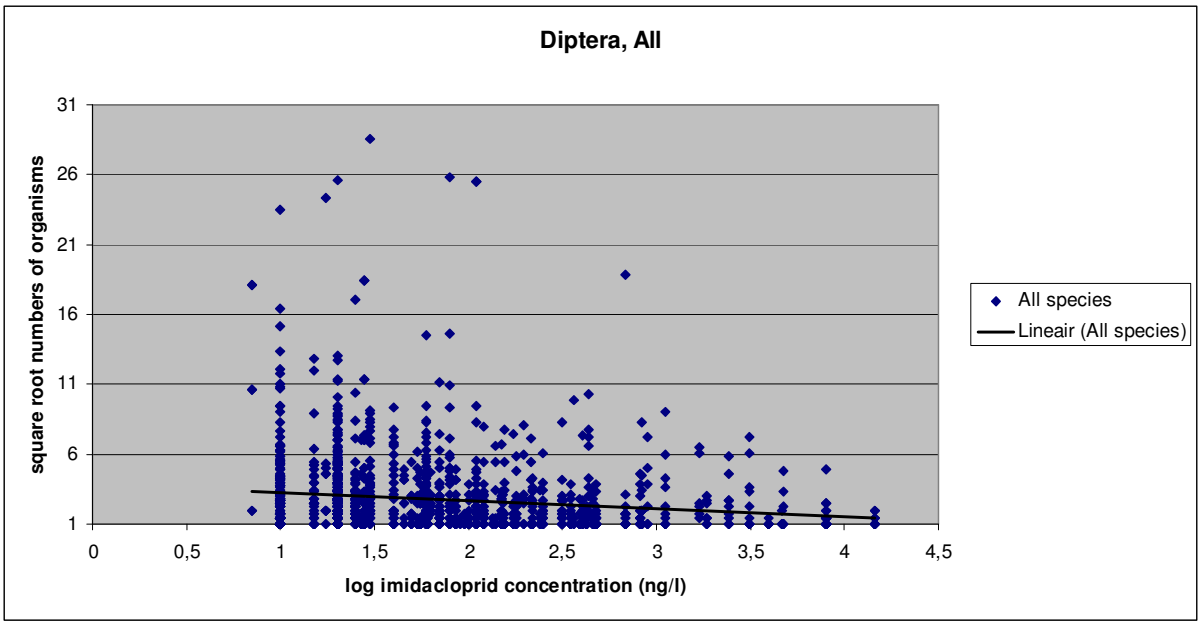


Figure 4. Scatter plots for Diptera, with linear trend lines. Top: the most abundant species, middle: the other species, bottom: all species. *d*=160-0 *dl*=0

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 trend line slope.

Regression analysis was also carried out to test the significance of the correlation shown by the trend lines 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	*C. gr sylvestris*	*G. pallens*	*E. albipennis*	*P. gr arcuatus*
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	*P. nubeculosum*	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 trend lines 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.

For each of the other orders, there is at least one declining trend line as well, 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 are 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 and signalling, and starvation of the individual or colony.

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. Six 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. All methods yielded significant results 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 trend lines 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 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 date 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
Allolobophora icterica, earth worm, Gevlekte worm (?)		LC_{50}: 2.81 mg/kg dry soil; 14 d exposure (Confidor, 200 g/l in 100% DMSO)			Capowiez et al., 2005
Amblyseius cucumeris, predatory mite, roofmijt	LC_{50}: 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
Anagrus nilaparvatae Pang et Wang, parasitoid wasp, parasitaire wesp	LC_{50}: 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
Anaphes iole Girault, fairyfly, parasitaire wesp		ST_{50}: 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)			
Aphelinus mali, wasp, sluipwesp	LC_{50}: 0.16 ppm; 24 h exposure to leaf dipped in solution for 10 s, not systemic (Confidor 35 wettable powder)				Cohen et al., 1996
Aporrectodea nocturna, earth worm, regenworm		LC_{50}: 3.74 mg/kg dry soil; 14 d exposure (Confidor, 200 g/l in 100% DMSO)			Capowiez et al., 2005
Artemia sp., Brine shrimps, Pekelkreeftjes (?)	LC_{50}: 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
Asellus aquaticus L., Water louse, Waterpissebed	LC_{50}: 8.5 mg/l; 48 h (Confidor SL 200)				Lukančič et al., 2010
Baetis rhodani, mayfly, haft of eendagsvlieg	LC_{50}: 8.49 µg/l; 48 h exposure; larvae only (Analytical grade powder)				Beketov and Liess, 2008
Bombus impatiens, Common eastern bumblebee, hommel	LC_{50}: 3.22 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)); 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)				
Bombus terrestris L., Large earth bumblebee or Buff-tailed bumblebee, Aardhommel	LD_{50}: 0.04 µg/bumblebee; 24 h after oral contact LD_{50}: 0.02 µg/bumblebee; 72 h after oral contact LD_{50}: 0.02 µg/bumblebee; 72 h after topical contact (Commercial product fed dispersed in water and syrup)				Marletto et al., 2003
Bombus terrestris,Large earth bumblebee or Buff-tailed bumblebee, Aardhommel		LC_{50} without foraging: 59 ppb; EC_{50}: 37 ppb LC_{50} with foraging: 20 ppb; EC_{50}: 3.7 ppb Experiment duration 11 wk, monitored weekly (Confidor® 20% SC)	10 ppb		Mommaerts et al., 2010
Ceriodaphnia dubia Richard, crustacean, watervlo	LC_{50}: 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
Chironomus tentans, Midge, Watermug	LC_{50}: 5.75µg/l; 96 h constant exposure, larvae	LC_{50}: 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_{50}: 5.40µg/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	
Chydorus sphaericus, planktonic cladoceran, watervlo	LC_{50}: 161950 µg/l; 24 h LC_{50}: 132673 µg/l; 48 h (Technical grade imidacloprid, 99.5% pure)			EC_{50} 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
Coleomegilla maculata lengi Timberlake, Pink spotted ladybird, lieveheersbeestje	LD_{50} adults: 0.074 µg a.i./insect; 48 hours after ventral application LD_{50} 3rd instar larvae: 0.034 µg a.i./insect; 48 h after ventral application LD_{50} adults: 60.8 mg/l; 48 hours exposure to foliage and *Leptinotarsa decemlineata* eggs dipped in solution LD_{50} 3rd instar larvae: 12.8 mg/l; 48 h, same treatment (Admire® 240 water flowable solution)	LD_{50} adults: 0.013 µg a.i./insect; 6 days after ventral application LD_{50} 3rd instar larvae: 0.008 µg a.i./insect; same treatment LD_{50} adults: 14.4 mg/l; 6 days exposure to foliage and *Leptinotarsa decemlineata* eggs dipped in solution LD_{50} 3rd instar larvae: 3.2 mg/l; 6 days, same treatment (Admire® 240 water flowable solution)			Lucas et al., 2004
Cypretta seurati,	LC_{50}: 732 µg/l; 24 h			EC_{50} was also determined,	Sánchez-Bayo &

planktonic crustacean, planktonisch schaaldier	LC_{50}: 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
Cypridopsis vidua, planktonic crustacean, planktonisch schaaldier	LC_{50}: >4000 µg/l; 24 h LC_{50}: 715 µg/l; 48 h (Technical grade imidacloprid, 99.5% pure)			LC_{50} 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
Cypridopsis vidua, planktonic crustacean, planktonisch schaaldier	LC_{50}: 3,951 µg/l; 24 h LC_{50}: 391 µg/l; 48 h LC_{50}: 7.1 µg/l; 96 h				Sánchez-Bayo, 2009
Cyrtorhinus lividipennis, mirid bug, wants	LC_{50}: 0.36 ppm; 24 h after dipping in solution; females (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000
Danio rerio, Zebrafish, Zebravis	LC_{50}: 241 mg/l; 96 h (Analytical grade) LC_{50}: 214 mg/l (Concentration of imi. in Confidor SL 200)				Tišler et al., 2009
Daphnia magna, water flea, watervlo	EC_{50}: 97.9 mg/l; 24 h EC_{50}: 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_{50}: 38 mg/l; 24 h EC_{50}: 30 mg/l; 48 h (Concentration of imi. in Confidor SL 200)			imidacloprid	
Daphnia magna, water flea, watervlo	LC_{50}: 64,873 µg/l; 48 h	LC_{50}: 9,500; 10 d			Sánchez-Bayo, 2009
Dendrobaena octaedra, earth worm, Koperworm (?)		LC_{50}: 5.7 mg/kg LC_{10}: 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
Diadegma insulare, parasitoid wasp, parasitaire wesp	LC_{50}: 0.081 mg a.i./ml (!); 30 min exposure to dipped leaves LC_{50}: 0.002 mg a.i./ml (!); 24 h exp. to dipped leaves (Provado 1.6 F)				Hill & Foster, 2000
Diaeretiella rapae, wasp, Bladluiswesp (?)	LC_{50}: 3,390 mg/l; 1 h exposure; F_0: adults collected from regularly sprayed field, thus considered resistant LC_{50}: 2,051 mg/l; 1 h; F_{11}: 11^{th} generation progeny reared from resistant adults LC_{50} : 132 mg/l; 1 h; F_{21}: 21^{st} generation progeny, considered susceptible LC_{50}: 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_0 parents than in F_{11} and F_{21} progeny, but was still significant in F_{11} progeny. Only PB synergism was significant in F_{21} progeny. LC_{50}: imi + 50 mg/l PB: 879 mg/l; 9 h; F_0	Wu et al., 2004

	after 1 h exposure; F_0 LC_{50}: 2,558 mg/l; 9 h; F_{11} LC_{50}: 105 mg/l; 9 h; F_{21} LC_{50}: 5.11 mg/l; 24 h: 23 h after 1 h exposure; other adult population collected from regularly sprayed field LC_{50}: 0.17 mg/l; 24 h; F_{21}			LC_{50}: imi + 50 mg/l triphenyl phosphate (TPP): 1,245 mg/l; 9h, F_0 LC_{50}: imi + 50 mg/l diethyl maleate (DEM): 1,026 mg/l; 9h; F_0 LC_{50}: imi + 100 mg/l PB: 420 mg/l LC_{50}: imi + 100 mg/l TPP: 815 mg/l; 9h; F_0 LC_{50}: imi + 100 mg/l DEM: 653 mg/l; 9h; F_0 Insecticide: synergist = 1:1 (v:v), and the terminal concentrations of PB, TPP, and DEM were 50 and 100 mg/liter, respectively.	
Eisenia fetida, Common brandling worm or Common dung-worm, Tijgerworm of Mestpier (?)	LC_{50}: 1.23 mg/l; 24 h, in solution LC_{50}: 0.77 mg/l; 48 h, in solution LC_{50}: 0.100 µg/cm^2; 24 h, direct contact with filter paper LC_{50}: 0.034 µg/cm^2; 48 h, direct contact with filter paper (Imidacloprid >95% pure)	LC_{50}: 3.48 mg/kg dry soil; 7 d, in artificial soil LC_{50}: 2.30 mg/kg dry soil; 14 d, in artificial soil			Luo et al., 1999 and Zang et al., 2000
Eisenia fetida, Common brandling worm or Common dung-worm, Tijgerworm of Mestpier (?)		LC_{50}: 25 mg/kg, with significant weight losses at 14 mg/kg (14 days?). No significant effects on microbial decomposition of			Kreutzweiser et al., 2008a

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

Harmonia axyridis, Multicoloured Asian lady beetle, Veelkleurig Aziatisch lieveheersbeestje	LC_{50} 1st inst.: <8.79 mg a.i./l; 48 h LC_{50} 2nd inst.: <8.79 mg a.i./l; 48 h LC_{50} 3rd inst.: 30.3 mg a.i./l; 48 h LC_{50} 4th inst.: 190.2 mg a.i./l; 48 h LC_{50} 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)	LC_{50} eggs: <8.79 mg a.i./l; 7 d LC_{50} pupae: >1000.0 mg a.i./l; 7 d			Youn et al., 2003
Hyalella azteca, crustacean, vlokreeft	LC_{50} juveniles: 65.43 µg/l; 96 h (99.2% pure imidacloprid) LC_{50} juveniles: 17.44µg/l; 96 h (Admire®)	LC_{50}: 7.08 µg/l; 28 d, constant exposure (7.01 µg/l after 10 d)	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		Stoughton et al., 2008
Ilyocypris dentifera, planktonic crustacean, planktonisch schaaldier	LC_{50}: 1122 µg/l; 24 h LC_{50}: 517 µg/l; 48 h (Technical grade			LC_{50} was lower under dark than under normal (16 h light : 8 h dark) laboratory conditions:	Sánchez-Bayo & Goka, 2006

	imidacloprid, 99.5% pure)			24 h: 759 µg/l 48 h: 214 µg/l	
Megachile rotundata, Alfalfa leafcutting bee, bij	LC_{50}: 0.17 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)); 48 h, direct contact toxicity, females and males (Technical grade, >95% purity)				Scott-Dupree et al., 2009
Nannotrigona perilampoides, stingless bee, angelloze bij	LD_{50}: 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
Orius laevigatus Fieber, predatory pirate bug, roofwants	LC_{50} residual contact, 5^{th} instar nymphs: 0.04 mg a.i./l, adults: 0.3 mg a.i./l. LC_{50} ingestion, 5^{th} instar nymphs: 1.1 mg a.i./l, adults: 2.1 mg a.i./l (Confidor 200 SL)				Delbeke et al., 1997
Oryzias latipes, Medaka fish or Japanese ricefish, Japanse 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 *Trichodina*, against 40% in the control group				Sánchez-Bayo & Goka, 2005
Osmia lignaria Cresson, Orchard mason bee or Blue orchard bee, bij	LC_{50}: 0.07 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)); 48 h, direct contact toxicity,				Scott-Dupree et al., 2009

	females and males (Technical grade, >95% purity)				
Palaemonetes pugio, Daggerblade grass shrimp, garnaal	LC_{50} larvae: 308.8 µg/l; 96 h exposure LC_{50} adults: 563.5 µg/l; 96 h exposure (Technical grade, 99.5% pure)		NOEC larvae: 100 µg/l; 96 h exposure	96 h LC_{50} 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
Pardosa pseudoannulata, thin-legged wolf spider, wolfspin	LC_{50}: 440 ppm; 48 h, individuals dipped in solution, 1st instars only (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000
Pheretima spp., Earth worms, regenwormen	LC_{50}: 155 mg/kg; 24 h exposure in artificial soil LC_{50}: 5 mg/kg; 48 h exposure in artificial soil	LC_{50}: 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
Phytoseiulus persimilis, predatory mite, roofmijt	LC_{50}: 1500, 1800 and 8500 ppm; 24 h after 5 seconds' dipping in solution LC_{50}: >10,000 ppm; 72 h direct contact with dipped leaf, females only, obtained from three companies (Admire 240 F)				Lee et al., 2002

Picromerus bidens, predatory bug, schildwants	LC_{50} 4th instar: 7.12 mg a.i./l; residual contact, mortality checked every day for four days (17.7% suspension concentrate)	LC_{50} 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
Podisus nigrispinus Dallas nymphs, predatory stinkbug, roofkever		LC_{50} 2nd instar: 34.40 mg; residual LC_{50} 5th instar: 147.66 mg; residual LC_{50} 2nd instar: 0.13 mg; ingestion LC_{50} 5th instar: 0.44 mg; ingestion All mortalities summarized after 5 days exposure (Imidacloprid 700 g/kg as Confidor WDGr)			Torres & Ruberson, 2004
Porcellio scaber, 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
Pteronarcys dorsata, stonefly, vlieg		LC_{50} (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
Rana limnocharis, Boie's wart frog or Cricket frog, kikker	LC_{50} tadpoles: 235 mg/l; 24 h exposure LC_{50} tadpoles: 165 mg/l; 48 h exposure LC_{50} tadpoles: 116 mg/l; 72 h exposure LC_{50} tadpoles: 82 mg/l; 96 h exposure (Imidacloprid >95% pure)				Feng et al., 2004
Rana nigromaculata Hallowell, Japanese pond frog or Dark-spotted frog, kikker	LC_{50} tadpoles: 268 mg/l; 24 h exposure LC_{50} tadpoles: 219 mg/l; 48 h exposure LC_{50} tadpoles: 177 mg/l; 72 h exposure LC_{50} tadpoles: 129 mg/l; 96 h exposure (Imidacloprid >95% pure)				Feng et al., 2004
Simulium latigonium, blackfly, vlieg	LC_{50}: 3.73 µg/l; 96 h exposure, larvae (Analytical grade powder)				Beketov and Liess, 2008
Simulium vittatum Zetterstedt cytospecies IS-7, blackfly, vlieg	LC_{50}: between 9.54 and 6.75 µg/l; 48 h, larvae (Analytical grade, ≥98% pure)		NOEC: likely 2 to 5 µg/l		Overmyer et al., 2005

Tetragnatha maxillosa, spider, spin	LC_{50}: 136 ppm; 48 h, individuals dipped in solution, 1st instars only (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000
Tipula spp., Craneflies or Daddy long legs, Langpootmuggen		LC_{50}: 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
Ummeliata insecticeps, spider, spin	LC_{50}: 995 ppm; 48 h, individuals dipped in solution, 1st instars only (Imidacloprid 10% wettable powder, 1% granules)				Tanaka et al., 2000

Thiacloprid (alphabetical list)

Species	Acute toxicity	Chronic toxicity	NOEC	Synergy effects	Source
Aleochara bilineata, rove beetle, kortschildkever		ER_{50}: < 187.5 g a.i./ha; 28 d contact toxicity, quartz sand (SC 480) LR_{50}: > 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
Anas platyrhynchos, 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)		
Aphidius rhopalosiphi, parasitoid wasp, parasitaire wesp	LR_{50}: 6.5 g a.i./ha; 48 h contact toxicity (SC 480) LR_{50}: 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
Asellus aquaticus, Water louse, Waterpissebed	LC_{50}: >698.5 µg/l; 24 h LC_{50}: 299 µg/l; 96 h (Analytical grade powder?)	LC_{50}: 153.4 µg/l; 19 d (Analytical grade powder?)			Beketov and Liess, 2007
Baetis rhodani, mayfly, haft of eendagsvlieg	LC_{50}: 4.60 µg/l; 96 h exposure, larvae (Analytical grade powder)				Beketov and Liess, 2008
Bombus terrestris, Large earth bumblebee or Buff-tailed bumblebee, Aardhommel		LC_{50} (without foraging; time?): 18 ppm EC_{50} (without foraging; time?): 12 ppm Experiment duration 11 wk, monitored weekly (Calypso® 48% SC)	1.2 ppm		Mommaerts et al., 2010
Coccinella septempunctata, Seven-spotted ladybird, Zevenstippelig lieveheersbeestje		LR_{50}: c 19.2 g a.i./ha; 15 d, contact with apple seedlings, larvae (SC 480) LR_{50}: 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_{50}: 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)		
Colinus virginianus, Northern bobwhite quail, Noordelijke boomkwartel of Virginische boomkwartel	LD_{50}: 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
Coturnix japonica, Japanese quail, Japanse kwartel	LD_{50}: 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
Culex pipiens, House mosquito, Gewone steekmug	LC_{50}: 7.35 µg/l; 24 h, larvae LC_{50}: 7.10; 96 h, larvae	LC_{50}: 5.76; 7 d, larvae LC_{50}: 6.04; 14 d, larvae			Beketov and Liess, 2007
Cyprinodon variegates, Sheepshead minnow, Edelsteentandkarper	LC_{50}: 19.7 mg a.i./l; 96 h static (Technical grade)				Schmuck, 2001
Daphnia magna, water flea, watervlo	LC_{50}: 7,200 µg/l; 24 h LC_{50}: 4,400; 96 h	LC_{50}: 4,400; 14 d LC_{50}: 4,100; 30 d			Beketov and Liess, 2007
Eisenia fetida, Common brandling worm or Common dung-worm, Tijgerworm of Mestpier		LC_{50}: 105 mg a.i./kg dry wt soil; 14 d (Technical grade) LC_{50}: 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_{50}: 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	
Gammarus pulex, crustacean, Brakwater vlokreeft	LC_{50}: 350 µg/l; 96 h exposure (Analytical grade powder)				Beketov and Liess, 2008
Gammarus pulex, crustacean, Brakwater vlokreeft	LC_{50}: >9,520.0 µg/l; 24 h LC_{50}: 580 µg/l; 96 h	LC_{50}: 190 µg/l; 17 d			Beketov and Liess, 2007
Hyaliodes vitripennis Say, predaceous mirid, roofwants	LC_{50}, adults: 0.0003 g a.i./l; 24 h LC_{50}, nymphs: 0.0015 g a.i./l; 24 h (Insects and apple leaf sprayed with 480 g/l SC Calypso ®)				Bostanian et al., 2005
Lepomis macrochirus, Bluegill, baars	LC_{50}: 25.2 mg a.i./l; 96 h static (Technical grade) LC_{50}: 38.7 mg a.i./l; 96 h static (SC 480)				Schmuck, 2001
Mysidopsis bahia, mysid shrimp, garnaal	LC_{50}: 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_{50}: 0.050 mg a.i./l; 96 h flow-through (SC 480)				
Nannotrigona perilampoides, stingless bee, angelloze bij	LD_{50}: 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
Notidobia ciliaris, caddisfly, schietmot	LC_{50}: 7.7 µg/l; 24 h, larvae only LC_{50}: 7 µg/l; 96 h, larvae only	LC_{50}: 6.8 µg/l; 15 d, larvae only			Beketov and Liess, 2007
Oncorhynchus mykiss, Rainbow trout, Regenboogforel	LC_{50}: 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
Pardosa spp., Thin-legged wolf spiders, wolfspinnen		ER_{50}: 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
Pimephales promelas, Fathead minnow, Amerikaanse dikkop-elrits	LC_{50}: > 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_0 male body length/weight); flow-through, 290 d		Schmuck, 2001

			(Technical grade)		
Poecilus cupreus, Ground beetle, loopkever		LR_{50}: > 211.2 g a.i./ha; 14 d contact toxicity, quartz sand (SC 480) LR_{50}: > 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
Simulium latigonium, blackfly, vlieg	LC_{50}: 10.1 µg/l; 24 h, larvae only LC_{50}: 7.8 µg/l; 96 h, larvae only	LC_{50}: 5.5 µg/l; 11 d, larvae only			Beketov and Liess, 2007
Sympetrum striolatum, Common darter dragonfly, Bruinrode heidelibel	LC_{50}: >113.3, µg/l; 24 h, larvae only LC_{50}: 47.6 µg/l; 96 h, larvae only	LC_{50}: 31.2 µg/l; 11 d, larvae only			Beketov and Liess, 2007
Typhlodromus pyri, predatory mite, Appelroofmijt		LR_{50}: 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
Bombus impatiens, Common eastern bumblebee, hommel	LC_{50}: 0.39 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)); 48 h direct contact toxicity,				Scott-Dupree et al., 2009

	females only (Technical grade, >95% purity)				
Megachile rotundata, Alfalfa leafcutting bee, bij	LC_{50}: 0.08 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)); 48 h direct contact toxicity, females and males (Technical grade, >95% purity)				Scott-Dupree et al., 2009
Osmia lignaria Cresson, Orchard mason bee or Blue orchard bee, bij	LC_{50}: 0.10 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)); 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
Amblyseius cucumeris, predatory mite, roofmijt	LC_{50}: 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
Anagrus nilaparvatae Pang et Wang, parasitoid	LC_{50}: 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)				
Anaphes iole Girault, fairyfly, parasitaire wesp		ST_{50}: 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
Anaphes iole Girault, fairyfly, parasitaire wesp	LC_{50}: 1.70 µg/ml; after 48 h direct contact; females only (Actara 25 WG 0.1 kg a.i./ha)				Williams & Price, 2004
Bombus terrestris, Large earth bumblebee or Buff-tailed bumblebee, Aardhommel		LC_{50} (without foraging; time?): 0.12 ppm; EC_{50} (without foraging; time?): 35 ppb Experiment duration 11 wk, monitored weekly (Actara® 25% WG)	10 ppb		Mommaerts et al., 2010
Harmonia axyridis, Multicoloured Asian lady beetle, Veelkleurig Aziatisch lieveheersbeestje	LC_{50} eggs: 382.31 mg a.i./l LC_{50} 1st inst.: 37.01 mg a.i./l LC_{50} 2nd inst.: 81.09 mg a.i./l LC_{50} 3rd inst.: 124.03 mg a.i./l LC_{50} 4th inst.: 249.23 mg a.i./l LC_{50} pupae: >2500.0 mg				Youn et al., 2003

	a.i./l LC_{50} adults: 150.46 mg a.i./l Instars and adults were exposed via topical application, eggs and pupae were dipped in solution for 10 s (Actara WG)				
Hyaliodes vitripennis Say, predaceous mirid, roofwants	LC_{50} adults: 0.0005 g a.i./l; 24 h LC_{50} nymphs: 0.0143 g a.i./l; 24 h (Insects and apple leaf sprayed with Actara® WG, 250 g/kg)				Bostanian et al., 2005
Nannotrigona perilampoides, stingless bee, angelloze bij	LD_{50}: 0.004 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
Phytoseiulus persimilis, predatory mite, roofmijt	LC_{50}: >1000 ppm; 24 h after 5 seconds' dipping in solution, and 72 h direct contact with dipped leaf; females only, obtained from three companies (Actara 240 F)				Lee et al., 2002
Podisus nigrispinus		LC_{50} 2nd instar: 18.39 mg			Torres & Ruberson,

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

LC_{50} = reported median lethal concentration
EC_{50} = effective concentration
ST_{50} = LT_{50}

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_{50} – 96 h direct contact – species in order of vulnerability

1 *Simulium latigonium*, blackfly, vlieg
LC_{50}: 0.00373 mg/l; 96 h exposure, larvae
(Analytical grade powder)

2 *Chironomus tentans*, Midge, Watermug
LC_{50}: 0.00575 mg/l; 96 h constant exposure, larvae
(99.2% pure imidacloprid)
LC_{50}: 0.00540 mg/l; 96 h constant exposure, larvae
(Admire®)

3 *Hyalella azteca*, crustacean, vlokreeft
LC_{50} juveniles: 0.06543 mg/l; 96 h
(99.2% pure imidacloprid)
LC_{50} juveniles: 0.01744 mg/l; 96 h
(Admire®)

4 *Gammarus pulex*, crustacean, Brakwater vlokreeft
LC_{50}: 0.270 mg/l; 96 h exposure
(Analytical grade powder)

5 *Palaemonetes pugio*, Daggerblade grass shrimp, garnaal
LC_{50} larvae: 0.3088 mg/l; 96 h exposure
LC_{50} 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_{50} tadpoles: 82 mg/l; 96 h exposure
(Imidacloprid >95% pure)

7 *Rana nigromaculata* Hallowell, Japanese pond frog or Dark-spotted frog, kikker
LC_{50} tadpoles: 129 mg/l; 96 h exposure
(Imidacloprid >95% pure)

8 *Danio rerio*, Zebrafish, Zebravis
LC_{50}: 241 mg/l; 96 h
(Analytical grade)
LC_{50}: 214 mg/l
(Concentration of imi. in Confidor SL 200)

LC_{50} – 48 h direct contact – species in order of vulnerability

1 *Osmia lignaria* Cresson, Orchard mason bee or Blue orchard bee, bij
LC_{50}: 0.07 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)) = 0.0007 mg/l; 48 h, direct contact toxicity, females and males
(Technical grade, >95% purity)

2 *Megachile rotundata*, Alfalfa leafcutting bee, bij
LC_{50}: 0.17 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)) = 0.0017 mg/l; 48 h, direct contact toxicity, females and males
(Technical grade, >95% purity)

3 *Ceriodaphnia dubia* Richard, crustacean, watervlo
LC_{50}: 0.00207 mg/l; 48 h
(Admire Pro; 42.8% active ingredient)

4 *Simulium vittatum* Zetterstedt cytospecies IS-7, blackfly, vlieg
LC_{50}: between 0.00954 and 0.00675 mg/l; 48 h, larvae
(Analytical grade, ≥98% pure)

5 *Baetis rhodani*, mayfly, haft of eendagsvlieg
LC_{50}: 0.00849 mg/l; 48 h exposure; larvae only
(Analytical grade powder)

6 *Bombus impatiens*, Common eastern bumblebee, hommel
LC_{50}: 3.22 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)) = 0.0322 mg/l; 48 h direct contact toxicity; females only
(Technical grade, >95% purity)

7 *Cypretta seurati*, planktonic crustacean, planktonisch schaaldier
LC_{50}: 0.301 mg/l; 48 h
(Technical grade imidacloprid, 99.5% pure)

8 *Cypridopsis vidua*, planktonic crustacean, planktonisch schaaldier
LC_{50}: 0.391 mg/l; 48 h

9 *Ilyocypris dentifera*, planktonic crustacean, planktonisch schaaldier
LC_{50}: 0.517 mg/l; 48 h
(Technical grade imidacloprid, 99.5% pure)

10 *Cypridopsis vidua*, planktonic crustacean, planktonisch schaaldier
LC_{50}: 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_{50}: 0.77 mg/l; 48 h, in solution
(Imidacloprid >95% pure)

12 *Gammarus fossarum* Koch, Stream scud, vlokreeft
LC_{50}: 0.8 mg/l (?); 48 h
(Confidor SL 200)

13 *Asellus aquaticus* L., Water louse, Waterpissebed
LC_{50}: 8.5 mg/l; 48 h
(Confidor SL 200)

14 *Coleomegilla maculata lengi* Timberlake, Pink spotted ladybird, lieveheersbeestje
LD_{50} 3rd 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_{50} 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_{50}: 64.873 mg/l; 48 h

17 *Chydorus sphaericus*, planktonic cladoceran, watervlo
LC_{50}: 132.673 mg/l; 48 h
(Technical grade imidacloprid, 99.5% pure)

18 *Rana limnocharis*, Boie's wart frog or Cricket frog, kikker
LC_{50} tadpoles: 165 mg/l; 48 h exposure
(Imidacloprid >95% pure)

19 *Rana nigromaculata* Hallowell, Japanese pond frog or Dark-spotted frog, kikker
LC_{50} tadpoles: 219 mg/l; 48 h exposure
(Imidacloprid >95% pure)

20 *Artemia* sp., Brine shrimps, Pekelkreeftjes (?)
LC_{50}: 361.23 mg/l; 48 h exposure under hyperosmotic conditions: 100% artificial salt water

LC_{50} – 24 h direct contact – species in order of vulnerability

1 *Aphelinus mali*, wasp, sluipwesp
LC_{50}: 0.16 ppm; 24 h exposure to leaf dipped in solution for 10 s, not systemic
(Confidor 35 wettable powder)

2 *Cypretta seurati*, planktonic crustacean, planktonisch schaaldier
LC_{50}: 0.732 mg/l; 24 h
(Technical grade imidacloprid, 99.5% pure)

3 *Ilyocypris dentifera*, planktonic crustacean, planktonisch schaaldier
LC_{50}: 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_{50}: 1.23 mg/l; 24 h, in solution
(Imidacloprid >95% pure)

5 *Diadegma insulare*, parasitoid wasp, sluipwesp
LC_{50}: 2 mg a.i./l; 24 h exp. to dipped leaves
(Provado 1.6 F)

6 *Cypridopsis vidua*, planktonic crustacean, planktonisch schaaldier
LC_{50}: 3.951 mg/l; 24 h

7 *Cypridopsis vidua*, planktonic crustacean, planktonisch schaaldier
LC_{50}: >4.0 mg/l; 24 h
(Technical grade imidacloprid, 99.5% pure

8 *Chydorus sphaericus*, planktonic cladoceran, watervlo
LC_{50}: 161.950 mg/l; 24 h
(Technical grade imidacloprid, 99.5% pure)

9 *Rana limnocharis*, Boie's wart frog or Cricket frog, kikker
LC_{50} tadpoles: 235 mg/l; 24 h exposure
(Imidacloprid >95% pure)

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

LC_{50} – 48 h after topical contact – species in order of vulnerability

1 *Harmonia axyridis*, Multicoloured Asian lady beetle, Veelkleurig Aziatisch lieveheersbeestje
LC_{50} 1st inst.: <8.79 mg a.i./l; 48 h
LC_{50} 2nd inst.: <8.79 mg a.i./l; 48 h
LC_{50} 3rd 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_{50}: 136 ppm; 48 h after topical application (dipping in solution); 1st instars
(Imidacloprid 10% wettable powder, 1% granules)

3 *Harmonia axyridis*, Multicoloured Asian lady beetle, Veelkleurig Aziatisch lieveheersbeestje
LC_{50} 4th inst.: 190.2 mg a.i./l; 48 h
LC_{50} 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_{50}: 440 ppm; 48 h after topical application (dipping in solution); 1st instars
(Imidacloprid 10% wettable powder, 1% granules)

5 *Gnathonarium exsiccatum*, spider, spin
LC_{50}: 801 ppm; 48 h after topical application (dipping in solution); 1st instars
(Imidacloprid 10% wettable powder, 1% granules)

6 *Ummeliata insecticeps*, spider, spin
LC_{50}: 995 ppm; 48 h after topical application (dipping in solution); 1st instars
(Imidacloprid 10% wettable powder, 1% granules)

LC_{50} – 24 h after topical contact – species in order of vulnerability

1 *Haplogonatopus apicalis*, Dryinid wasp, sluipwesp
LC_{50}: 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_{50}: 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_{50}: 1500, 1800 and 8500 ppm; 24 h after 5 seconds' dipping in solution
(Admire 240 F)

4 *Amblyseius cucumeris*, predatory mite, roofmijt
LC_{50}: 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_{50} – 96 h exposure – species in order of vulnerability

1 *Baetis rhodani*, mayfly, haft of eendagsvlieg
LC_{50}: 0.00460 mg/l; 96 h exposure, larvae
(Analytical grade powder)

2 *Notidobia ciliaris*, caddisfly, schietmot
LC_{50}: 0.007 mg/l; 96 h, larvae only

3 *Culex pipiens*, House mosquito, Gewone steekmug
LC_{50}: 0.00710 mg/l; 96 h, larvae

4 *Simulium latigonium*, blackfly, vlieg
LC_{50}: 0.0078 mg/l; 96 h, larvae only

5 *Mysidopsis bahia*, mysid shrimp, garnaal
LC_{50}: 0.031 mg a.i./l; 96 h flow-through
(Technical grade)

6 *Sympetrum striolatum*, Common darter dragonfly, Bruinrode heidelibel
LC_{50}: 0.0476 mg/l; 96 h, larvae only

7 *Mysidopsis bahia*, mysid shrimp, garnaal
LC_{50}: 0.050 mg a.i./l; 96 h flow-through
(SC 480)

8 *Asellus aquaticus*, Water louse, Waterpissebed
LC_{50}: 0.299 mg/l; 96 h
(Analytical grade powder?)

9 *Gammarus pulex*, crustacean, Brakwater vlokreeft
LC_{50}: 0.350 mg/l; 96 h exposure
(Analytical grade powder)

10 *Gammarus pulex*, crustacean, Brakwater vlokreeft
LC_{50}: 0.580 mg/l; 96 h

11 *Cyprinodon variegates*, Sheepshead minnow, Edelsteentandkarper
LC_{50}: 19.7 mg a.i./l; 96 h static
(Technical grade)

12 *Oncorhynchus mykiss*, Rainbow trout, Regenboogforel
LC_{50}: 30.5 mg a.i./l; 96 h static
(Technical grade)

13 *Lepomis macrochirus*, Bluegill, baars
LC_{50}: 38.7 mg a.i./l; 96 h static
(SC 480)
LC_{50}: 25.2 mg a.i./l; 96 h static
(Technical grade)

14 *Pimephales promelas*, Fathead minnow, Amerikaanse dikkop-elrits
LC_{50}: > 104 mg a.i./l; 96 h static
(Technical grade)

15 *Daphnia magna*, water flea, watervlo
LC_{50}: 4.400 mg/l; 96 h

LC_{50} – 24 h exposure – species in order of vulnerability

1 *Culex pipiens*, House mosquito, Gewone steekmug
LC_{50}: 0.00735 mg/l; 24 h, larvae

2 *Notidobia ciliaris*, caddisfly, schietmot
LC_{50}: 0.0077 mg/l; 24 h, larvae only

3 *Simulium latigonium*, blackfly, vlieg
LC_{50}: 0.0101 mg/l; 24 h, larvae only

4 *Sympetrum striolatum*, Common darter dragonfly, Bruinrode heidelibel
LC_{50}: >0.1133, mg/l; 24 h, larvae only

5 *Hyaliodes vitripennis* Say, predaceous mirid, roofwants
LC_{50}, 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_{50}: >0.6985 mg/l; 24 h
(Analytical grade powder?)

7 *Hyaliodes vitripennis* Say, predaceous mirid, roofwants
LC_{50}, 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_{50}: 7.200 mg/l; 24 h

9 *Gammarus pulex*, crustacean, Brakwater vlokreeft
LC_{50}: >9.520 mg/l; 24 h

Clothianidin

LC_{50} - 48 h direct contact – species in order of vulnerability

1 *Megachile rotundata*, Alfalfa leafcutting bee, bij
LC_{50}: 0.08 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)) = 0.0008 mg/l; females and males

2 *Osmia lignaria* Cresson, Orchard mason bee or Blue orchard bee, bij
LC_{50}: 0.10 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)) = 0.001 mg/l; females and males

3 *Bombus impatiens*, Common eastern bumblebee, hommel
LC_{50}: 0.39 (concentration expressed as percentage of solution (wt:vol) ($x10^{-3}$)) = 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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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 *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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

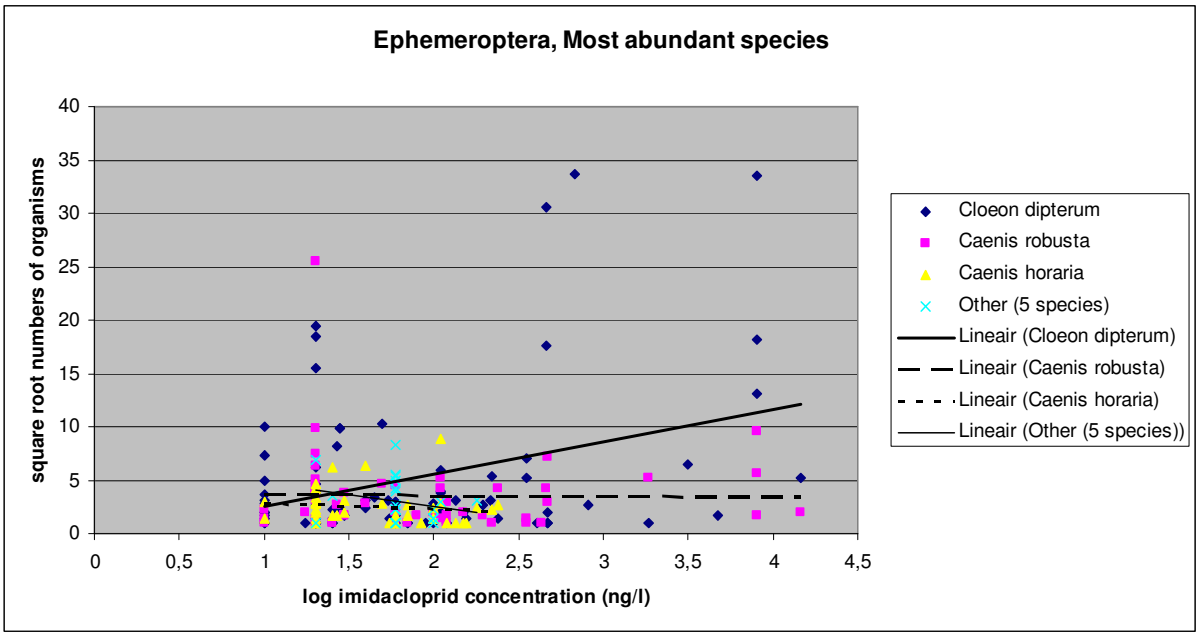
Category	0 km radius av. abundance	0 km radius *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
0-55 ng/l	46.4	15	31.1	36	48.4	96
> 55 ng/l	512.5	26	327.2	41	151.6	94
Significance					$p = 0.49540$	

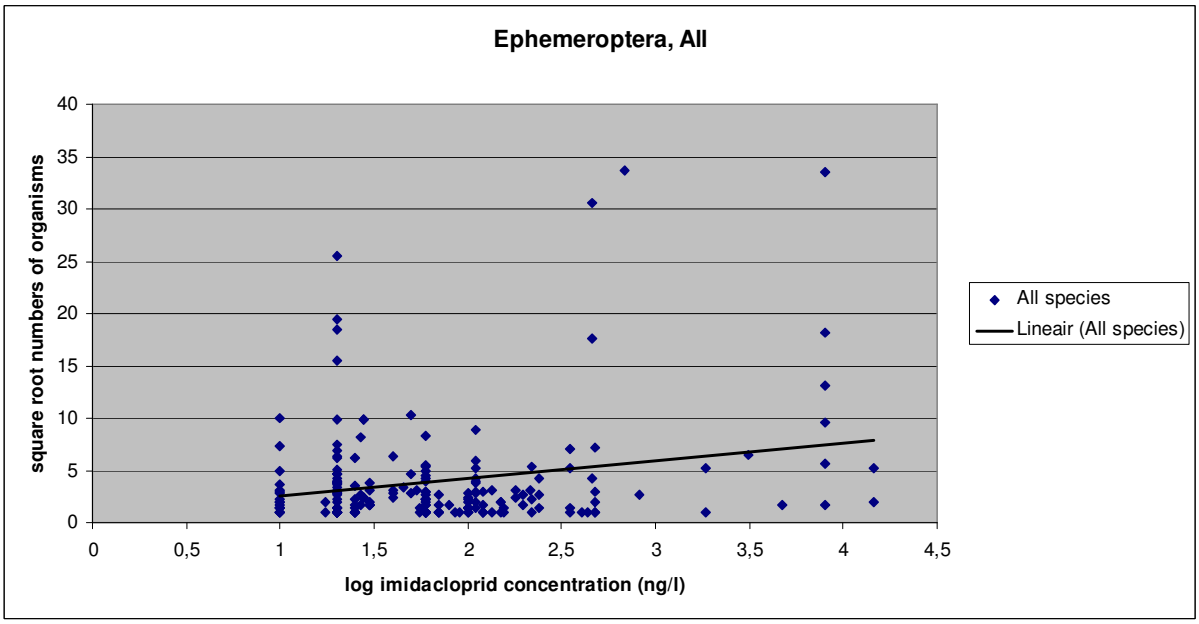
Odonata, d=160-0 dl=0

Category	0 km radius av. abundance	0 km radius *n*	1 km radius av. abundance	1 km radius *n*	2 km radius av. abundance	2 km radius *n*
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

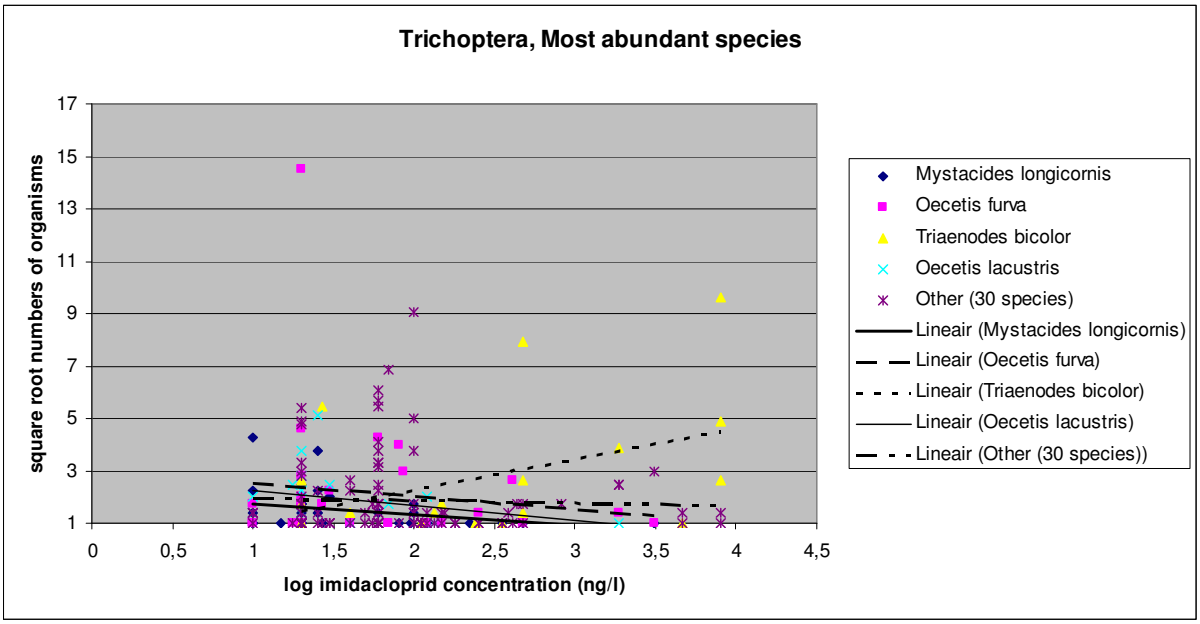
Ephemeroptera

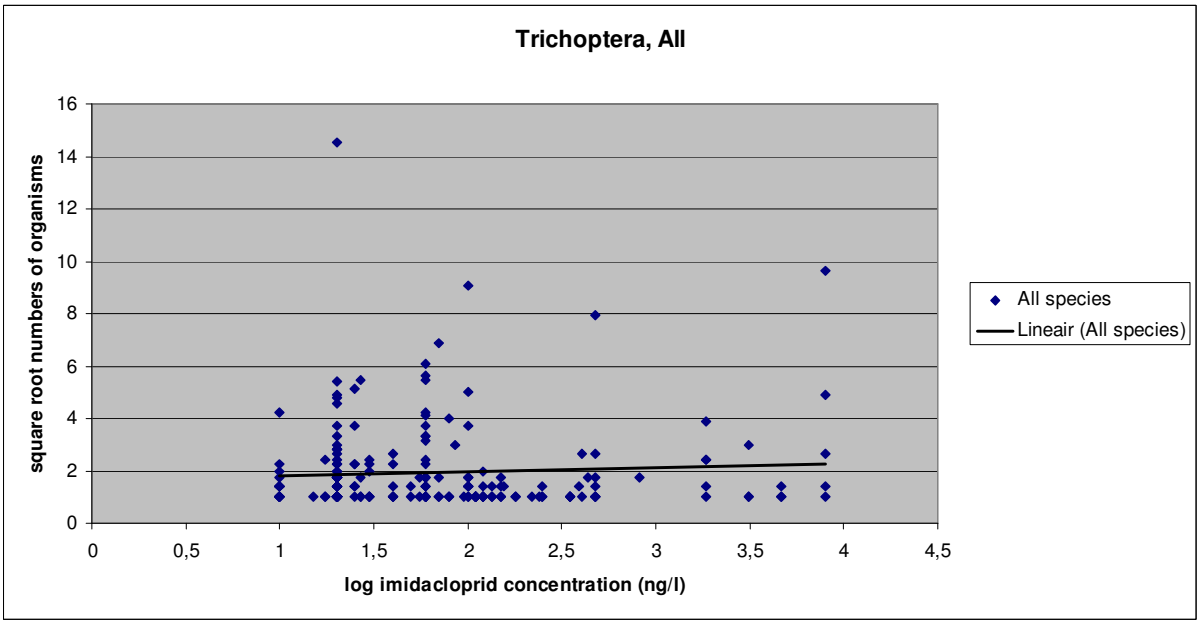




Ephemeroptera			
Species	*C. dipterum*	*C. robusta*	*C. horaria*
Regression	*r* = 0.34176	*r* = -0.01519	*r* = -0.12311
Significance	*p* (uncorr) = 0.00168	*p* (uncorr) = 0.91491	*p* (uncorr) =0.45526
Species	Other (5 species)	All	
Regression	*r* = -0.23093	*r* = 0.22903	
Significance	*p* (uncorr) =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

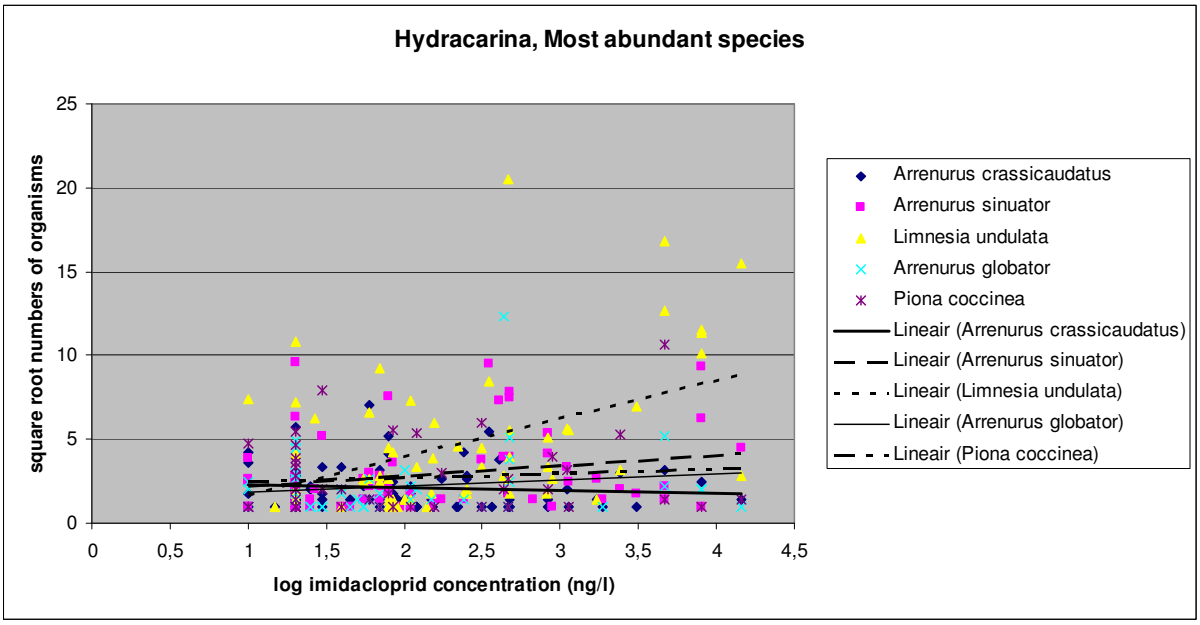
Trichoptera

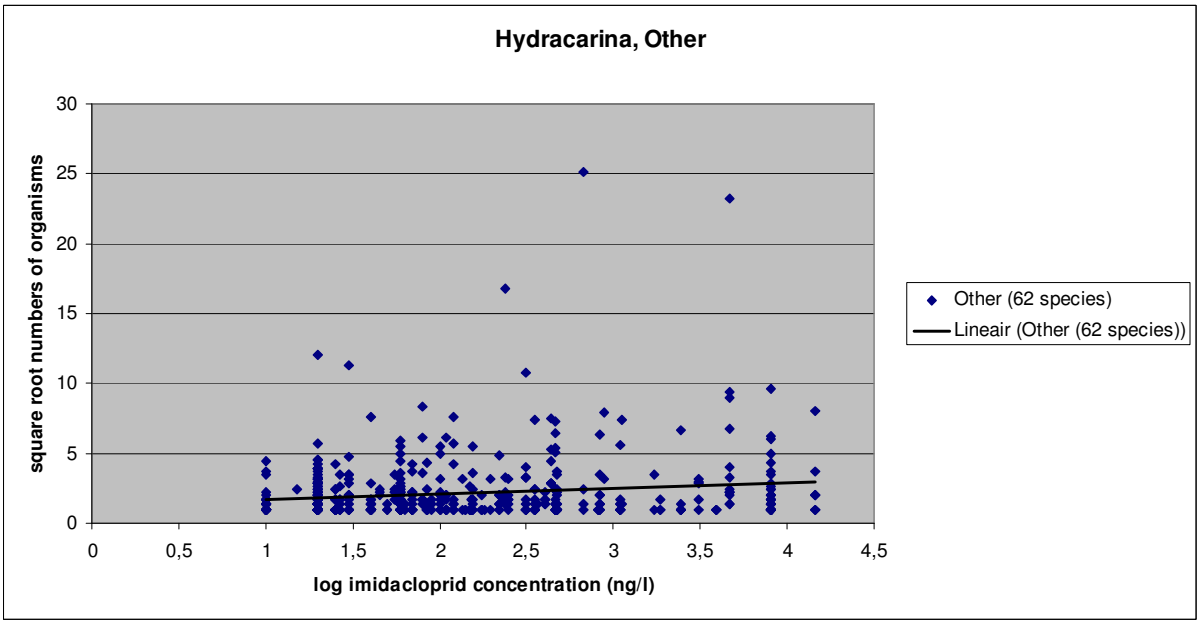


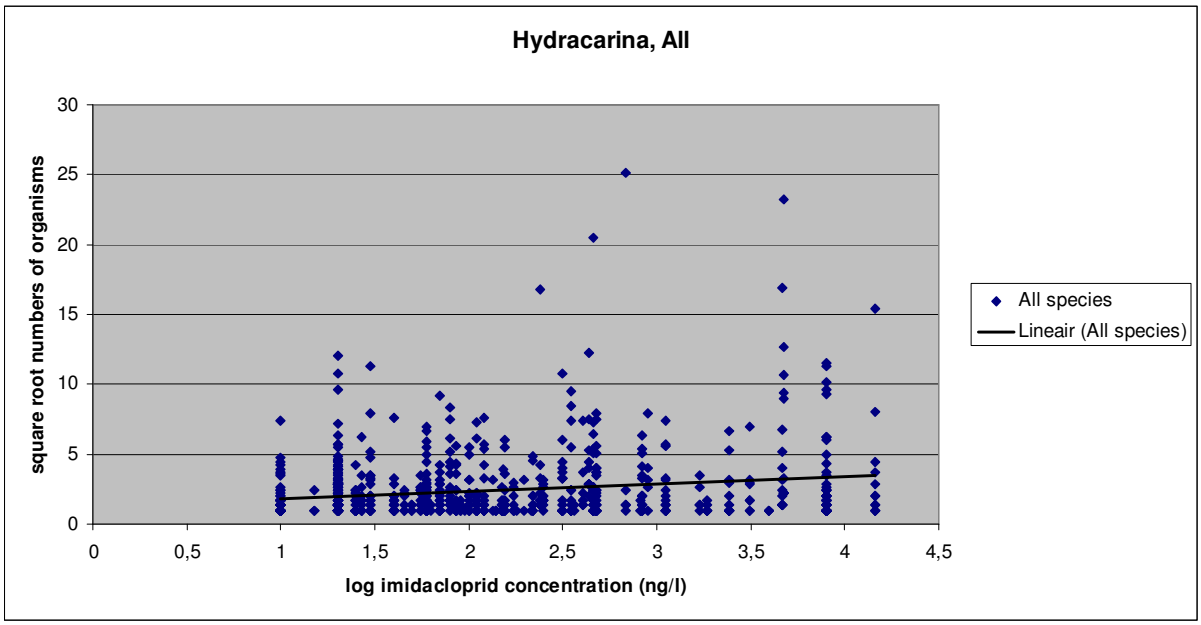


Trichoptera			
Species	*M. longicornis*	*O. furva*	*T. bicolor*
Regression	*r* = -0.3126	*r* = -0.12221	*r* = 0.45666
Significance	*p* (uncorr) = 0.08152	*p* (uncorr) = 0.52001	*p* (uncorr) = 0.03744
Species	*O. lacustris*	Other (30 species)	All
Regression	*r* = -0.27616	*r* = -0.039507	*r* = 0.059028
Significance	*p* (uncorr) = 0.23856	*p* (uncorr) = 0.68337	*p* (uncorr) = 0.39248

Hydracarina

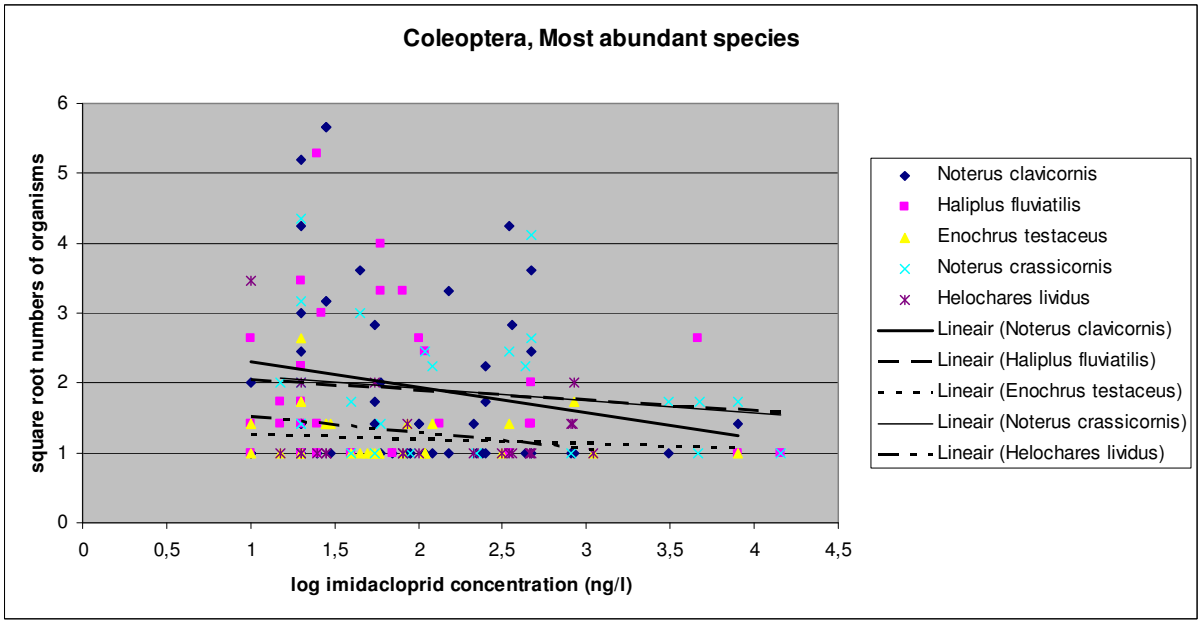


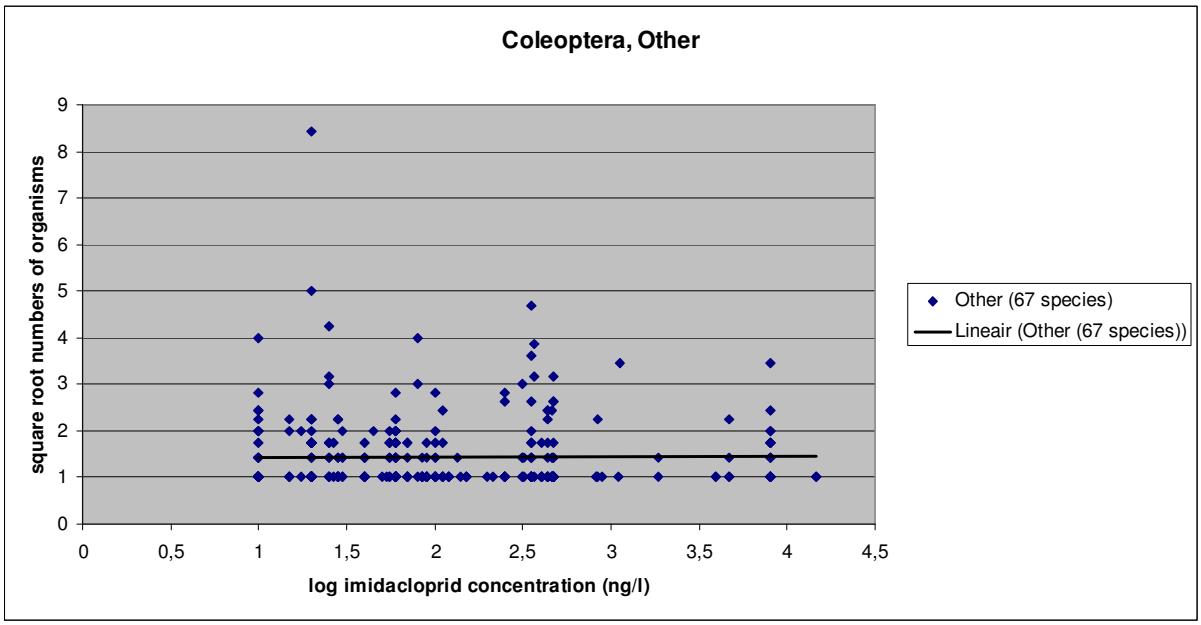


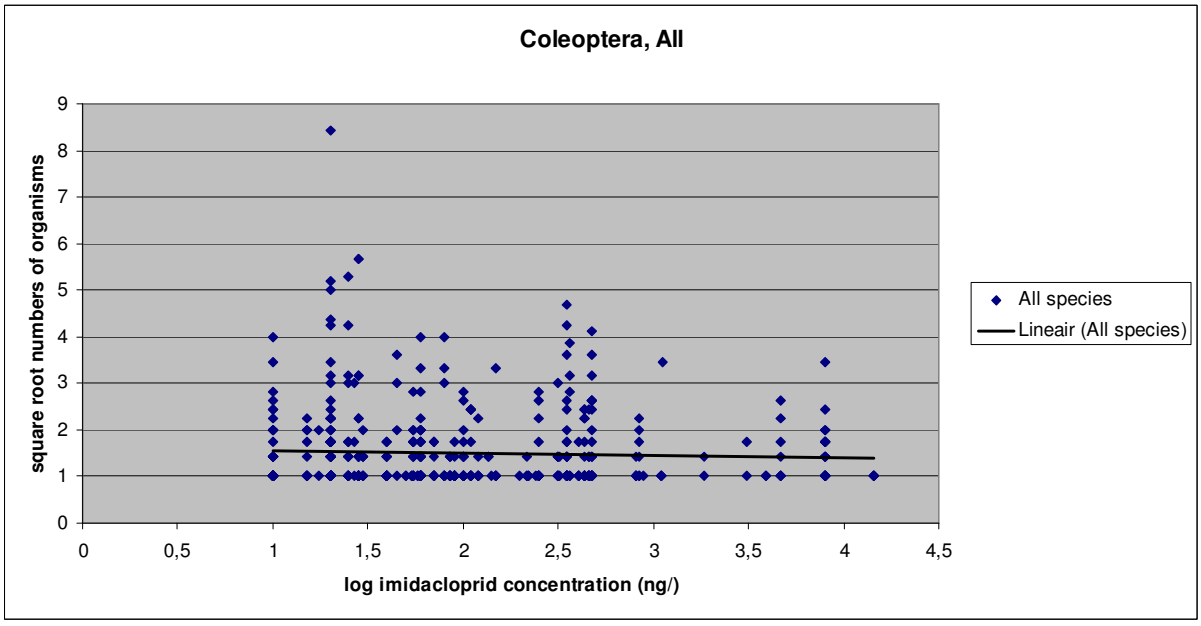


Hydracarina				
Species	*A. crassicaudatus*	*A. sinuator*	*L. undulata*	*A. globator*
Regression	r = -0.09980	r = 0.23492	r = 0.43865	r = 0.15638
Significance	p (uncorr) = 0.39105	p (uncorr) = 0.05028	p (uncorr) = 0.00032	p (uncorr) = 0.30498
Species	*P. coccinea*	Other (62 species)	All	
Regression	r = 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

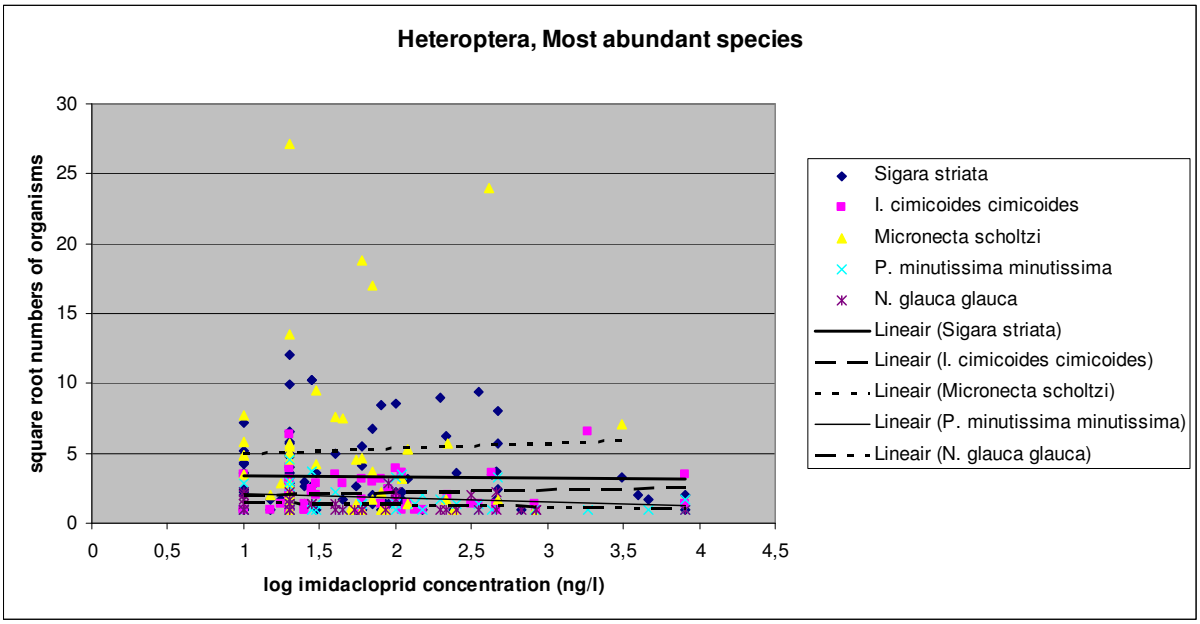


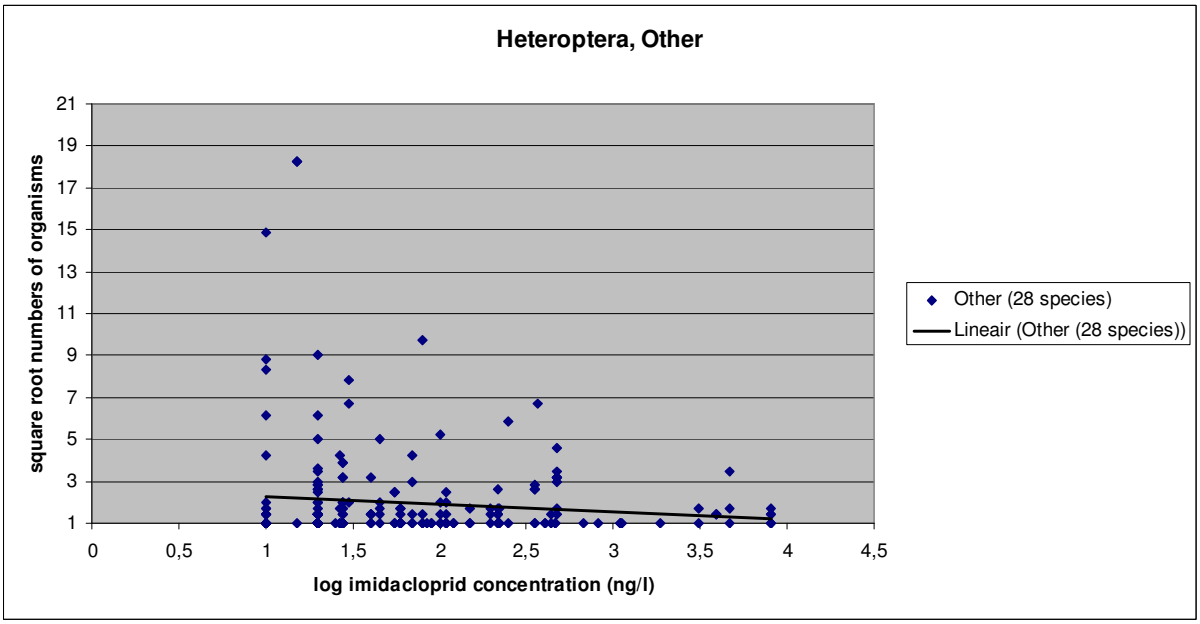


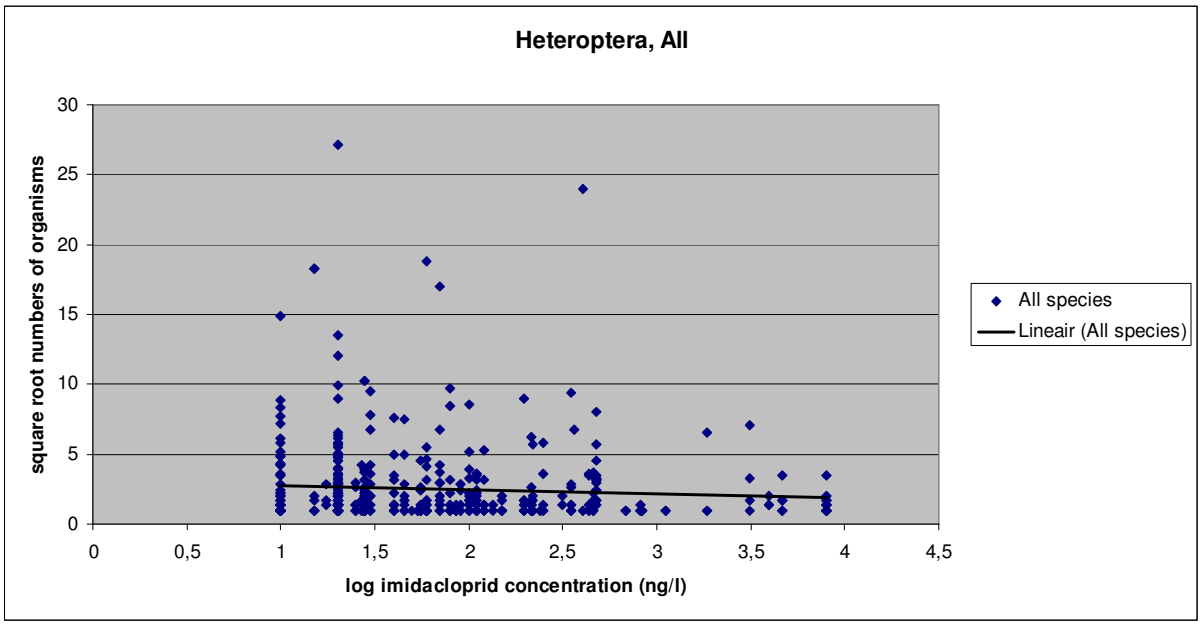


Coleoptera				
Species	*N. clavicornis*	*H. fluviatilis*	*E. testaceus*	*N. crassicornis*
Regression	*r* = -0.18148	*r* = -0.11729	*r* = -0.11127	*r* = -0.16467
Significance	*p* (uncorr) = 0.20719	*p* (uncorr) = 0.51566	*p* (uncorr) = 0.55124	*p* (uncorr) = 0.41178
Species	*H. lividus*	Other (67 species)	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

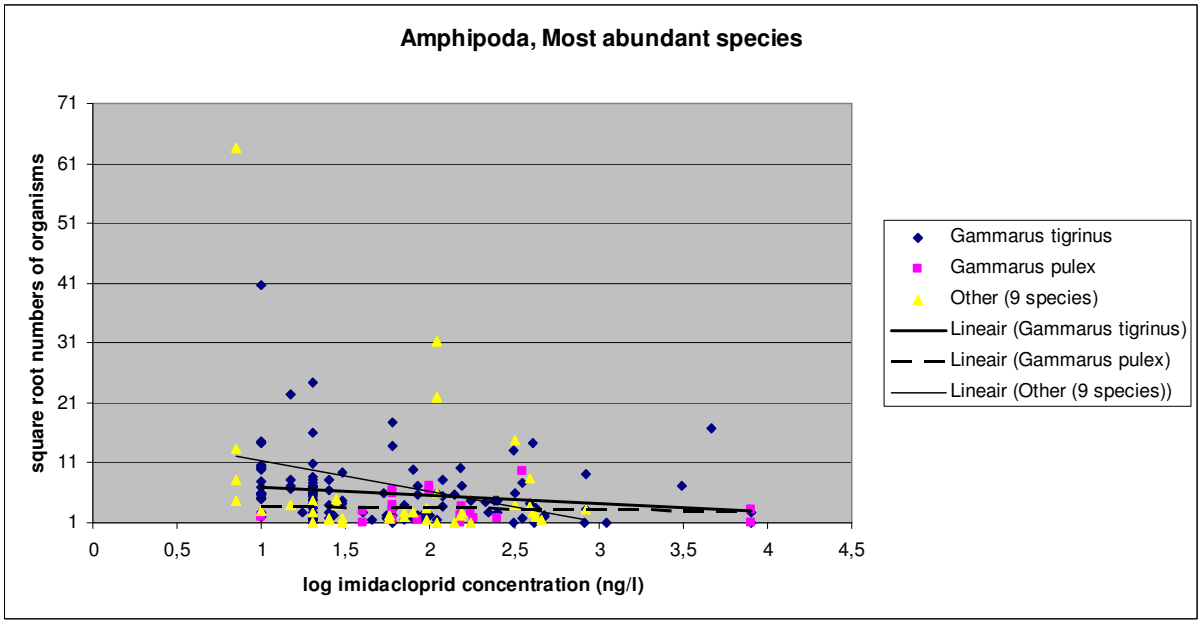


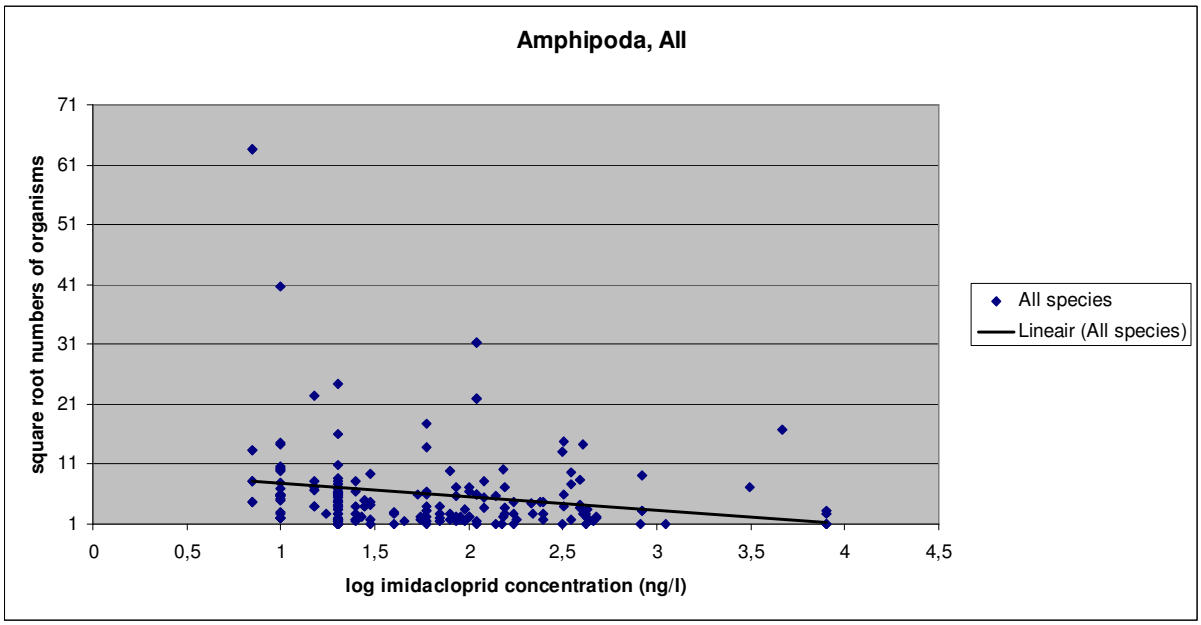




	Heteroptera			
Species	*S. striata*	*I. cimicoides cim.*	*M. scholtzi*	*P. minutissima min.*
Regression	*r* = -0.018234	*r* = 0.10923	*r* = 0.035921	*r* = -0.23284
Significance	*p* (uncorr) = 0.86397	*p* (uncorr) = 0.39806	*p* (uncorr) = 0.80643	*p* (uncorr) = 0.15373
Species	*N. glauca glauca*	Other (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	

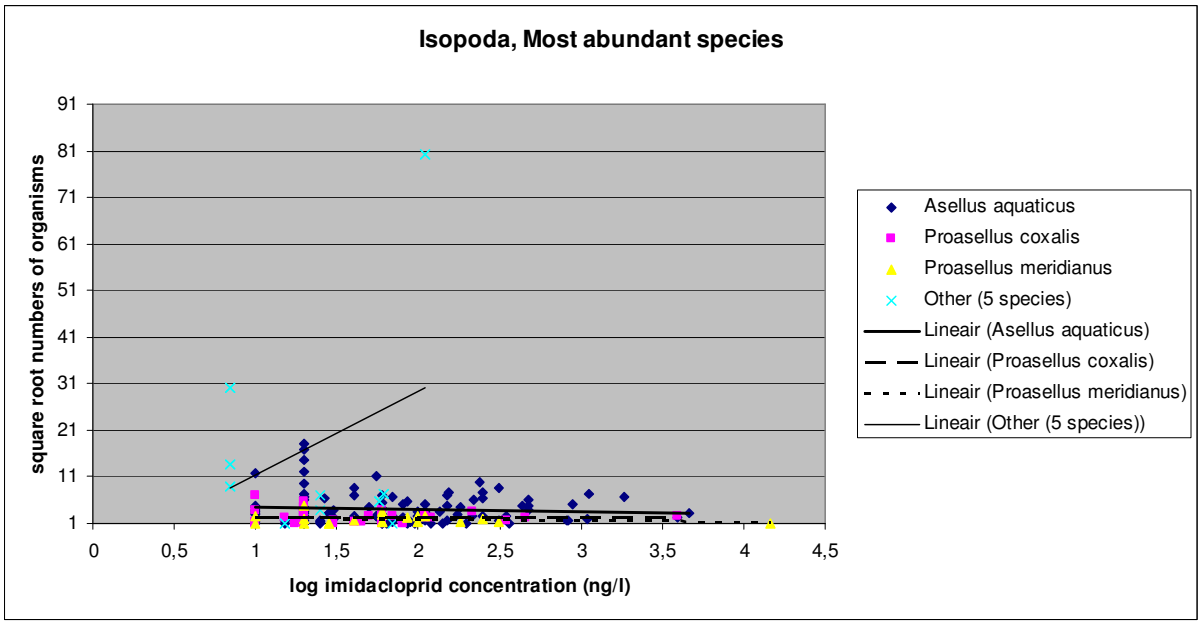
Amphipoda

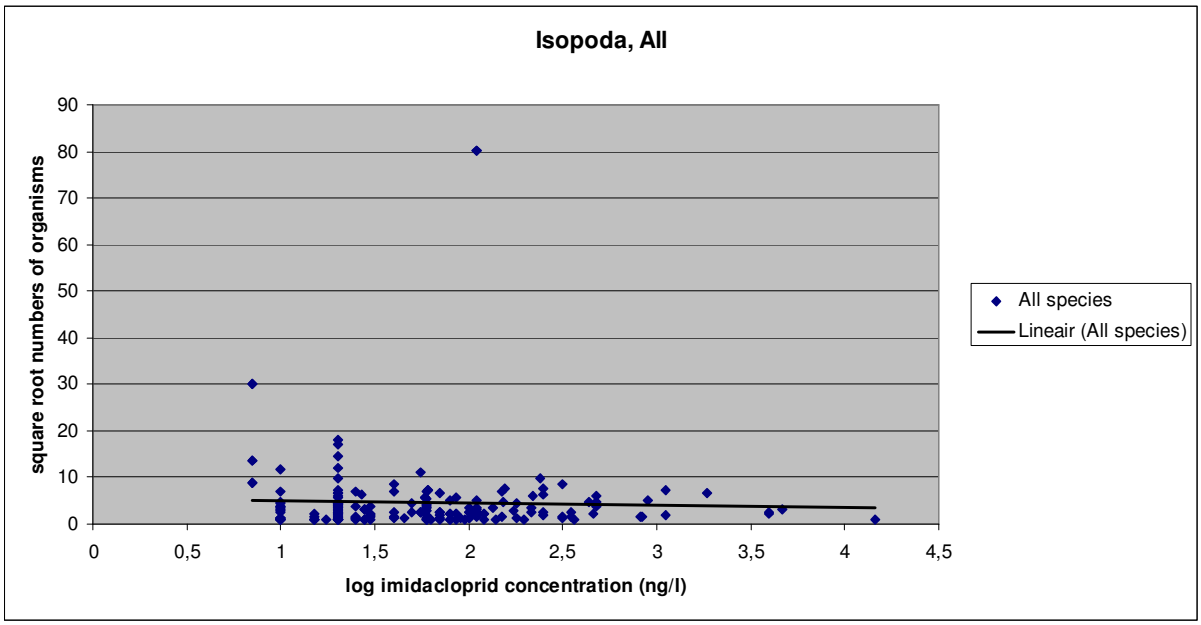




	Amphipoda			
Species	*G. tigrinus*	*G. pulex*	Other (9 species)	All
Regression	r = -0.16554	r = -0.053635	r = -0.23453	r = -0.18314
Significance	p (uncorr) = 0.06842	p (uncorr) = 0.8223	p (uncorr) = 0.08480	p (uncorr) = 0.00100

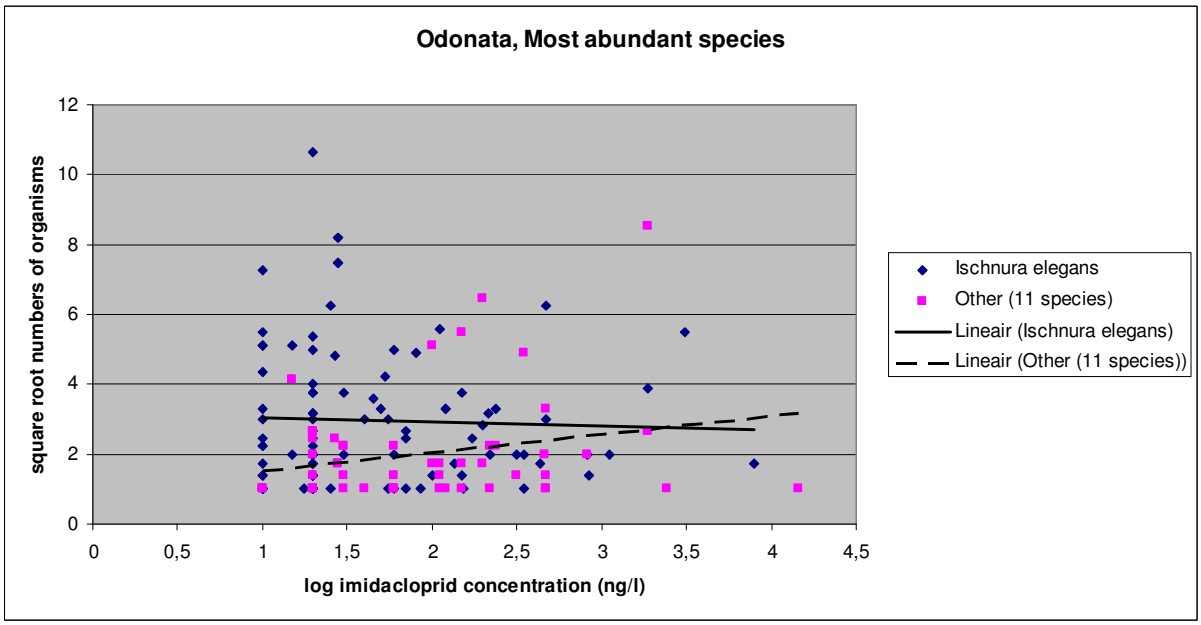
Isopoda

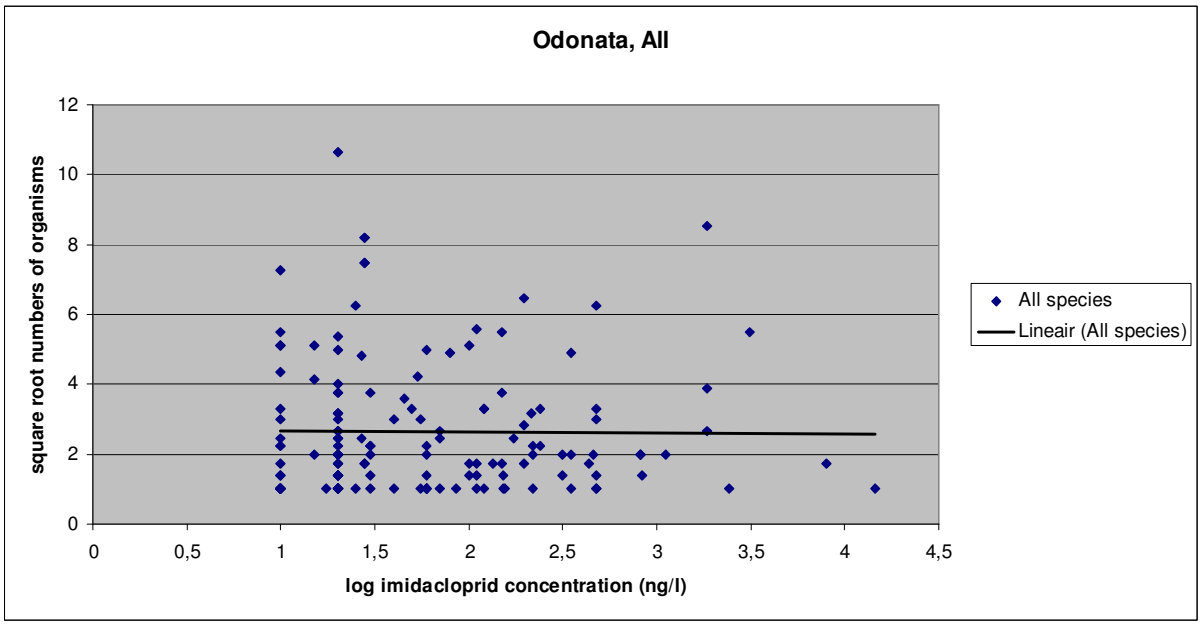




Isopoda			
Species	*A. aquaticus*	*P. coxalis*	*P. meridianus*
Regression	*r* = -0.078004	*r* = -0.031175	*r* = -0.21133
Significance	*p* (uncorr) = 0.41578	*p* (uncorr) = 0.84657	*p* (uncorr) =0.35779
Species	Other (5 species)	All	
Regression	*r* = 0.34309	*r* = -0.035866	
Significance	*p* (uncorr)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

Odonata





	Odonata		
Species	*I. elegans*	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