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Running Head: Water Quality Standards for Imidacloprid

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ABSTRACT

The neonicotinoid insecticide imidacloprid is among the pesticides that most frequently exceed current water quality standards in Dutch surface waters. Recent research shows that effects of imidacloprid on water organisms occur at concentrations below these standards. Mayflies appear to be particularly sensitive with chronic No Observed Effect Concentrations in the nanogram per liter range. The aim of this study was to derive updated water quality standards in accordance with the methodology of the European Water Framework Directive by evaluating the available recent literature on acute and chronic ecotoxicity of imidacloprid to aquatic organisms in laboratory and semi-field experiments. It is concluded that the standard for long-term exposure should be lowered to 8.3 nanogram per liter, the standard for short-term concentration peaks can be maintained at the current value of 0.2 microgram per liter. The European Commission set restrictions to the use of imidacloprid-based products to reduce the risks for bees and the Dutch national authorities issued emission reduction measures to protect aquatic life. Future monitoring data will ultimately reveal if these measures are sufficient to meet the newly proposed standards.

Key Words: imidacloprid, water quality standards, aquatic toxicity.

INTRODUCTION

The neonicotinoid insecticide imidacloprid is among the pesticides that most frequently exceed current water quality standards in Dutch surface waters (De Snoo and Vijver 2012). Products based on imidacloprid are used for a variety of crops, including maize, beets, and various greenhouse crops. The compound is systemic, meaning that after uptake it is distributed throughout the whole plant, and exerts its toxicity to sucking and biting insects via sap or leaves consumption. The products can be applied in various ways, *e.g.*, via seed and bulb treatment, by addition to nutrient solution or compost, by dripping or pouring, and via spray application. Authorized uses also include household biocide applications for ant and fly control and veterinary use in flea collars.

In 2013, the European Commission restricted the use of imidacloprid and two other neonicotinoid pesticides because the European Food Safety Authority (EFSA) identified potential high risks for bees due to exposure to dust from treated seeds, and from residues in pollen, nectar, or guttation fluid (EFSA 2013a,b,c). However, the European restrictions do not apply to imidacloprid use in greenhouses and full-field applications after flowering, and will not affect potential emissions to surface water from these sources, nor from the biocidal and veterinary applications, although the latter are likely to consist of lower tonnages.

In 2007, a literature review was carried out to update the then indicative Dutch environmental risk limit for imidacloprid and to derive water quality standards according to the European Water Framework Directive (WFD) (Posthuma-Doodeman 2008). A water quality standard in this context means the concentration of a chemical in surface water below which no unacceptable effects are expected to occur. The WFD distinguishes two types of water quality

standards. One is a long-term Environmental Quality Standard (EQS), expressed as an Annual Average concentration (AA-EQS) and normally based on chronic ecotoxicity data. This standard aims to protect the ecosystem against adverse effects resulting from long-term exposure. The other is a standard that aims to protect the ecosystem from short-term concentration peaks, referred to as a Maximum Acceptable Concentration EQS (MAC-EQS) and based on acute ecotoxicity data (EC 2011a). The AA-EQS should not only protect aquatic organisms, but should also provide protection for indirect exposure of humans and predatory birds or mammals via consumption of fish or shellfish. However, following WFD methodology, these routes are not relevant for imidacloprid in view of the absence of bioaccumulation potential (log K_{ow} 0.57; EC 2006). The current Dutch AA-EQS is 0.067 µg/L, based on the lowest No Observed Effect Concentration (NOEC) of 0.67 µg/L from a semi-chronic test with the midge *Chironomus tentans* (Anatra-Cordone and Durkin 2005) and applying an assessment factor of 10. The MAC-EQS is 0.2 µg/L, based on the NOEC from a mesocosm experiment (EC 2006) with an assessment factor of 3 (Posthuma-Doodeman 2008). The study with *C. tentans* was the only valid non-acute toxicity test with imidacloprid on insects that was available at that time.

During the past years, a large number of studies on aquatic ecotoxicity of imidacloprid have been published, probably because of the debate on the presumed relationship between the use of neonicotinoids and worldwide bee health decline. Among these studies are chronic laboratory tests with sensitive aquatic arthropod species such as *Hyalella azteca*, *C. tentans* (Stoughton *et al*. 2008), and *C. riparius* (Pestana *et al*. 2009a). The NOEC values published for these species are in the range of the NOEC used for standard setting. However, Alexander *et al*. (2007) showed that mayflies (Ephemeroptera) might be much more sensitive than the taxa tested

so far. The acute LC50 of 0.65 µg/L obtained for *Epeorus longimanus* is similar to the chronic NOEC for *C. tentans*, suggesting that much lower endpoints could be expected for mayflies when tested chronically. This was confirmed by Roessink *et al*. (2013), who found EC10-values of 24 and 33 ng/L for *Caenis horaria* and *Cloeon dipterum* after 28 days of exposure. Based on this information, the Dutch Ministry of Infrastructure and the Environment decided to update the water quality standards again and commissioned the National Institute for Public Health and the Environment (RIVM) to (re-)evaluate the available literature, including micro- and mesocosm studies, and, if necessary, to propose new values. This paper describes the process of data collection, evaluation, and standard derivation, and discusses the implications for water quality assessment for The Netherlands and other countries where imidacloprid is used.

METHODS AND MATERIALS

The methodology for deriving water quality standards is described in the *Technical Guidance for Deriving Environmental Quality Standards under the Water Framework Directive* (EC 2011a). The WFD-guidance builds on the guidance developed by the European Chemicals Agency (ECHA 2008) within the context of the European regulation for Registration, Evaluation and Authorisation of Chemicals (REACH). Additional national guidance was used for those aspects that were not (fully) addressed in the WFD-guidance (Brock *et al*. 2011; Smit *et al*. 2013; Van Vlaardingen and Verbruggen 2007). Basically, the derivation consists of a four-step approach: collection of literature, evaluation of the scientific reliability, selection of relevant endpoints, and derivation of the EQSs. Depending on the available data, the AA-EQS and MAC-EQS can be derived in three ways: by applying an Assessment Factor (AF) to the lowest

endpoint (AF-approach), by statistical extrapolation using Species Sensitivity Distributions (SSD-approach), and on the basis of micro-or mesocosm studies (model ecosystem approach). When enough data are available, all three methods have to be performed and the selection of the final value should be made based on expert judgment, taking into consideration the remaining uncertainty associated with, *e.g.*, the number of data available and the extrapolation of laboratory data to the field situation. Preference is given to the results from the SSD-approach or from model ecosystem-studies, since these entail a more robust approach towards assessing ecosystem effects (EC 2011a). In the present study, all three methods have been considered.

The starting point for collection of data was the 2008-report that includes data from the Draft Assessment Report prepared within the context of the former European pesticides directive 91/414/EEC (EC 2006) and public scientific literature until 2007. Additional literature published from 2007– August 2013 was collected using SCOPUS (http://www.scopus.com/). The Competent Authority Report (CAR) prepared for the evaluation of imidacloprid under the former European biocides directive 98/8/EC was also consulted (EC 2011b) as well as a Swiss report on water quality standards (Oekotoxzentrum 2013). The registration holder in The Netherlands for products based on imidacloprid (Bayer CropScience) provided an additional study (Roessink and Hartgers 2013).

All references were checked for relevant endpoints related to population health (*e.g.*, mortality, growth, reproduction) or ecosystem effects and evaluated with respect to scientific validity. For this, studies were rated with a Reliability index (Ri) of 1 to 4, following Klimisch *et al*. (1997). Ri 1 (reliable without restrictions) generally applies to studies according to international test guidelines, preferably performed according to Good Laboratory Practice (GLP)

with full documentation of data. Ri 2 (reliable with restrictions) relates to studies or data (mostly not performed according to GLP) in which the test parameters do not totally comply with the specific testing guideline or for which no guideline is available, but which are nevertheless well documented and scientifically acceptable. Ri 3 (not reliable) concerns studies with inadequate methodology and/or reporting, while Ri 4 is used for studies that do not give sufficient experimental detail, *e.g.*, data listed in summaries or reviews without further information. Laboratory studies were summarized in tables with explanatory notes regarding the reliability assessment (see Supporting Information 1).

Micro- and mesocosm studies were summarized and evaluated according to De Jong *et al.* (2008), who present a detailed checklist for summarizing and evaluating this type of studies. Key items in the evaluation are the representativeness of the aquatic community with respect to trophic levels, taxa richness, and abundance of potentially sensitive species, the experimental set-up, the exposure regime and the statistical and ecological evaluation of the observed effects in relation to the mode of action of the compound (see Supporting Information 2).

Because imidacloprid is susceptible to photolysis (EC 2006), special attention was paid to maintenance of exposure concentrations. The available laboratory data are inconclusive on the occurrence of photolysis under laboratory conditions. In some cases lower toxicity was found under light conditions as compared to darkness (*e.g.*, Sánchez-Bayo and Goka 2006), probably caused by a decrease in concentrations of imidacloprid as a result of photodegradation. Therefore, endpoints from studies performed under light were only accepted if analytical verification of test concentrations was included. Endpoints were based on actual concentrations if these deviated more than 20% from nominal.

For the AF- and SSD-approach, a single endpoint per species should be used as input, representing the most sensitive relevant parameter reported (EC 2011a). For any species, whenever multiple reliable values were available for the same endpoint obtained in similar tests with species from comparable life stages, the geometric mean of these values was taken as single endpoint per species. For any species, whenever reliable endpoints were available from tests with different durations, the most relevant duration was selected based on existing guidelines (Smit *et al*. 2013). For the purpose of quality standard derivation, tests with active substances are preferred. The reason for this is that potential side effects of formulations are assumed to be limited to edge-of-field surface waters immediately after emission, and may thus be less relevant for larger water bodies. To decide whether or not similar results from ecotoxicity studies with formulated products and active substance could be pooled into a geometric mean, an arbitrary cut-off criterion was used. If the difference between (no) effect values was a factor of 3 or less, the data were pooled. Otherwise the value for the active substance was taken forward, even when this was higher than that obtained for the product. However, if the most critical test result for a species was obtained in a test with a formulated product, and no value was available for a comparable endpoint from a test with the active substance, the result obtained with the formulation was used. Detailed information on data treatment can be found in EC (2011a), Brock *et al*. (2011), Smit *et al*. (2013), and Van Vlaardingen and Verbruggen (2007).

RESULTS AND DISCUSSION

Laboratory Toxicity Data

A total of 215 acute and 106 chronic ecotoxicity results were collected, the vast majority from studies with freshwater organisms (for full dataset, see Supporting Information 1). Valid acute data are presented in Table 1, including L(E)C50 values for 29 species of freshwater bacteria, algae, crustaceans, insects, fish and annelids, and for a marine crustacean, mollusk (unbound value) and fish. A total of 19 valid chronic NOEC or L(E)C10 values were obtained for algae, crustaceans, insects, a marine mollusk (unbound value), and fish (Table 2). Toxicity data for freshwater and marine species were pooled since there are no indications of a difference in sensitivity between freshwater and marine organisms of the relevant taxonomic groups (EC 2011a).

Acute toxicity data are presented in Figure 1, where bound L(E)C50-values for different taxonomic groups are plotted on a log-scale. From the data in Table 1 and Figure 1 it is clear that there is a large variation in sensitivity among the species tested, between taxa as well as within taxa. Within a taxon, even closely -related species show large differences in sensitivity towards imidacloprid, despite similar life-forms and feeding strategies (see, *e.g.*, *Daphnia magna* and *Ceriodaphnia dubia*, *Gammarus pulex*, and *G. roeseli*). Crustaceans and insects are overall most sensitive. Based on the single value for *Lumbriculus variegatus*, annelids may also belong to the sensitive taxa. Within the group of aquatic insects, Ephemeroptera (represented by the mayflies *Caenis horaria*, *Cloeon dipterum*, and *Epeorus longimanus*) and Diptera (represented by the midges *Chironomus dilutus* and *C. tentans*, and the blackfly *Simulium vittatum*) are most sensitive. The midge *Chaoborus obscuripes* seems to be an exception with a rather high acute

EC50 in comparison to the other midges, but the chronic toxicity result for this species is low (see Table 2).

The selected bound chronic results per species are presented in Figure 2. The previously used semi-chronic 10-days NOEC for *C. tentans* of 0.67 µg/L could be replaced by a NOEC of 0.42 µg/L from a test with a longer duration (28 days). The LC10-values of 14.5 µg/L for *Pteronarcys dorsata* and 34 µg/L for *Tipula* sp. originate from a 14-days test, which is shorter than the minimum test duration for chronic tests with arthropods. However, because larvae were tested it was considered justified to include the data in the chronic dataset. The NOEC of ≥ 5.0 µg/L for *Sericostoma vittatum* was also obtained with larvae, but this test lasted only 6 days. Since the result is a '≥-value', the result was not used directly in the calculation of the AA-EQS but is included in the table to show that valid data for this particular species are present.

The chronic data show a similar, high variation in sensitivity among species as observed in the acute dataset. Again, crustaceans and insects represent the sensitive species groups, but the ranking of individual species as regards their relative sensitivity differs between the acute and chronic dataset. Based on acute and chronic data, *D. magna* is least sensitive while *C. dipterum*, *C. horaria* and *C. tentans* are the most sensitive. In between, species switch positions when comparing the acute and chronic data. This emphasizes the importance of testing a range of species within a taxon. In addition, the comparison of acute and chronic effect concentrations points at the high Acute-to-Chronic Ratio (ACR) for imidacloprid. For those species for which both an acute L(E)C50 and a chronic NOEC or L(E)C10-value are available, the ratio between the two values ranges from 16 for *C. tentans*, to 143 for *C. obscuripes*. This indicates that the factor of 10, which is usually assumed to cover the difference between acute L(E)C50-values and

chronic NOECs, underestimates the effects of prolonged exposure to imidacloprid. The time-cumulative effect of imidacloprid was pointed out by Tennekes (2010) and Tennekes and Sánchez-Bayo (2013) and a high ACR was also demonstrated for other species (Charpentier *et al*. 2014). Although these studies mostly refer to lethal effects, they underpin the conclusion of Roessink *et al*. (2013), that acute studies are not appropriate to assess the effects of long-term exposure to imidacloprid. It also indicates that semi-field studies should be critically evaluated with respect to exposure time, because effects may be underestimated if exposure duration has been too short. In general, chronic studies are considered indispensable for derivation of any AA-EQS and consideration should be given to critical ecological traits of the test species compared to relevant field species.

Microcosms, Mesocosms and Other Studies

A total of 15 bioassay experiments and micro/mesocosm studies were collected. Some of them were indoor, single, or multiple species tests under more realistic conditions (Böttger *et al.* 2013; Kreutzweiser *et al*. 2007, 2008), but did not examine the effects on whole aquatic communities. If valid, results of such tests were added to the laboratory dataset. Other (semi-)field studies were not included because they were performed in rice paddy test systems with application types that are not relevant to the Dutch situation, *e.g.*, by using nursery boxes or lysimeters with treated seedlings (Hayasaka *et al*. 2012a,b; Jinguji *et al*. 2013; Sánchez Bayo and Goka 2005, 2006b). It is noted, however, that these studies confirm the outcome of the other valid and relevant micro/mesocosm studies. These latter are summarized in Table 3 and briefly discussed below (for full summaries, see Supporting Information 2).

Study 1. This outdoor pond study with two applications of Confidor 200 SL at a 21-days interval was included in the European authorization of imidacloprid (EC 2006; Brock 2005; Ratte and Memmert 2003). Effects were found on community parameters such as taxa richness, diversity, similarity and principal response of the community, with Chironomidae and Baetidae being the most sensitive. The NOEC was established as 0.6 µg a.s./L based on initial concentrations. Decline of concentrations was moderately fast, the DT50 for dissipation from the water phase ranged from 5.8 to 13.0 days (average DT50 8.2 days) and 12–20% of the nominal concentrations was present in the water phase just before the second application. According to criteria given by Brock *et al*. (2011) and EFSA (2013d), this study may be used to derive acute and chronic risk limits, because exposure was characterized by peak exposure (relevant for the MAC-EQS), while concentrations of imidacloprid in between applications were sufficiently maintained (relevant for the AA-EQS). However, according to the European assessment, the variability in insect species sensitivity to imidacloprid was not fully covered in this study, and the most sensitive taxon of the current laboratory dataset, Ephemeroptera, was not adequately represented. EFSA (2008) advised to use a safety factor of 1–3 when deciding on authorization of products based on imidacloprid.

Study 2. Colombo *et al*. (2013) treated outdoor pond enclosures with three applications of technical imidacloprid at 0.6 to 40 µg/L at a 7-days interval. Clear effects on abundance and emergence of several macroinvertebrate taxa were observed at the two highest initial concentrations of 17.3 and 40 µg/L. Ephemeroptera were most sensitive and showed effects on emergence at 3.2 µg/L, no significant effects were present at 1.4 µg/L. Imidacloprid disappeared

rapidly from the water phase with a DT50 of 28 hours, consequently the study was only considered for derivation of the MAC-EQS.

Study 3. Alexander *et al*. (2008) exposed benthic communities in outdoor artificial streams to a single 12-hours pulse of Admire 240 g/L at 0.1 to 10 µg a.s./L or to a 20-days continuous treatment with 0.1 to 1 µg a.s./L. The 12-hours NOEC for the pulse treatment was established as 3.9 µg a.s./L (actual measured) based on effects on emergence and abundance of the mayfly species *Epeorus* spp. (Ephemeroptera: Heptageniidae). For *Baetis* spp. (Ephemeroptera: Baetidae), the NOEC was ≥ 9.1 µg a.s./L (actual measured). For the continuous treatment, the 20-days NOEC for emergence of *Epeorus* spp. was 0.1 µg a.s./L, the NOEC for *Baetis* spp. was 0.3 µg a.s./L, based on measured concentrations. In both treatments, significant effects on adult thorax and/or head length were observed at the lowest concentration of 0.1 µg a.s./L. Although the ecological implications of reduced head- or thorax length are not clear, the authors point at a potential impact on, *e.g.*, mating success. The exposure duration of 12 hours for pulse treatment and 20 days for continuous treatment is shorter than the duration of the laboratory tests used for derivation of the MAC- and AA-EQS, respectively. Moreover, species and community interactions were not reported. Consequently, the study could only be used as additional information.

Study 4. Pestana *et al*. (2009b) exposed benthic macroinvertebrates and periphyton in outdoor artificial stream mesocosms to three 24-hour pulses of Admire 240 g/L at 2 and 20 µg a.s./L at an interval of 7 days. Observations were made after the last pulse. The high dose caused a significant reduction (69%) in combined Ephemeroptera, Plecoptera and Tricoptera taxa, Oligochaetes were sensitive as well. Coleoptera were less affected (ca. 29% reduction). No

effects were seen on Chironomidae. The NOEC was set to the average measured concentration of imidacloprid over the 24-hours exposure time at the low dose, which was 1.63 µg/L. This treatment level was considered for derivation of the MAC-EQS, taking account of the fact that exposure duration was shorter than in the laboratory studies used for MAC-derivation.

Study 5. Berghahn *et al*. (2012) and Mohr *et al.* (2012) incubated straw litterbags in reference streams. After colonization the collected invertebrates were exposed to two series of three weekly 12-hour pulses of imidacloprid (99.9% pure) at 12 µg/L in indoor stream mesocosms. They observed significant effects on several insect taxa, with Ephemeroptera (affected after single pulse), Trichoptera (id.), Chironomidae and Gammaridae being most sensitive. Consequently the NOEC of this study was set to < 12 µg/L. The systems were re-stocked with aquatic organisms before the second pulse series. This is a kind of recolonization that under natural conditions is only possible when an undisturbed community is present upstream. This makes the study less relevant for EQS-derivation. Again, the exposure duration was shorter than in the laboratory studies used for MAC-derivation.

Study 6. Roessink and Hartgers (2013) treated outdoor enclosures that were additionally stocked with *C. dipterum*-larvae with two applications of imidacloprid SL 200 at 0.097 to 3.8 µg/L at a 21-days interval. Abundance was followed until 37 days after application. The timing of application (October) did not allow for assessment of reproduction and emergence. About 40% of the initial concentration was present in the water phase just before the second application, exposure can therefore be considered chronic. A decrease in abundance was observed in one of the replicates of the 3.8 µg a.s./L treatment. Although this decrease was not significant and not consistent with the other replicates, the authors considered it as a treatment -

related effect and set the NOEC to 1.52 µg a.s./L nominal. This is much higher than the 28-days laboratory EC10 for immobility of 0.033 µg/L reported for the same species by Roessink *et al.* (2013) (see Table 2). A possible explanation could be that the summer generation that was used in the laboratory test is more sensitive than animals preparing for overwintering. A comparison between spring and autumn collected animals was made in an acute study with *G. roeseli* (Böttger *et al.* 2012), but no conclusions could be drawn from this experiment because test water and feeding were varied as well. It was concluded that the NOEC of outdoor study 5 should not be used to replace the lower endpoints observed for mayflies in the other laboratory and outdoor experiments.

Derivation of the MAC-EQS for Peak Exposure

AF-approach

According to the WFD-guidance, the MAC-EQS may initially be derived by applying an assessment factor of 100 to the lowest acute L(E)C50-value; this factor can be lowered to 10 if the compound has a known mode of toxic action and representative species for the most sensitive taxonomic group are included in the dataset (EC 2011a). This is the case for imidacloprid and using the lowest EC50 of 0.65 µg/L for *E. longimanus*, this results in a MAC-EQS_{AF} of 0.065 µg/L (65 ng/L).

SSD-approach

For using SSDs, the WFD- and REACH-guidance require that the database contains preferably more than 15, but at least 10 datapoints, from different species covering at least eight

specified taxonomic groups (EC 2011a; ECHA 2008). The acute dataset does not fully cover the specified taxa, since data on aquatic macrophytes are missing. However, because imidacloprid is an insecticide with a specific mode of action, and other primary producers are clearly not sensitive, it was considered justified to use the SSD-approach without macrophytes. Shown in Figure 3 is the acute SSD constructed with the program *ETX* 2.0 (Van Vlaardingen *et al*. 2004) fitting all available acute data to a log-normal distribution. It is apparent that there is a distinction between bacteria, algae and fish at the upper right hand side of the distribution, and crustaceans and insects at the lower left hand side. The overall fit is poor and the assumption of a normal distribution is rejected by the tests included in the *ETX*-package (Anderson-Darling, Kolmogorov-Smirnov, Cramer von Mises).

If a clear distinction in sensitivity exists, the WFD-guidance offers the option to construct an SSD for the taxa that are most sensitive in line with the mode of action. Because the data for insects and crustaceans overlap, both groups were included in such a specific SSD. An exception was made for *D. magna*. According to EFSA (2013d), when differences in sensitivity are 1 or 2 orders of magnitude (factor 10–100), care should be taken for a bias in the effect assessment due to insensitive species. The endpoint for *D. magna* was left out because the EC50 is more than 3000 times higher than the geometric mean of all arthropods (including *D. magna*). The resulting SSD is shown in Figure 4. The median estimate of the HC5 is 0.36 µg/L (95% confidence interval 0.09 and 0.97 µg/L). This is almost a factor of 2 lower than the lowest available endpoint (0.65 µg/L for *E. longimanus*). The WFD-guidance recommends to apply a default assessment factor of 10 to the HC5 when L(E)50 data are used in a generic SSD; this factor should account for the extrapolation from a 50% effect level to the no-effect level associated with the MAC-

EQS, and cover remaining uncertainty regarding the extrapolation from a laboratory-based SSD to the field situation.

No guidance is given, however, which assessment factor should be used in case a specific SSD is constructed for the potentially most sensitive species group(s). A lower assessment factor may be sufficient because including particularly sensitive species reduces uncertainty, but the factor should still correct for the extrapolation from 50% effect to no effect, and for the extrapolation from lab to field. Taking this into account, Brock *et al*. (2011) proposed an assessment factor of 6 for this situation. Using this value a MAC-EQS_{SSD} of 0.06 µg/L was derived, which is slightly lower than the value obtained by the AF-approach. Given the position of the two lowest data points on the right hand side of the SSD-curve (see Figure 4), the HC5 is probably worst case.

Considering the fact that at the level of the MAC-EQS no effects should occur after short-term exposure, using acute L(E)C10-values instead of L(E)C50-values would be most appropriate for derivation of this standard. For the 10 aquatic arthropods tested by Roessink *et al*. (2013), the acute LC10 ranges from 2.55 to > 10,000 µg/L, while the acute EC10 ranges from 0.1 to 223 µg/L. The HC5 based on acute EC10-values was reported as 0.084 µg/L by the authors. Leaving the EC10 for *Gammarus pulex* and *Micronecta* sp. out of consideration because of high control mortality, the remaining eight EC10-values would lead to an HC5 of 0.05 µg/L. This value is very similar to the above derived MAC-EQS_{SSD} of 0.06 µg/L based on acute L(E)C50-values with an assessment factor of 6.

Mesocosm-approach

ACCEPTED MANUSCRIPT

The available micro- and mesocosm studies were summarized above. Five studies were considered reliable and potentially useful for derivation of the MAC-EQS: outdoor pond study 1 (two applications, moderately fast decline of imidacloprid concentrations between applications), outdoor pond enclosure study 2 (three applications, fast dissipation from the water phase), outdoor stream study 3 (single 12-hours pulse application), outdoor stream study 4 (repeated 24-hours pulse application), and indoor stream study 5 (repeated 12-hours pulse application). Below, the use of these studies for derivation of the MAC-EQS is discussed in the context of exposure duration and ecological reality.

When using mesocosm data for derivation of water quality standards it should first be decided how to express the NOEC from such a study. When concentrations decline during the experiment, using the initial concentration may underestimate the risk since in reality the organisms have been exposed to lower concentrations. EFSA (2013d) advises to use the time window of the critical laboratory tests for calculation of the time weighted average (TWA) concentration after the highest peak in the NOEC-treatment. Similarly, based on the duration of acute ecotoxicity tests, Brock *et al.* (2011) proposed to use the 48-hours TWA concentration in the NOEC-treatment for derivation of the MAC-EQS_{MESO}. For the outdoor pond study (study 1), the NOEC was set to 0.6 µg/L (initial), which is equivalent to a 48-hours TWA of 0.51 µg/L. Expressed as a 48-hours TWA concentration, the NOEC of the outdoor pond enclosure (study 2) equals 0.82 µg/L. The NOECs from stream mesocosms with single or repeated 12–24 hours pulse applications were 3.9, (study 3), 1.63 (study 4) and < 12 µg/L (study 5), respectively, based on concentrations during the pulses. The WFD-guidance proposes to put an assessment factor of 5 on the lowest NOEC of a single valid mesocosm. Based on a comparison of multiple studies,

Brock *et al*. (2011) argued that lower factors are sufficient and suggested an assessment factor of 2–3 in case of a single application design, and a factor of 1–2 when multiple applications are used. The lower factors of these ranges (2 for single applications, 1 for multiple applications) may be applied when more studies are available, as is the case here. These factors are in line with recommendations of EFSA (2013d).

According to the EU risk assessment (EC 2006), pond study 1 did not fully address the variability in insect species sensitivity, and Ephemeroptera were not adequately represented. This taxon was, however, included in the other studies, but the exposure duration in the stream studies (studies 3–5) was shorter than the minimum standard test duration for arthropods of 48 hours. Together, this would be a reason not to use the lowest assessment factor. Both pond study 1 and pond enclosure study 2 involved multiple applications, which would be a reason for a lower assessment factor. In pond study 1, however, the application interval was large and effects were already present after the 1st application. This was also the case in the indoor stream study that delivered the NOEC of < 12 µg/L (study 5). The NOEC of 1.63 µg/L (stream study 4) was obtained after multiple applications, but it cannot be judged if a single pulse would have resulted in a higher NOEC.

In addition, the NOEC for effects on thorax and/or head length of *Baetis* ssp. and *Epeorus* ssp. was < 0.1 µg/L. Although the ecological consequences are not clear, this as a reason for concern. Based on these arguments, it was decided to use the lowest NOEC of 0.51 µg/L with the higher assessment factor of 3 proposed by Brock *et al*. (2011) and set the MAC-EQS_{MESO} to 0.17 µg/L. This is still higher than the NOEC for thorax/head length, and also higher than the 96-hours laboratory EC10 for *C. dipterum* of 0.1 µg/L reported by Roessink *et al*. (2013). However,

the other 96-hours EC10-values reported by Roessink *et al*. (2013) are a factor of 2 or more higher, and the lowest 96-hours LC10 of 2.55 µg/L for *C. horaria* is a factor of 15 higher than this MAC-EQS_{MESO}.

Selection of the MAC-EQS

The MAC-EQS_{AF} is 0.065 µg/L, the MAC-EQS_{SSD} is 0.06 µg/L, and the MAC-EQS_{MESO} is 0.17 µg/L. The difference between lowest and highest value is a factor of 2.8. The SSD-based MAC-EQS is similar to the value obtained with the AF-approach. As indicated above, the MAC-EQS should preferably be based on the SSD- or mesocosm-approach. The MAC-EQS_{SSD} of 0.06 µg/L is similar to the HC5 based on acute EC10-data, but it is lower than the lowest acute EC10 of 0.1 µg/L reported by Roessink *et al*. (2013) and more than a factor of 5 lower than the other acute EC10-values. Considering the acute LC10-values, the difference is more than a factor of 40. As shown above, the MAC-EQS_{MESO} of 0.17 µg/L is protective for almost all species when considering the acute EC10-values of Roessink *et al*. (2013) and 15 times lower than the lowest acute LC10. Since the mesocosms represent the most ecologically relevant way of exposure and effects testing, preference was given to the mesocosm-based MAC-EQS, and it is concluded that the current Dutch MAC-EQS of 0.2 µg/L can be maintained. This value is twice as high as the Swiss proposal for the MAC-EQS of 0.1 µg/L (Oekotoxzentrum 2013), based on the acute EC50 for *Cypretta seuratti* (see Table 1) with an assessment factor of 10. The Swiss assessment did not include SSDs as an option, the mesocosm studies were not considered because they were performed with formulated products rather than with the active substance.

Derivation of the AA-EQS for Long-term Exposure

AF-approach

According to the WFD- and REACH-guidance (EC 2011a; ECHA 2008), an assessment factor of 10 can be applied to the lowest EC10 of 0.024 µg/L for the mayfly *C. horaria* because chronic NOEC or L(E)C10-values are available for algae, *Daphnia*, and fish, and the acutely most sensitive taxon is included in the chronic dataset. This results in an AA-EQS_{AF} of 0.0024 µg/L (2.4 ng/L).

SSD-approach

The taxa represented in the chronic dataset (Table 2) do not meet the criteria of the WFD-guidance for constructing a generic SSD. However, based on the same considerations as presented above for the derivation of the MAC-EQS, constructing a specific SSD was considered for derivation of the AA-EQS. Insects and crustaceans were combined into one dataset for arthropods, and *D. magna* was left out since the NOEC for this species is over 900 times higher than the geometric mean of all arthropods. The SSD is shown in Figure 5. The median estimate of the HC5 is 0.025 µg/L (95% confidence interval 0.002–0.1 µg/L), which is similar to the lowest NOEC (0.024 µg/L for *C. horaria*). The WFD- and REACH guidance recommend to apply a default assessment factor of 5-1 to the HC5 when chronic NOEC/L(E)10 data are used in a generic SSD (EC 2011a; ECHA 2008). However, a lower assessment factor may be appropriate in case a specific SSD is constructed for the potentially most sensitive species groups. For this, a default assessment factor of 3 was proposed by Brock *et al*. (2011), which is consistent with EFSA (2013d). The dataset is limited and does not meet the requirements of a generic SSD and

the number of data points for sensitive taxa is only just above the minimum of 10. Although the data cover the species groups that have consistently been shown to be sensitive, the high ACR is an indication that if other acutely sensitive species would have been tested chronically, a number of relatively low endpoints might be added to the chronic dataset. This would potentially lead to a lower HC5 and favors the use of a higher assessment factor. On the other hand, the results of the mesocosm- and related studies, although not considered adequate as a direct basis for AA-EQS (see below), indicate that the assessment factor of 3 as proposed by Brock *et al*. (2011) might be sufficiently protective for the sensitive aquatic taxa. Using this factor, the AA-EQS_{SSD} is 0.0083 µg/L (8.3 ng/L).

Mesocosm-approach

Two studies were available in which chronic exposure was sufficiently maintained: outdoor pond study 1 and outdoor stream study 3 (see Table 3). Following EFSA (2013d), the NOEC of the pond study was expressed as the 28-days TWA-concentration, being 0.23 µg/L, based on the duration of the critical laboratory test with *C. horaria*. Mayflies were not adequately represented in this study, and a lower NOEC of 0.1 µg/L was derived for the Ephemeroptera *Epeorus* spp. and *Baetis* spp. in the stream study. Species or community interactions were not included in this study and the duration of exposure was 20 days, which is shorter than in the critical laboratory studies (28 days). Given the high ACR of imidacloprid for insects, longer exposure may have led to increased effects. In addition, the NOEC for effects on thorax and/or head length of *Baetis* sp. and *Epeorus* sp. was <0.1 µg/L. In view of the available

information, it was not considered justified to use the mesocosm studies directly for derivation of the AA-EQS.

Selection of the AA-EQS

The AA-EQS$_{AF}$ is 0.0024 µg/L (2.4 ng/L), the AA-EQS$_{SSD}$ is 0.0083 µg/L (8.3 ng/L). The difference is a factor of 3.5. The WFD-guidance gives preference to an SSD-based AA-EQS since this is a more robust approach towards ecosystem effects; it was therefore decided to set the AA-EQS to 0.0083 µg/L (8.3 ng/L). This is a factor of 8 lower than the current Dutch AA-EQS (0.067 µg/L). Being a factor of 3 below the lowest laboratory NOEC for mayflies and a factor of 12 lower than the NOEC that was observed for the same taxon in the stream mesocosm, the new AA-EQS is considered protective for effects on the most sensitive taxa in the current dataset. The value is in line with the Swiss proposed EQS of 0.013 µg/L (13 ng/L) (Oekotoxzentrum 2013). The U.S. Environmental Protection Agency (USEPA) uses a chronic toxicity benchmark of 1.05 µg/L (OPP 2014), which seems rather high given the fact that the acute LC50 for some insect species is below this value (see Table 2). Canada uses a value of 0.23 µg/L, based on a 28-day EC15 for *C. riparius* with a safety factor of 10 (CCME 2007). The LOEC used to derive the Canadian standard is based on initial concentrations in the water phase of a water/sediment study (EC 2006), and these are likely to overestimate the actual exposure concentrations in the water phase during the test. When based on actual concentrations in the water phase, the LOEC would probably be much lower. All cited standards have been derived before the mayfly data of Roessink *et al.* (2013) became available.

Implications of the New Standards

Monitoring data for imidacloprid in The Netherlands are presented in the Dutch Pesticide Atlas (CML and RWS-WVL 2014). Concentrations at individual sampling locations frequently exceed current water quality standards. In 2012, the MAC-EQS of 0.2 µg/L was exceeded at 45 out of 451 locations (10%), the AA-EQS of 0.067 µg/L was exceeded at 54 out of 451 monitoring locations (12%). Exceedance is detected the whole year round, but less in winter (CML and RWS-WVL 2014). Kreuger *et al.* (2010) measured pesticide residues in samples from six water courses in a greenhouse horticulture area in Sweden and detected imidacloprid in 39% of the samples, the highest concentration being 15 µg/L. Concentrations of 39 and 89 µg/L were found in drainage water from greenhouses. Widespread occurrence of imidacloprid is also confirmed for regions outside Europe. In a survey of rivers around Sydney, Sánchez-Bayo and Hyne (2014) detected imidacloprid in 93% of the samples, with concentrations up to 4.6 µg/L in the vicinity of a turf farm. Starner and Goh (2012) analyzed 75 surface water samples from agricultural areas in California in 2010-2011, and detected imidacloprid in 89% of the samples. Maximum concentrations were between 1.38 and 3.29 µg/L and the authors report that 19% of the samples exceeded the USA toxicity benchmark of 1.05 µg/L, while 73% and 88% of the samples exceeded the current Dutch AA-EQS of 0.067 µg/L and MAC-EQS of 0.2 µg/L, respectively (Starner and Goh 2012). Comparing concentrations in single samples with the AA-EQS is not fully justified, since this should be done on the basis of the annual average concentration per location. However, at one sampling location Starner and Goh (2012) found concentrations between 0.162 and 0.488 µg/L in monthly samples taken from May to August, suggesting that exposure was above the critical level for a longer period of time. Similarly,

Lamers *et al*. (2011) detected imidacloprid on six consecutive sampling dates between April and June when monitoring river water in a rice cultivation area in Northern-Vietnam. Imidacloprid concentrations of about 0.5 μg/L were reported shortly after pesticide application, and mean measured concentrations were around 0.2 μg/L, which is well above the proposed AA-EQS.

The available monitoring data indicate that the proposed water quality standards for imidacloprid are likely to be exceeded unless measures are taken to reduce emissions. Based on some of the recently published studies on aquatic arthropods that are also included in this paper, the Dutch board for the authorization of plant protection products and biocides (Ctgb) lowered the Regulatory Acceptable Concentration (RAC) and restricted the use of several imidacloprid-based products (Ctgb 2014a,b). Treatment of discharge water from greenhouses and further drift reduction measures for field applications were made compulsory. If applied correctly, these measures may lead to reduced emissions to surface water. However, due to differences in methodology and dataset, the RAC was set to a chronic HC5 of 0.027 μg/L without an assessment factor, and is thus a factor of 3 higher than the revised AA-EQS proposed in this study. Moreover, simultaneous or consecutive use of different products with the same active substance on different crops is not accounted for in the authorization procedure. This means that if a safe use is identified according to the provisions for authorization, this is no guarantee that the new WFD-water quality standards will be met in the field. The overall impact of the newly proposed standard on the assessment of Dutch surface water quality thus remains unclear until new monitoring data are available.

Van Dijk *et al*. (2013) linked the observed decline in abundance of some aquatic invertebrate taxa in The Netherlands to contamination of surface water by imidacloprid, and used

these ecological observations to motivate that a lower water quality standard be needed. In a recent response, Vijver and Van den Brink (2014) concluded that the status of aquatic ecosystems in the highly managed landscape of The Netherlands is the result of a complex suite of stressors, of which pesticides are one factor. Imidacloprid, although important in terms of ecological risks, is one of many pesticides being applied. They argue that water quality standards should not be solely based on field observations but should largely rely on the results of controlled experiments, in order to separate stress from a single pesticide from other stressors (*ibid*.). The present study confirms, based on the analysis of such experiments, that the current water quality standard for imidacloprid should indeed be lowered.

It is noted that both pesticide authorization and water quality assessment according to the WFD are performed on a substance-by-substance basis, and do not take into account the presence of other pesticides. In case of neonicotinoids, this is of particular importance because different active substances share a common mode of action. An initial assessment of the impact of combined exposure may be made by adding up the risk ratios of different pesticides found at a single location when comparing monitoring data with quality standards (Syberg *et al*. 2009; Teuschler and Herzberg 1995). If such an analysis points at a potential risk caused by a combination of multiple pesticides, risk mitigation should be focused on the package of compounds, rather than on single substances. For greenhouse applications, the treatment of discharge water issued for imidacloprid-based products will probably also lead to reduced emissions of other substances and potentially lower the combined exposure to pesticides.

CONCLUSIONS

Based on an up-to-date evaluation of acute and chronic laboratory studies and semi-field experiments, it is concluded that the water quality standard for long-term exposure to imidacloprid should be set to 8.3 ng/L. The standard for short-term peak exposure of 0.2 μg/L can be maintained. Based on these values, it is expected that imidacloprid will remain a problematic substance for Dutch water quality. Future monitoring data will ultimately reveal if the measures that were taken to reduce emissions are sufficient to meet the newly proposed standards. Since imidacloprid is only one of the large number of pesticides used, the presence of other pesticides should be taken into account when assessing water quality.

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Table 1. Selected aquatic ecotoxicity data for imidacloprid from acute toxicity studies with freshwater and marine species (indicated with sw). L(E)C50 in µg imidacloprid/L.

Taxon/species	**L(E)C50 [µg/L]**	**Reference**
Bacteria		
Vibrio fischerii	58876 [a]	Tišler *et al*. (2009)
V. qinghaiensis sp.	79255	Zhou *et al*. (2010)
Algae		
Desmodesmus subspicatus	389000 [b]	Tišler *et al*. (2009)
Pseudokirchneriella subcapitata	>100000 [c]	EC (2006)
Crustaceans		
Americamysis bahia (sw)	35.9 [d]	Anatra-Cordone and Durkin (2005), EC (2006)
Asellus aquaticus	119 [e]	Roessink *et al*. (2013)
Ceriodaphnia dubia	2.07	Roessink *et al*. (2013)
Chydorus sphaericus	832	Sánchez-Bayo and Goka (2006a)
Cypretta seuratti	1	Sánchez-Bayo and Goka (2006a)
Cypridopsis vidua	10 [e]	Sánchez-Bayo and Goka (2006a)
Daphnia magna	52455 [f]	EC (2006), Tišler *et al*. (2009)
Gammarus pulex	110 [e]	Ashauer *et al*. (2011)
Gammarus roesseli	1.94 [g]	Böttger *et al*. (2012)
Hyallella azteca	55	Stoughton *et al*. (2008)

Taxon/species	L(E)C50 [µg/L]	Reference
Ilyocypris dentifera	3 [e]	Sánchez-Bayo and Goka (2006a)
Insects		
Caenis horaria	1.77 [e]	Roessink *et al*. (2013)
Chaoborus obscuripes	284 [e]	Roessink *et al*. (2013)
Chironomus dilutus	2.65	LeBlanc *et al*. (2012)
Chironomus tentans	6.9 [h]	Stoughton *et al*. (2008)
Cloeon dipterum	1.02 [e]	Roessink *et al*. (2013)
Epeorus longimanus	0.65 [i]	Alexander *et al*. (2007)
Limnephilidae	1.79 [e]	Roessink *et al*. (2013)
Notonecta spp.	18.2 [e]	Roessink *et al*. (2013)
Plea minutissima	35.9 [e]	Roessink *et al*. (2013)
Sialis lutaria	50.6 [e]	Roessink *et al*. (2013)
Simulium vittatum	8.1 [j]	Overmyer *et al*. (2005)
Fish		
Danio rerio	227099 [k]	Tišler *et al*. (2009)
Leuciscus idus melanotus	237000	EC (2006)
Oncorhynchus mykiss	211000	EC (2006)
Cyprinodon variegatus (sw)	161000	Anatra-Cordone and Durkin (2005), EC (2006)
Molluscs		

Taxon/species	L(E)C50 [µg/L]	Reference
Crassostrea virginica (sw)	>145000 [l,m]	Anatra-Cordone and Durkin (2005), EC (2006)
Annelids		
Lumbriculus variegatus	6.2	Alexander *et al.* (2007)

a: geometric mean of 61900 and 56000 µg/L for tests with active and formulation; marine species tested in freshwater; b: test with active, endpoint for formulation >3 times lower; c: unbound values are not used for EQS-derivation, value included to show that species has been tested; d: geometric mean of 37.7, 34.1 and 36 µg/L from tests with active and formulation; e: lowest relevant endpoint, immobility; f: geometric mean of 30000, 85000, and 56600 µg/L, 48 h tests with formulation and active, endpoint immobility; g: most sensitive life-stage: spring collected early adults; h: geometric mean of 10.5 and 5.75 µg/L, lowest relevant endpoint from tests with active; i: endpoint from most relevant test duration; j: geometric mean of 6.75, 8.25 and 9.54 µg/L; k: geometric mean of 241000 and 214000 µg/L, tests with active and formulation; l: highest concentration without 50% effect; m: unbound values are not used for EQS-derivation, value included to show that species has been tested. For details on individual tests, see Supporting Information 1.

Table 2. Selected aquatic ecotoxicity data for imidacloprid from chronic toxicity studies with freshwater and marine species (indicated with sw). NOEC or L(E)C10 in µg imidacloprid/L.

Taxon/species	**NOEC/L(E)10 [µg/L]**	**Reference**
Algae		
Desmodesmus subspicatus	106000 [a]	Tišler *et al*. (2009)
Pseudokirchneriella subcapitata	<100000 [b]	EC (2006)
Crustaceans		
Asellus aquaticus	1.35 [c]	Roessink *et al*. (2013)
Daphnia magna	1768 [d]	Jemec *et al*. (2007)
Gammarus pulex	2.95 [c]	Roessink *et al*. (2013)
Hyallella azteca	0.47 [e,f]	Stoughton *et al*. (2008)
Insects		
Caenis horaria	0.024 [c]	Roessink *et al*. (2013)
Chaoborus obscuripes	1.99 [c,f]	Roessink *et al*. (2013)
Chironomus riparius	< 0.4 [c,g]	Pestana *et al*. (2009a)
Chironomus tentans	0.42 [f]	Stoughton *et al*. (2008)
Cloeon dipterum	0.033 [c]	Roessink *et al*. (2013)
Plea minutissima	2.03 [c]	Roessink *et al*. (2013)
Pteronarcys dorsata	14.5 [h,i]	Kreutzweiser *et al*. (2007, 2008)
Sericostoma vittatum	≥ 5.0 [f,i]	Pestana *et al*. (2009a)

Taxon/species	NOEC/L(E)10 [µg/L]	Reference
Sialis lutaria	1.28 [c]	Roessink *et al*. (2013)
Tipula sp.	34 [f,i]	Kreutzweiser *et al*. (2008)
Fish		
Danio rerio	300000	Tišler *et al*. (2009)
Oncorhynchus mykiss	1200 [j]	Anatra-Cordone and Durkin (2005)
Molluscs		
Crassostrea virginica (sw)	>23300 [b,k]	Anatra-Cordone and Durkin (2005) EC (2006)

a: test with active, endpoint for formulation >10 times lower; b: unbound values are not used for EQS-derivation, value included to show that species has been tested; c: lowest relevant endpoint, immobility; d: lowest relevant endpoint, number of neonates; geometric mean of 1250 and 2500; e: endpoint from most relevant test duration; f: lowest relevant endpoint, mortality; g: lowest relevant endpoint, development rate; h: geometric mean of 15.8 and 13.3, 14-d LC10; i: test duration semi-chronic; j: lowest relevant endpoint, growth; k: lowest concentration without effects. For details on individual tests, see Supporting Information 1.

Table 3. Summary of results from mesocosm and related tests. NOEC represents treatment level without significant effects, expressed in µg imidacloprid/L.

Study type	Treatment	NOEC [µg/L]	Critical effect	Reference
1. Outdoor pond	2 x 0.6 - 23.5 µg a.s./L, 21-days interval	0.6 [a]	community effects, mainly Ephemeroptera (Baetidae), Diptera (Chironomidae)	EC (2006), Brock (2005) Ratte and Memmert (2003)
2. Outdoor pond enclosure	2 x 0.6 - 40 µg a.s./L, 7-days interval	1.4 [a]	abundance of Chironomidae, Ephemeroptera	Colombo *et al.* (2013)
3. Outdoor stream	12-hours pulse 0.1 - 10 µg a.s./L	3.9 [b]	emergence of *Epeorus* spp. (Ephemeroptera)	Alexander *et al.* (2008)
		< 0.1 [b]	adult male thorax length of *Baetis* and *Epeorus* spp.	
	20-hours continuous 0.1 - 10 µg a.s./L	0.1 [b]	emergence of *Epeorus* spp. (Ephemeroptera)	
		< 0.1 / 0.1 [b]	adult male thorax length of *Baetis* and *Epeorus* spp.	
4. Outdoor	3 x 24-h pulse 2 or 20 µg a.s./L,	1.63 [b]	effects on Ephemeroptera, Plecoptera and Tricoptera,	Pestana *et al.* (2009b)

stream	7-days interval		Oligochaetes at next dose (20 µg a.s./L)	
5. Indoor stream	3 x 12-h pulse of 12 µg a.s./L, 7-days interval; treatment repeated after ca. 50 d	< 12 [b]	abundance and emergence of Ephemeroptera (affected after single pulse), Trichoptera (id.), Chironomidae and Gammaridae	Berghahn *et al*. (2012) Mohr *et al*. (2012)
6. Outdoor enclosure	2 x 0.097 - 3.8 µg a.s./L, 21-days interval	1.52 [a]	abundance of *Cloeon dipterum* larvae	Roessink and Hartgers (2013)

a.s. = active substance. a: actual initial concentration, b: average actual during treatment, For details of individual tests, see Supporting Information 2.

Figure 1. Representation of acute toxicity of imidacloprid to water organisms. Acute L(E)C50-values for bacteria, algae, crustaceans, insects, fish and annelids are plotted on the Y-axis. Note that the Y-axis is presented on a log-scale.

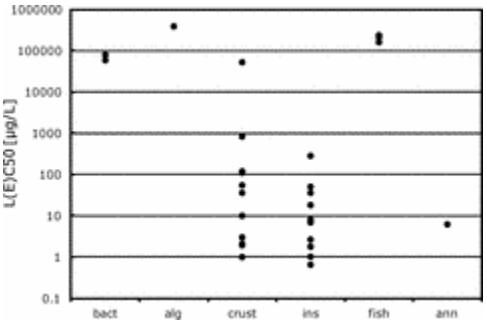


Figure 2. Representation of chronic toxicity of imidacloprid to water organisms. Chronic NOEC or L(E)C10-values for algae, crustaceans, insects and fish are plotted on the Y-axis. Note that the Y-axis is presented on a log-scale.

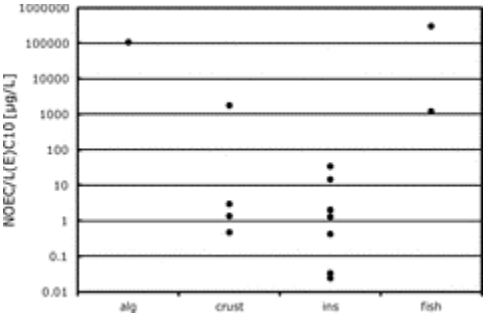


Figure 3. Species Sensitivity Distribution for imidacloprid based on acute toxicity data for all available aquatic species. The X-axis represents the L(E)C50-values in µg/L for algae (∗), annelids (×), bacteria (△), crustaceans (□), insects (●) and fish (○), the Y-axis represents the fraction of species potentially affected.

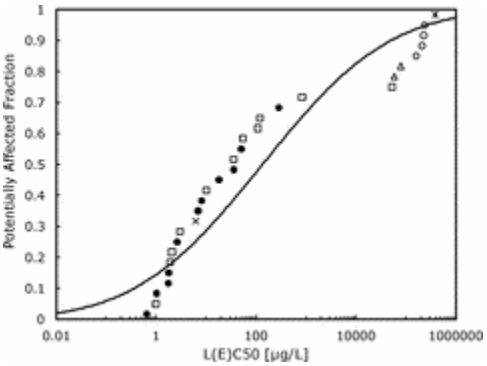


Figure 4. Species Sensitivity Distribution for imidacloprid based on acute toxicity data for aquatic arthropods combined, endpoint for *Daphnia magna* omitted. The X-axis represents L(E)C50-values for crustaceans (□) and insects (●) µg/L, the Y-axis represents the fraction of species potentially affected. The dashed line represents the Hazardous Concentration for 5% of the species (HC5 = 0.36 µg/L).

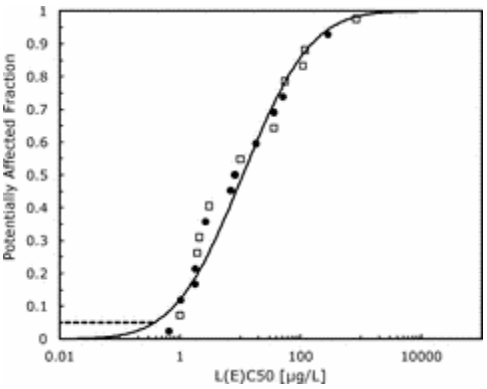
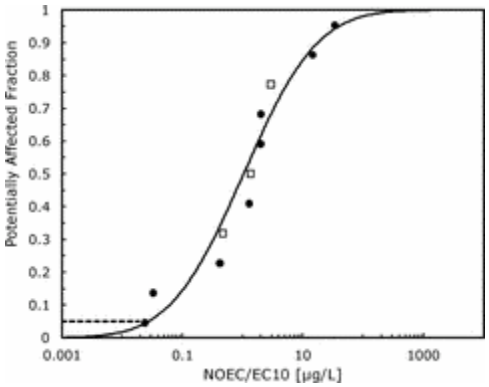


Figure 5. Species Sensitivity Distribution for imidacloprid based on chronic toxicity data for aquatic arthropods combined, endpoint for *Daphnia magna* omitted. The X-axis represents NOEC/L(E)C10 -values for crustaceans (□) and insects (●) in µg/L, the Y-axis represents the fraction of species potentially affected. The dashed line represents the Hazardous Concentration for 5% of the species (HC5 = 0.025 µg/L).



Supporting information 1. Detailed ecotoxicity data.

Legend to column headings	
A	test water analysed Y(es)/N(o)
Test type	S = static; R = renewal; F = flow through; c = closed
Purity	refers to purity of active substance or content of active substance in formulation; ag = analytical grade; tg = technical grade
Test water	am = artificial medium; dtw = dechlorinated tap water; dw = deionised/dechlorinated/distilled water; nw = natural water; rw = reconstituted water; rtw = reconstituted tap water; tw = tap water
T	temperature
Ri	Reliability index according to [1]. Valid studies (Ri 2 or higher) are considered for EQS-derivation, depending on relevance and considering notes on data treatment (section 1.3.4)

Table S1.1 Acute ecotoxicity of imidacloprid for freshwater organisms

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Bacteria																
Vibrio fischeri	strain NRRL-B-11,177	Y	S	imidacloprid	ag				15	30 min	EC50	bioluminescence	61900	2	1	[2]
Vibrio fischeri	strain NRRL-B-11,177	Y	S	Confidor	200 g/L				15	30 min	EC50	bioluminescence	56000	2	1	[2]
Vibrio qinghaiensis sp.	Q67	N	S	imidacloprid	99.5%				22	15 min	EC50	bioluminescence	79255	2	2	[2]
Cyanobacteria																
Anabaena flos-aquae		Y	S	NTN 33893 2F	21.6					96 h	EC50		32800	4	4	[3]
Algae																
Desmodesmus subspicatus		Y	S	imidacloprid	ag				21	72 h	EC50	growth rate	389000	2	5	[4]
Desmodesmus subspicatus		Y	S	Confidor	200 g/L				21	72 h	EC50	growth rate	116000	2	6	[4]
Pseudokirchneriella subcapitata		N	S	Confidor					24	72 h	EC50	growth	> 1E6	3	7	[5]
Pseudokirchneriella subcapitata		N	S	imidacloprid	98.6					72 h	EC50	biomass	> 100000	2	8	[6]
Pseudokirchneriella subcapitata		N	S	imidacloprid	98.6					72 h	EC50	growth rate	> 100000	2	8	[6]
Scenedesmus subspicatus	10000 cells/mL	N	S	imidacloprid	tg			8.2-9.1	23	72 h	EC50	biomass	> 10000	3	9	[6]
Scenedesmus subspicatus	10000 cells/mL	N	S	imidacloprid	tg			8.2-9.1	23	72 h	EC50	growth rate	> 10000	3	9	[6]
Scenedesmus subspicatus	10000 cells/mL	N	S	imidacloprid	92.8			8.1-9.2	23	96 h	EC50	growth rate	> 10000	3	10	[7]
Crustacea																
Asellus aquaticus	field collected	N		Confidor	200 g/L	am			10	1 h	NOEC	respiration	100	3	11	[8]
Asellus aquaticus	field collected	N		Confidor	200 g/L					24 h	EC50	immobility	800	3	12	[8]
Asellus aquaticus	field collected	N		Confidor	200 g/L					48 h	LC50	mortality	8500	3	12	[8]
Asellus aquaticus	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	119	2	13	[9]
Asellus aquaticus	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	24.7	2	13	[9]
Asellus aquaticus	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	316	2	13	[9]
Asellus aquaticus	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	61.6	2	13	[9]
Ceriodaphnia dubia	< 24 h	Y	S	Admire Pro	42.8%	dw	80-100		25	48 h	LC50	mortality	2.07	2	14	[10]
Ceriodaphnia dubia	< 24 h old	N	R	Admire	200 g/L	dtw		7.8-7.9	21	48 h	EC50	immobility	571.62	3	15	[11]
Ceriodaphnia reticulata	< 24 h old	N	R	Admire	200 g/L	dtw		7.8-7.9	21	48 h	EC50	immobility	5552.9	3	15	[11]
Chydorus sphaericus	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	LC50	mortality	132700	3	16	[12]
Chydorus sphaericus	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	EC50	immobility	2209	3	16	[12]
Chydorus sphaericus	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	EC50	immobility	832	2	17	[12]
Cypretta seuratti	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	LC50	mortality	301	3	16	[12]
Cypretta seuratti	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	EC50	immobility	16	3	16	[12]
Cypretta seuratti	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	EC50	immobility	1	2	17	[12]
Cypridopsis vidua	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	LC50	mortality	715	3	16	[12]
Cypridopsis vidua	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	LC50	mortality	273	2	18	[12]
Cypridopsis vidua	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	EC50	immobility	3	3	16	[12]
Cypridopsis vidua	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	EC50	immobility	10	2	17	[12]

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Daphnia magna	< 24 h	N	S	imidacloprid	tg	nw			20	48 h	LC50	mortality	17360	3	19	[13]
Daphnia magna	< 24 h	N	S	imidacloprid	tg	nw			27	48 h	LC50	mortality	10440	3	20	[13]
Daphnia magna	< 24 h	Y	S	imidacloprid	95.4		160-180	8.3-8.4	20	48 h	EC50	immobility	85000	2	21	[6,14]
Daphnia magna	< 24 h	Y	S	imidacloprid	95.4					48 h	EC50	immobility	> 32000	3	22	[14]
Daphnia magna	24 h	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	LC50	mortality	64870	3	16	[12]
Daphnia magna	24 h	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	EC50	immobility	6029	3	16	[12]
Daphnia magna	< 24 h	N	S	Confidor					20	48 h	EC50	immobility	64600	4	23	[5]
Daphnia magna	< 24 h	N	S	imidacloprid						48 h	EC50	immobility	97000	3	24	[15
Daphnia magna	4-5 d	N	S	imidacloprid						24 h	EC50	feeding activity	3700	3	25	[15]
Daphnia magna	< 24 h	Y	S	imidacloprid	ag				21	48 h	EC50	immobility	56600	2	26	[4]
Daphnia magna	< 24 h	Y	S	Confidor	200 g/L				21	48 h	EC50	immobility	30000	2	27	[4]
Daphnia magna	< 24 h old	N	R	Admire	200 g/L	dtw		7.8-7.9	21	48 h	EC50	immobility	43265	3	28	[11]
Daphnia magna	< 24 h, 0.94 mm	Y	R	imidacloprid	99.0%	am		7.4-8.2	20	7 d	NOEC	body length	1200	2	29	[16]
Daphnia magna	< 24 h, 0.94 mm	Y	R	imidacloprid	99.0%	am		7.4-8.2	20	7 d	NOEC	time until maturation	4000	2	30	[16]
Daphnia magna	< 24 h, 0.94 mm	Y	R	imidacloprid	99.0%	am		7.4-8.2	20	7 d	NOEC	# offspring	1300	2	30	[16]
Daphnia magna	< 24 h, 0.94 mm	Y	R	imidacloprid	99.0%	am		7.4-8.2	20	7 d	EC50	body length	21727	2	31	[16]
Daphnia magna	< 24 h, 0.94 mm	Y	S	imidacloprid	99.0%	am		7.4-8.2	20	24 h	EC50	feeding	1830	2	32	[16]
Daphnia magna	< 24 h, 0.94 mm	Y	S	imidacloprid	99.0%	am		7.4-8.2	20	24 h	LC50	mortality	> 100000	2	33	[16]
Daphnia pulex	< 24 h old	N	R	Admire	200 g/L	dtw		7.8-7.9	21	48 h	EC50	immobility	36872	3	28	[11]
Gammarus fossatum	field collected	N		Confidor	200 g/L	am			10	1 h	NOEC	respiration	≥ 10	3	11	[8]
Gammarus fossatum	field collected	N		Confidor	200 g/L					24 h	EC50	immobility	70	3	12	[8]
Gammarus fossatum	field collected	N		Confidor	200 g/L					48 h	LC50	mortality	800	3	12	[8]
Gammarus pulex	adults, field collected	Y	S	14C-imidacloprid	> 95%	am	250		13	48 h	EC50	immobility	110	2	34	[17]
Gammarus pulex	adults, field collected	Y	S	14C-imidacloprid	> 95%	am	250		13	96 h	EC50	immobility	131	2	34	[17]
Gammarus pulex	field collected	N	S	imidacloprid	ag	am	180	7.4	15	48 h	LC50	mortality	270	3	35	[18]
Gammarus pulex	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	18.3	3	36	[9]
Gammarus pulex	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	3.63	3	36	[9]
Gammarus pulex	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	263	3	36	[9]
Gammarus pulex	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC10	mortality	99.5	3	36	[9]
Gammarus roeseli	field collected in autumn; juveniles; 6 mm	Y	S	imidacloprid		am		7.7	17	96 h	EC50	immobility	129.5	2	37	[19]
Gammarus roeseli	field collected in autumn; juveniles; 6 mm	Y	S	imidacloprid		am		7.7	17	96 h	EC10	immobility	98.4	2	38	[19]
Gammarus roeseli	field collected in autumn; juveniles; 6 mm	Y	S	imidacloprid		am		7.7	17	96 h	EC50	immobility	86.14	2	39	[19]
Gammarus roeseli	field collected in autumn; juveniles; 6 mm	Y	S	imidacloprid		am		7.7	17	96 h	EC10	immobility	6	2	40	[19]
Gammarus roeseli	field collected in autumn; juveniles; 6 mm	Y	S	imidacloprid		aw		7.8	12	96 h	EC50	immobility	14.2	2	41	[19]
Gammarus roeseli	field collected in autumn; juveniles; 6 mm	Y	S	imidacloprid		aw		7.8	12	96 h	EC10	immobility	1.4	2	42	[19]
Gammarus roeseli	field collected in spring; early adults; 9 mm	Y	S	imidacloprid		aw		7.8	12	96 h	EC50	immobility	1.94	2	43	[19]
Gammarus roeseli	field collected in spring; adults; 11 mm	Y	S	imidacloprid		aw		7.8	12	96 h	EC50	immobility	28.9	2	44	[19]
Gammarus roeseli	field collected in spring; adults; 11 mm	Y	S	imidacloprid		aw		7.8	12	96 h	EC10	immobility	2.6	2	45	[19]
Gammarus roeseli	field collected in spring; adults; 11 mm	Y	S	imidacloprid		aw		7.8	12	96 h	EC50	immobility	14.8	2	46	[19]
Gammarus roeseli	field collected in spring; adults; 11 mm	Y	S	imidacloprid		aw		7.8	12	96 h	EC10	immobility	1	3	47	[19]
Gammarus roeseli	field collected	N	S	not spec.		rw			17.7	26 h	NOEC	drift	≥ 12	3	48	[20]

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Hyalella azteca	2-3 mm juveniles	Y	S	imidacloprid						96 h	LC50	mortality	526	2	49	[3,6]
Hyalella azteca	2-3 mm juveniles	Y	S	imidacloprid						96 h	EC50	immobility	55	2	50	[3,6]
Hyalella azteca	2-3 mm juveniles	Y	S	imidacloprid						96 h	NOEC	immobility	0.35	2	50	[3,6]
Hyalella azteca	juveniles, 2-9 d	Y	R	imidacloprid	99.2%	ftw	133	8.2	24	96 h	LC50	mortality	65.4	3	51	[21]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	96 h	LC50	mortality	17.4	3	52	[21]
Hyalella azteca	juveniles, 2-9 d	Y	S	Admire	240 g/L	ftw	133	8.2	24	96 h	NOEC	mortality	≥ 11.93	2	53	[21]
Hyalella azteca	juveniles, 2-9 d	Y	S	Admire	240 g/L	ftw	133	8.2	24	96 h	NOEC	growth	1.15	2	54	[21]
Hyalella azteca	juveniles, 2-9 d	Y	S	Admire	240 g/L	ftw	133	8.2	24	96 h	EC50	growth	9.83	3	55	[21]
Hyalella azteca	juveniles, 2-9 d	Y	S	Admire	240 g/L	ftw	133	8.2	24	96 h	LC50	mortality	9.74	3	56	[21]
Hyalella azteca	juveniles, 2-9 d	Y	S	Admire	240 g/L	ftw	133	8.2	24	96 h	NOEC	mortality	3.53	2	57	[21]
Hyalella azteca	juveniles, 2-9 d	Y	S	Admire	240 g/L	ftw	133	8.2	24	96 h	NOEC	growth	≥ 11.93	2	58	[21]
Ilyocypris dentifera	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	LC50	mortality	517	3	16	[12]
Ilyocypris dentifera	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	LC50	mortality	214	2	18	[12]
Ilyocypris dentifera	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	EC50	immobility	3	3	16	[12]
Ilyocypris dentifera	collected from rice fields	N	S	imidacloprid	tg	tw		7.5-7.8	22	48 h	EC50	immobility	3	2	17	[12]
Moina macrocopa	< 24 h old	N	R	Admire	200 g/L	dtw		7.8-7.9	21	48 h	EC50	immobility	45271	3	28	[11]
Insecta																
Aedes aegypti	4th instar	N	S	imidacloprid	97.4	dw			25	72 h	LC50	mortality	84	3	59	[22]
Aedes aegypti	larvae, 3 d	N		imidacloprid		tw				72 h	LC50	mortality	819.5	3	60	[23]
Aedes aegypti (L.)	1st instar, 24 h old	N	S	imidacloprid	tg	am			20	48 h	LC50	mortality	45	3	19	[13
Aedes aegypti (L.)	1st instar, 24 h old	N	S	imidacloprid	tg	am			27	48 h	LC50	mortality	44	3	19	[13]
Aedes albopictus	4th instar, strain MAmAal	N	S	imidacloprid	97.7	tw			25	24 h	LC50	mortality	600	3	59	[24]
Aedes albopictus	4th instar, strain HAmAal	N	S	imidacloprid	97.7	tw			25	24 h	LC50	mortality	300	3	59	[24]
Aedes albopictus	4th instar, strain VBFmAal	N	S	imidacloprid	97.7	tw			25	24 h	LC50	mortality	800	3	59	[24]
Aedes albopictus	4th instar, strain SFmAal	N	S	imidacloprid	97.7	tw			25	24 h	LC50	mortality	600	3	59	[24]
Aedes albopictus	4th instar, strain Ikaken	N	S	imidacloprid	97.7	tw			25	24 h	LC50	mortality	500	3	59	[24]
Baetis rhodani	larvae, field collected	N	S	imidacloprid	ag	am	180	7.4	15	48 h	LC50	mortality	8.49	3	35	[18]
Baetis rhodani	large larvae, field collected, 0.51 mg, 5.77 mm	N	S	imidacloprid	ag	am		7.8-8.1	12	96 h	LC50	mortality	41.23	3	61	[25]
Baetis rhodani	large larvae, field collected, 0.51 mg, 5.77 mm	N	S	imidacloprid	ag	am		7.8-8.1	12	96 h	EC50	immobility	5.21	3	61	[25]
Baetis rhodani	small larvae, field collected, 0.10 mg, 3.25 mm	N	S	imidacloprid	ag	am		7.8-8.1	12	96 h	LC50	mortality	3.85	3	61	[25]
Baetis rhodani	small larvae, field collected, 0.10 mg, 3.25 mm	N	S	imidacloprid	ag	am		7.8-8.1	12	96 h	EC50	immobility	1.72	3	61	[25]
Caenis horaria	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	6.68	2	13	[9]
Caenis horaria	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC10	mortality	2.55	2	13	[9]
Caenis horaria	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	1.77	2	13	[9]
Caenis horaria	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	0.325	2	13	[9]
Centroptilum triangulifer	larvae, <24 h old	N	S	imidacloprid	ag	am		7.4-7.5	19-22	72 h	LC50	mortality	8.88	3	62	[25]
Centroptilum triangulifer	larvae, <24 h old	N	S	imidacloprid	ag	am		7.4-7.5	19-22	72 h	EC50	immobility	4.98	3	62	[25]
Chaoborus obscuripes	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	284	2	13	[9]
Chaoborus obscuripes	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	223	2	13	[9]
Chaoborus obscuripes	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	294	2	13	[9]
Chaoborus obscuripes	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC10	mortality	178	2	13	[9]
Cheumatopsyche brevilineata	1st instar larvae, strain M, < 24 h	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	6.6	3	63	[26]
Cheumatopsyche brevilineata	2nd instar larvae, strain M	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	11	3	63	[26]
Cheumatopsyche brevilineata	3rd instar larvae, strain M	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	21	3	63	[26]

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Cheumatopsyche brevilineata	4th instar larvae, strain M	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	21	3	64	[26]
Cheumatopsyche brevilineata	5th instar larvae, strain M	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	38	3	64	[26]
Cheumatopsyche brevilineata	1st instar larvae, strain K, < 24 h	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	7	3	64	[26]
Cheumatopsyche brevilineata	2nd instar larvae, strain k	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	10	3	64	[26]
Cheumatopsyche brevilineata	3rd instar larvae, strain k	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	20	3	64	[26]
Cheumatopsyche brevilineata	4th instar larvae, strain k	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	20	3	64	[26]
Cheumatopsyche brevilineata	5th instar larvae, strain k	N	S	imidacloprid	ag	dtw			20	24 h	EC50	immobility	37.9	3	65	[26]
Chironomus dilutus	larvae, 10 d old	Y		Admire 240F		dgw			23	96 h	LC50	mortality	2.65	2	66	[27]
Chironomus riparius	larvae, 6 d, 2nd instar	N	S	Confidor	200 g/L	am	250		20	96 h	EC50	immobility	12.94	3	67	[28]
Chironomus riparius	larvae, 6 d, 2nd instar	N	S	Confidor	200 g/L	am	250		20	24 h	NOEC	respiration	< 0.4	3	68	[28]
Chironomus riparius	larvae, 4 d, 2nd instar	Y	R	Confidor	200 g/L	rw				96 h	NOEC	growth	0.74	3	69	[29]
Chironomus riparius	larvae, 4 d, 2nd instar	Y	R	Confidor	200 g/L	rw				96 h	NOEC	locomotion, ventilation	0.74	3	70	[29]
Chironomus riparius	larvae, 4 d, 2nd instar	Y	R	Confidor	200 g/L	rw				96 h	NOEC	growth	≥ 2.15	3	71	[29]
Chironomus riparius	larvae, 4 d, 2nd instar	Y	R	Confidor	200 g/L	rw				96 h	NOEC	locomotion, ventilation	≥ 2.15	3	71	[29]
Chironomus riparius	larvae, 7 d	N	S	Confidor	200 g/L	rw				48 h	LC50	mortality	19.9	3	72	[29]
Chironomus riparius	1st instar larvae	Y	S	imidacloprid	99.9					24 h	LC50	mortality	55.2	3	73	[6]
Chironomus riparius	larvae, 10 d old, late 3rd instar	Y	R	Confidor	200 g/L	rw				96 h	NOEC	locomotion	0.55	3	74	[30]
Chironomus riparius	larvae, 10 d old, late 3rd instar	Y	R	Confidor	200 g/L	rw				96 h	NOEC	ventilation	0.3	3	74	[30]
Chironomus riparius	larvae, 10 d old, late 3rd instar	Y	R	Confidor	200 g/L	rw				96 h	NOEC	ACh activity	0.55	3	74	[30]
Chironomus riparius	late instar	N	S	not spec.		rw			17.7	26 h	NOEC	drift	< 12	3	75	[20]
Chironomus tentans	2nd instar	Y	R	imidacloprid	95%					96 h	LC50	mortality	10.5	2	76	[3]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	96 h	LC50	mortality	5.4	3	77	[21]
Chironomus tentans	larvae, 7 d	Y	R	imidacloprid	99.2%	ftw	140	8.2	24	96 h	LC50	mortality	5.75	2	78	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	96 h	NOEC	mortality	≥ 3.47	2	79	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	96 h	NOEC	mortality	≥ 3.47	2	79	[21]
Cloeon dipterum	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	1.02	2	13	[9]
Cloeon dipterum	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	0.100	2	13	[9]
Cloeon dipterum	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	26.3	2	13	[9]
Cloeon dipterum	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC10	mortality	6.16	2	13	[9]
Cloeon dipterum	large larvae, 0.65 mg	N	S	imidacloprid	ag	am		7.9-8.0	9	96 h	LC50	mortality	104.63	3	80	[25]
Cloeon dipterum	large larvae, 0.65 mg	N	S	imidacloprid	ag	am		7.9-8.0	9	96 h	EC50	immobility	43.03	3	80	[25]
Cloeon dipterum	small larvae, 0.13 mg	N	S	imidacloprid	ag	am		7.9-8.0	9	96 h	LC50	mortality	100	3	80	[25]
Cloeon dipterum	small larvae, 0.13 mg	N	S	imidacloprid	ag	am		7.9-8.0	9	96 h	EC50	immobility	43.33	3	80	[25]
Cloeon dipterum	late instar; field collected	N	S	not spec.		rw			17.7	26 h	NOEC	drift	≥ 12	3	48	[20]
Culex quinquefasciatus	4th instar, VBFmCq	N	S	imidacloprid	0.977	tw			25	24 h	LC50	mortality	300	3	59	[31]
Culex quinquefasciatus	4th instar, HAmCq	N	S	imidacloprid	0.977	tw			25	24 h	LC50	mortality	200	3	59	[31]
Culex quinquefasciatus	4th instar, MAmCq	N	S	imidacloprid	0.977	tw			25	24 h	LC50	mortality	400	3	59	[31]
Culex quinquefasciatus	4th instar, S-Lab	N	S	imidacloprid	0.977	tw			25	24 h	LC50	mortality	40	3	59	[31]
Culex quinquefasciatus	4th instar larvae	N	S	imidacloprid		tw			27	24 h	LC50	mortality	5	3	81	[32]
Epeorus assimilis	large larvae, 9.74 mg	N	S	imidacloprid	ag	am		7.6-7.9	13	96 h	LC50	mortality	52.33	3	82	[25]
Epeorus assimilis	large larvae, 9.74 mg	N	S	imidacloprid	ag	am		7.6-7.9	13	96 h	EC50	immobility	1.07	3	82	[25]
Epeorus assimilis	small larvae, 7.15 mg	N	S	imidacloprid	ag	am		7.2-7.8	4	96 h	LC50	mortality	20.89	3	83	[25]
Epeorus assimilis	small larvae, 7.15 mg	N	S	imidacloprid	ag	am		7.2-7.8	4	96 h	EC50	immobility	5.06	3	83	[25]
Epeorus longimanus	larvae, early instar, 3 mm, collected from field	Y	S	Admire	240 g/L	dgw		8.1	20	24 h	LC50	mortality	2.1	2	84	[33]
Epeorus longimanus	larvae, late instar, 7.5 mm, collected from field	Y	S	Admire	240 g/L	dgw		8.1	20	24 h	LC50	mortality	2.1	2	85	[33]

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Epeorus longimanus	larvae, late instar, 7.5 mm, collected from field	Y	S	Admire	240 g/L	dgw		8.1	20	96 h	LC50	mortality	0.65	2	86	[33]
Epeorus longimanus	larvae, late instar, 7.5 mm, collected from field	Y	S	Admire	240 g/L	dgw		8.1	20	24 h	NOEC	feeding rate	1	2	87	[33]
Epeorus longimanus	larvae, late instar, 7.5 mm, collected from field	Y	S	Admire	240 g/L	dgw		8.1	20	24 h	NOEC	feeding rate	< 0.1-0.5	3	88	[33]
Habrophlebia lauta	large larvae, field collected, 0.65 mg	N	S	imidacloprid	ag	am		7.8-8.6	13	96 h	LC50	mortality	179.92	3	89	[25]
Habrophlebia lauta	large larvae, field collected, 0.65 mg	N	S	imidacloprid	ag	am		7.8-8.6	13	96 h	EC50	immobility	34.65	3	89	[25]
Habrophlebia lauta	small larvae, field collected, 0.17 mg	N	S	imidacloprid	ag	am		7.8-8.6	13	96 h	LC50	mortality	57.62	3	89	[25]
Habrophlebia lauta	small larvae, field collected, 0.17 mg	N	S	imidacloprid	ag	am		7.8-8.6	13	96 h	EC50	immobility	31.18	3	89	[25]
Hydropsyche pellucidula	larvae, 3.44 mg	N	S	imidacloprid	ag	am		7.7-8.0	12	96 h	LC50	mortality	44.93	3	90	[25]
Hydropsyche pellucidula	larvae, 3.44 mg	N	S	imidacloprid	ag	am		7.7-8.0	12	96 h	EC50	immobility	23.07	3	90	[25]
Leuctra sp.	larvae, 0.64 mg	N	S	imidacloprid	ag	am		7.5-8.0	8	96 h	LC50	mortality	247.09	3	91	[25]
Leuctra sp.	larvae, 0.64 mg	N	S	imidacloprid	ag	am		7.5-8.0	8	96 h	EC50	immobility	8.57	3	91	[25]
Limnephilidae	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	1.79	2	13	[9]
Limnephilidae	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	0.532	2	13	[9]
Limnephilidae	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	25.7	2	13	[9]
Limnephilidae	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC10	mortality	9.86	2	13	[9]
Micronecta spp.	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	10.8	3	92	[9]
Micronecta spp.	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	9.41	3	92	[9]
Micronecta spp.	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	28.2	3	92	[9]
Micronecta spp.	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC10	mortality	8.857	3	92	[9]
Notonecta spp.	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	18.2	2	13	[9]
Notonecta spp.	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	3.00	2	13	[9]
Notonecta spp.	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	> 10000	2	13	[9]
Notonecta spp.	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC10	mortality	> 10000	2	13	[9]
Plea minutissima	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	35.9	2	13	[9]
Plea minutissima	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	30.4	2	13	[9]
Plea minutissima	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	37.5	2	13	[9]
Plea minutissima	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC10	mortality	32.3	2	13	[9]
Pteronarcys comstocki	nymphs, 20 mm	Y	S	Admire	240 g/L	gw			14.5	3 x 24 h	NOEC	feeding rate	1.63	2	93	[34]
Pteronarcys comstocki	nymphs, 20 mm	Y	S	Admire	240 g/L	gw			20	24 h	NOEC	O2 consumption	2	2	94	[34]
Sericostoma vittatum	larvae, field collected	N	S	Confidor	200 g/L	am	250		20	96 h	EC50	immobility	47.22	3	95	[28]
Sericostoma vittatum	larvae, field collected	N	S	Confidor	200 g/L	am	250		20	24 h	NOEC	respiration	1.9	3	96	[28]
Sericostoma vittatum	larvae, field collected	Y	R	Confidor	200 g/L	am	250		20	72 h	NOEC	burrowing behaviour	2.5	2	97	[28]
Sialis lutaria	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC50	immobility	50.6	2	13	[9]
Sialis lutaria	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	EC10	immobility	15.7	2	13	[9]
Sialis lutaria	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC50	mortality	>10000	2	13	[9]
Sialis lutaria	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	96 h	LC10	mortality	>10000	2	13	[9]
Simulium latigonium	larvae, collected from mesocosm	N	S	imidacloprid	ag	am	180	7.4	15	48 h	LC50	mortality	3.73	3	35	[18]
Simulium vittatum	5th instar	Y	S	imidacloprid	98%	rw		7.3-7.7	20	48 h	LC50	mortality	6.75	2	98	[35]
Simulium vittatum	5th instar	Y	S	imidacloprid	98%	rw		7.3-7.7	20	48 h	LC50	mortality	8.25	2	99	[35]
Simulium vittatum	5th instar	Y	S	imidacloprid	98%	rw		7.3-7.7	20	48 h	LC50	mortality	9.54	2	99	[35]
Siphonoperla sp.	larvae, 0.55 mg	N	S	imidacloprid	ag	am		7.5-8.0	8	96 h	LC50	mortality	883.89	3	91	[25]
Siphonoperla sp.	larvae, 0.55 mg	N	S	imidacloprid	ag	am		7.5-8.0	8	96 h	EC50	immobility	8.63	3	91	[25]
Amphibia																
Rana limnocharis	1 month old	N	R	imidacloprid	> 95%	dw			20	96 h	LC50	mortality	82000	3	100	[3,36]
Rana N. Hallowell	1.5 months old	N	R	imidacloprid	> 95%	dw			20	96 h	LC50	mortality	129000	3	100	[3,36]

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Pisces																
Danio rerio		Y	S	imidacloprid	ag	nw	140	8.4	21	96 h	LC50	mortality	241000	2	101	[4]
Danio rerio		Y	S	Confidor	200 g/L	nw	140	8.4	21	96 h	LC50	mortality	214000	2	101	[4]
Leuciscus idus melanotus		Y	S	imidacloprid	95.3		230	8.1	21	96 h	LC50	mortality	237000	2	102	[6]
Lepomis macrochirus	27 mm, 0.46 g	Y	S	imidacloprid	95		46	7.4	22	96 h	LC50	mortality	> 105000	3	103	[3,6]
Oncorhynchus mykiss	5.3 cm, 1.3 g	N	S	imidacloprid	95.3		230	8.0-8.1	15.4	96 h	LC50	mortality	211000	2	104	[6]
Oncorhynchus mykiss	4.4 cm, 1.07 g	Y	S	imidacloprid	95		40-48	7.0-7.9	12	96 h	LC50	mortality	> 83000	3	105	[6]
Annelida																
Lumbriculus variegatus	2.5 cm, 1.2 mg	Y	S	Admire	240 g/L	dgw		8.1	20	96 h	EC50	immobility	6.2	2	106	[33]
Lumbriculus variegatus	2.5 cm, 1.2 mg	Y	S	Admire	240 g/L	dgw		8.1	20	24 h	NOEC	egestion rate	≥ 10	3	107	[33]
Lumbriculus variegatus	2.5 cm, 1.2 mg	Y	S	Admire	240 g/L	dgw		8.1	20	24 h	NOEC	egestion rate	0.1-1	3	108	[33]
Tubifex tubifex	adult, 4 cm long, Ø 1-2 cm	N	S	imidacloprid		am	62	7	20	24 h	EC50	locomotory behaviour	90	3	109	[37]
Tubifex tubifex	adult, 4 cm long, Ø 1-2 cm	N	S	imidacloprid		am	62	7	20	24 h	LC50	mortality	320	3	109	[37]

Notes

1 Marine species, but tested in distilled water. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively.

2 Solvent 1% DMSO, solvent control included; no analysis of test concentrations, but short exposure time

4 Based on mean measured concentrations. Study previously assigned Ri2, but information on test conditions and test parameter are not available.

5 Test probably performed according to ISO guideline. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively. Cells counted only at start and 72 h, initial cell density not reported.

6 Test probably performed according to ISO guideline. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively. Toxicity of formulation is more than 3 times higher than that of active substance, preference is given to test with active. Sovent of formulation included in control tests. Cells counted only at start and 72 h, initial cell density not reported.

7 Concentrations not measured, test under continuous light. No details on test water. Endpoint given as growth inhibition, not clear if growth rate or biomass is meant, test was performed according to OECD 1984 which gives both options.

8 Test according to OECD 201. Limit test. Previously assigned Ri3 because endpoint was based on nominal. However, additional information shows that measured concentrations were 100-102% of nominal, endpoint based on nominal concentrations.

8 Test according to OECD 201. Limit test. Previously assigned Ri3 because endpoint was based on nominal. However, additional information shows that measured concentrations were 100-102% of nominal, endpoint based on nominal concentrations.

9 Test according to OECD 201. Concentrations not measured, test performed under light.

10 Test according to OECD 201. Concentrations not measured, test performed under light. Refers to same test as above.

11 Concentrations not measured, not clear if performed under darkness. No details on test water and conditions. Endpoint refers to both ratio of electron transport system activity and respiration.

12 Concentrations not measured, not clear if performed under darkness. No details on test water and conditions.

13 Concentration in dosing solution 97.5%, recovery in concurrent chronic tests was 91.9% on average

14 Mean measured concentration 88% of nominal (range 76-105%), endpoint based on measured concentrations. Concurrent study indicated little degradation over 8 d. Test conditions taken from Deardorff and Stark, 2009.

15 Daily renewal of test solutions, but concentrations not measured and performed under 16:8 h L:D as recommended in OECD 202

16 Concentrations not measured, test performed under 16:8 h L:D.

17 Concentrations not measured, but performed under darkness. Most sensitive endpoint for this species.

18 Concentrations not measured, but performed under darkness.

19 Concentrations not measured, test performed under light. Solvent control included.

20 Concentrations not measured, test performed under light. Temperature too high.

21 Test according to OECD 202. Endpoint based on mean measured concentrations.

22 Test according to OECD 202. Precipitation at two highest concentrations (56 and 100 mg/L), these were not included in EC50 estimation.

23 Concentrations not measured, but test performed in the dark. No details on test water. No details on test compound. Test performed with Daphtoxkit.

24 Test according to OECD 202, no further details on test water and conditions. Concentrations not measured, not clear if performed under darkness.

25 No details on test water and conditions. Concentrations not measured, not clear if performed under darkness. Feeding activity determined from algal growth.

26 Test according to ISO. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively.
27 Test according to ISO. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively. Formulation is more toxic than active substance, preference is given to test with active.
28 Daily renewal of test solutions, but concentrations not measured; performed under 16:8 h L:D as recommended in OECD 202
29 Measured concentrations <10 mg/L within 6.3% of nominal, maximum deviation at >10 mg/L was 26.4%; endpoints reported as nominal; NOEC taken from table S3 in supporting info; endpoint reliable, but in view of exposure duration not relevant for MAC- and/or AA-EQS
30 Reduced feeding; measured concentrations <10 mg/L within 6.3% of nominal, maximum deviation at >10 mg/L was 26.4%; endpoints reported as nominal; NOEC taken from table 3; endpoint reliable, but in view of exposure duration not relevant for MAC- and/or AA-EQS
31 Measured concentrations <10 mg/L within 6.3% of nominal, maximum deviation at >10 mg/L was 26.4%; endpoints reported as nominal; EC50 estimated using data from table S3 in supporting info, using non-linear fit of log-logistic concentrations respons model in Graphpad Prism, bottom fixed to 0; endpoint reliable, but in view of exposure duration not relevant for MAC- and/or AA-EQS
32 Measured concentrations <10 mg/L within 6.3% of nominal, maximum deviation at >10 mg/L was 26.4%; endpoints reported as nominal; test reliable, but consequence of endpoint for population effects not clear
33 Measured concentrations <10 mg/L within 6.3% of nominal, maximum deviation at >10 mg/L was 26.4%; endpoints reported as nominal
34 Acclimation 5 d. Animals fed during test. 12h:12h light;dark. Endpoint based on mean measured concentrations. Hardness calculated from information in Naylor et al., 1989.
35 Concentrations not measured, test performed under 10:14 h L:D
36 Concentration in dosing solution 97.5%, recovery in concurrent chronic tests was 91.9% on average; control mortality 33%, result considered as indicative by authors
37 Exp 1 in paper. Feeding with conditioned alder leaf discs; measured concentrations differed <1% from nominal, result expressed as nominal; EC50 obtained from author upon request, fits with reported difference of factor 9.2 with exp 3 and checked with digitised graph
38 Exp 1 in paper. Feeding with conditioned alder leaf discs; measured concentrations differed <1% from nominal, result expressed as nominal; EC10 obtained from author upon request
39 Exp 2 in paper. No feeding; measured concentrations differed <1% from nominal, result expressed as nominal; EC50 obtained from author upon request, fits with reading from digitised graph
40 Exp 2 in paper. No feeding; measured concentrations differed <1% from nominal, result expressed as nominal; EC10 obtained from author upon request
41 Exp 3 in paper. Feeding with conditioned alder leaf discs; test medium: filtered stream water from control stream mesocosms; measured concentrations differed <1% from nominal, result expressed as nominal
42 Exp 3 in paper. Feeding with conditioned alder leaf discs; test medium: filtered stream water from control stream mesocosms; measured concentrations differed <1% from nominal, result expressed as nominal; EC10 obtained from author; EC10 >2 times lower than lowest test concentration, reason for Ri 3
43 Exp 4 in paper. Feeding with conditioned alder leaf discs; test medium: filtered stream water from control stream mesocosms; measured concentrations differed <1% from nominal, result expressed as nominal
44 Exp 5 in paper. Feeding with conditioned alder leaf discs; test medium: filtered stream water from control stream mesocosms; measured concentrations differed <1% from nominal, result expressed as nominal; EC50 obtained from author upon request, fits with reading from digitised graph
45 Exp 5 in paper. Feeding with conditioned alder leaf discs; test medium: filtered stream water from control stream mesocosms; measured concentrations differed <1% from nominal, result expressed as nominal; EC10 obtained from author upon request; EC10 marginally lower than lowest test concentration/2, value considered acceptable
46 Exp 6 in paper. No feeding; test medium: filtered stream water from control stream mesocosms; measured concentrations differed <1% from nominal, result expressed as nominal; EC50 obtained from author upon request, fits with reading from digitised graph
47 Exp 6 in paper. No feeding; test medium: filtered stream water from control stream mesocosms; measured concentrations differed <1% from nominal, result expressed as nominal; EC10 obtained from author upon request; EC10 factor of 6 lower than lowest test concentration, reason for Ri 3
48 Exposure in carrousel drift meter; stream velocity 0.2 m/s at top, <<0.1 m/s at bottom; 16:8 L:D, concentrations not measured; no passive drift observed
49 DAR only reports endpoints, but summary available in Anatra-Cordone and Durkin, 2005. Endpoint based on measured concentrations. Original study also cited in Stoughton et al., 2008
50 DAR only reports endpoints, but summary available in Anatra-Cordone and Durkin, 2005.
51 Mean measured concentration 64-99% of nominal, results based on mean measured concentrations. Rinsed cheesecloth present. Animals fed during test. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. NOEC is close to LC50 (54.24 µg/L), and LOEC is far above LC50 (243.68 µg/L), this indicates large variation between replicates. No figure available to check concentration response pattern, LC50 not considered reliable.
52 Mean measured concentration 66-96% of nominal, results based on mean measured concentrations. Rinsed cheesecloth present. Animals fed during test. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. NOEC and LOEC much higher than LC50 (48.75 and 263.12 µg/L). This indicates large variation between replicates. No figure available to check concentration response pattern, LC50 not considered reliable.
53 Pulse exposure followed by observation in clean water for 10 d. Mean measured concentration 115-119% of nominal, results based on mean measured concentrations. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. Nitex screen present. Animals fed during test. NOEC reported as 11.93 µg/L, but since LOEC is reported as >11.93 µg/L, NOEC should read ≥ 11.93 µg/L.
54 Pulse exposure followed by observation in clean water for 10 d. Mean measured concentration 115-119% of nominal, results based on mean measured concentrations. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. Nitex screen present. Animals fed during test.
55 Pulse exposure followed by observation in clean water for 10 d. Mean measured concentration 115-119% of nominal, results based on mean measured concentrations. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. Nitex screen present. Animals fed during test. Number of test concentrations (3) too low for reliable estimate of EC50.
56 Pulse exposure followed by observation in clean water for 28 d. Mean measured concentration 115-119% of nominal, results based on mean measured concentrations. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. Nitex screen present. Animals fed during test. Number of test concentrations (3) too low for reliable estimate of LC50.
57 Pulse exposure followed by observation in clean water for 28 d. Mean measured concentration 115-119% of nominal, results based on mean measured concentrations. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. Nitex screen present. Animals fed during test.

58 Pulse exposure followed by observation in clean water for 28 d. Mean measured concentration 115-119% of nominal, results based on mean measured concentrations. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. Nitex screen present. Animals fed during test. NOEC reported as 11.93 µg/L, but since LOEC is reported as >11.93 µg/L, NOEC should read ≥ 11.93 µg/L.
59 Concentrations not measured, test performed under light.
60 Concentrations not measured. No information on test water and conditions.
61 Concentrations not measured, performed under light (1818 lux)
62 Concentrations not measured, performed under ambient light
13 Concentration in dosing solution 97.5%, recovery in concurrent chronic tests was 91.9% on average
63 Concentrations not measured. Acetone used as solvent, max. 0.1% (v/v). Animals not fed. Test performed under continuous fluorescent light. EC50 read from graph.
64 Concentrations not measured. Acetone used as solvent, max. 0.1% (v/v). Glass beads added to test vessel. Animals not fed. Test performed under continuous fluorescent light. EC50 read from graph.
65 Concentrations not measured. Acetone used as solvent, max. 0.1% (v/v). Glass beads added to test vessel. Animals not fed. Test performed under continuous fluorescent light
66 Test performed in dechlorinated groundwater with 0.5 cm washed silicasand; 16:8 h L:D; analysis of low and high exposure concentration, values in between calculated from regression
67 Endpoint based on nominal concentrations, taking into account measured concentration in stock. Hardness calculated from information in Naylor et al., 1989. Assumed that reported "laboratory conditions" (20 ºC, 16:8 h L:D) also refer to conditions of the test.
68 Endpoint based on nominal concentrations. LOEC given as 0.4 µg/L in table (NOEC < 0.4 µg/L), as 1.2 µg/L in text (NOEC 0.4 µg/L). Figure indocates that NOEC is most likely < 0.4 µg/L. Hardness calculated from information in Naylor et al., 1989. Assumed that reported "laboratory conditions" (20 ºC, 16:8 h L:D) also refer to conditions of the test.
69 Measured concentrations are reported as 0.39, 0.74 and 2.15 µg/L (78, 49 and 48% of nominal) at the end of the constant exposure test. Not clear which time period is meant, probably the end of the 10-days exposure period in which half of the test solutions was renewed every 48 h. Exposure over the actual test period not known. Acid-washed inorganic fine sediment present. 43% reduction in growth as compared to control at next higher concentration.
70 Measured concentrations are reported as 0.39, 0.74 and 2.15 µg/L (78, 49 and 48% of nominal) at the end of the constant exposure test. Not clear which time period is meant, probably the end of the 10-days exposure period in which half of the test solutions was renewed every 48 h. Exposure over the actual test period not known. Acid-washed inorganic fine sediment present. ca. 15% reduction in activity as compared to control at next higher concentration.
71 Pulse exposure for 96 h, followed by observation in clean water for 6 d. Measured concentrations are reported as 0.39, 0.74 and 2.15 µg/L (78, 49 and 48% of nominal) at the end of the constant exposure test. Not clear which time period is meant, probably the end of the 10-days exposure period in which half of the test solutions was renewed every 48 h. Exposure over the actual test period not known. Acid-washed inorganic fine sediment present.
72 Range finding experiment for chronic study. Endpoint most likely based on nominal concentrations.
73 Test system equivalent to OECD 202. Measured initial concentrations 95.6-102 % of nominal, concentrations at end not measured. Probably performed under light.
74 Pulse exposure for 96 h, followed by observation in clean water for 48 h; half of the test solutions was renewed after 48 h; measured concentrations are reported as 0.30, 0.55 and 1.20 µg/L (40, 63 and 60% of nominal) at the end of the exposure period; endpoint reported on the basis of measured concentration; no data on initial concentrations and not clear if measured concentrations refer to 48 or 96 h; exposure over the actual test period not known; acid-washed inorganic fine sediment present.
75 Exposure in carrousel drift meter; stream velocity 0.2 m/s at top, <<0.1 m/s at bottom; 16:8 L:D, concentrations not measured; organisms active after 12 h, almost immobile after 26 h
76 DAR reports only 10-d endpoints from this study, 96-h values cited by Anatra-Cordone and Durkin, 2005. Endpoint based on measured concentrations.
77 Mean measured concentration 78-103% of nominal, results based on mean measured concentrations. Silica sand present. Animals fed during test. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. NOEC is close to LC50 (5.11 µg/L), and LOEC is far above LC50 (23.59 µg/L), this indicates large variation between replicates. No figure available to check concentration response pattern, LC50 not considered reliable.
78 Mean measured concentration 78-103% of nominal, results based on mean measured concentrations. Silica sand present. Animals fed during test. Number of test concentrations (4) low, but considered acceptable for LC50 calculation. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling.
79 Pulse exposure for 96 h, followed by observation in clean water for 10 d. Mean measured concentration 113-123% of nominal, results based on mean measured concentrations. Not fully clear if performed under renewal or static conditions, renewal assumed from description of sampling. Silica sand present. Animals fed during test. Survival measured as emergence. No significant difference at highest concentration according to figure. NOEC reported as 3.47 µg/L, but since LOEC is reported as >3.47 µg/L, NOEC should read ≥ 3.47 µg/L.
80 Concentrations not measured, performed under light (1167 lux)
81 Test performed according to WHO protocol. Plastic cups. Acetone control included. Concentrations not measured, not clear if performed in the dark. Experiment to investigate efficacy of different imidacloprid analogues, only pure imidacloprid is reported here.
82 Concentrations not measured, performed under light (3090 lux)
83 Concentrations not measured, performed under light (2300 lux)
84 Test performed in dechlorinated groundwater. Average of three tests. Result based on nominal, actual concentrations 90-120% of nominal.
85 Test performed in dechlorinated groundwater. Result based on nominal, actual concentrations 90-120% of nominal.
86 Test performed in dechlorinated groundwater. Result based on nominal, actual concentrations 90-120% of nominal.
87 Test performed in dechlorinated groundwater. Result based on nominal, actual concentrations 90-120% of nominal. NOEC refers to effect on feeding rate during 24-h exposure period.
88 Test performed in dechlorinated groundwater. Result based on nominal, actual concentrations 90-120% of nominal. NOEC refers to effect on feeding rate over 4 d recovery period after exposure for 24 h. No consistent pattern, NOECs were 0.5, <0.1, <0.1 and 0.1 µg/L on the consecutive recovery days.
89 Concentrations not measured, performed under light (1153 lux)
90 Concentrations not measured, performed under light 3200 lux), 3 individuals appeared to be H. saxonica

91 Concentrations not measured, performed under light (1748 lux)
92 Concentration in dosing solution 97.5%, recovery in concurrent chronic tests was 91.9% on average; control mortality 20%, result considered as indicative by authors
93 In-situ bioassay at outflow of outdoor stream-mesocosms that received three 24-h pulses of 2 or 20 µg/L imidacloprid at 7-d time interval. Average peak concentrations during the pulses were 1.63 and 17.60 µg/L (81 and 88% of nominal). Significant inhibition by 71% at 17.6 µg/L, 27% inhibition at 1.63 µg/L. test reliable, but consequence of endpoint for population effects not clear
94 Oxygen consumption measured during last 4 h of 24 h exposure period. Concentrations not measured, but test performed under darkness and same stocks used as for mesocosm experiment in which concentrations were >80% of nominal. Most likely performed in groundwater; test reliable, but consequence of endpoint for population effects not clear
95 Endpoint based on nominal concentrations, taking into account measured concentration in stock. Hardness calculated from information in Naylor et al., 1989. Assumed that reported "laboratory conditions" (20 ºC, 14:10 h L:D) also refer to conditions of the test. Animals acclimated for 14 d.
96 Endpoint based on nominal concentrations. Not clear if performed under darkness. Hardness calculated from information in Naylor et al., 1989. Assumed that reported "laboratory conditions" (20 ºC, 14:10 h L:D) also refer to conditions of the test. Animals acclimated for 14 d.
97 Partial renewal (100 out of 150 mL) every 48 h. Endpoint reported as LOEC 7.8 µg/L nominal, NOEC is thus 3.9 µg/L nominal. Based on measured concentration in old solutions (66-63% of nominal), actual NOEC recalculated as 2.5 µg/L. Inorganic fine sediment present. Hardness calculated from information in Naylor et al., 1989. Test performed under 14:10 h L:D. Animals acclimated for 14 d. Endpoint measured as number of animals visible on sediment or in water. test reliable, but consequence of endpoint for population effects not clear
98 Endpoint based on average of measured concentrations at start and end; test performed under 16:8 h L:D; acetone control at level of highest amount added
99 Endpoint based on average of measured concentrations at start and end. Test performed under 16:8 h L:D.
100 Concentrations not measured, not clear whether performed under darkness.
101 Test in stream water. Initial concentrations 94-100% of nominal, concentrations remained stable during experiment. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively.
102 Test according to OECD guidelines. Previously assigned Ri3 because endpoint was based on nominal. However, additional information shows that measured concentrations were >85% of nominal, except for highest concentration (1000 mg/L, 54 % recovery). Acceptable recovery at next two lower concentrations where already 100% mortality was observed, endpoint based on nominal.
103 Test according to FIFRA guidelines. DMF 0.1 mL/L, solvent control included. Endpoint based on mean measured concentrations (86-94% of nominal). Previously assigned Ri2, but surface film and precipitate were (partly transiently) noted in the 42, 64 and 105 mg/L test solutions.
104 Test according to OECD 203. Previously assigned Ri3 because endpoint was based on nominal. However, additional information shows that measured concentrations were >80% of nominal. Endpoint based on nominal.
105 Test according to FIFRA guidelines. DMF 0.1 mL/L, solvent control included. Endpoint based on mean measured concentrations (75-101% of nominal). Previously assigned Ri2, but surface film and precipitate were (partly transiently) noted in the 42, 64 and 83 mg/L test solutions.
106 Test performed in dechlorinated groundwater. Result based on nominal, actual concentrations <LOD at 0.1 µg/L, 38 and 69% of nominal at 0.5 and 1.0 µg/L, and 94-100% at 5.0-240 µg/L. Acceptable recovery at level of EC50.
107 Test performed with sediment slurry (16% OM) contaminated with imidaloprid solutions in dechlorinated groundwater. Result based on nominal, actual concentrations of solutions <LOD at 0.1 µg/L, 38 and 69% of nominal at 0.5 and 1.0 µg/L, and 94-100% at 5.0-240 µg/L. Actual concentration in overlying water during test not known. NOEC refers to effect on egestion rate during 24-h exposure period.
108 Test performed with sediment slurry (16% OM) contaminated with imidaloprid solutions in dechlorinated groundwater. Result based on nominal, actual concentrations of solutions <LOD at 0.1 µg/L, 38 and 69% of nominal at 0.5 and 1.0 µg/L, and 94-100% at 5.0-240 µg/L. Actual concentration in overlying water during test not known. NOEC refers to effect on egestion rate over 4 d recovery period after exposure for 24 h. NOECs tend to increase over time, and were 0.1, 0.5, 1 and 1 µg/L on the consecutive recovery days.
109 Hardness calculated from given Ca and Mg concentrations. Concentrations not measured, test performed under 12:12 h L:D. No aeration. Locomotion recorded automatically every 10 min for 4 min. Regression coefficient of concentration-response relationship is low (0.49)

Table S1.2 Chronic ecotoxicity of imidacloprid for freshwater organisms

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Cyanobacteria																
Anabaena flos-aquae		Y	S	NTN 33893 2F	21.6					96 h	NOEC		24900	4	1	[3]
Algae																
Desmodesmus subspicatus		Y	S	imidacloprid	ag				21	72 h	EC10	growth rate	106000	2	2	[4]
Desmodesmus subspicatus		Y	S	Confidor	200 g/L				21	72 h	EC10	growth rate	5600	2	3	[4]
Pseudokirchneriella subcapitata		N	S	imidacloprid	98.6					72 h	NOEC	growth rate	< 100000	2	4	[6]
Pseudokirchneriella subcapitata		N	S	imidacloprid	98.6					72 h	NOEC	biomass	< 100000	2	4	[6]
Scenedesmus subspicatus		N	S	imidacloprid	tg			8.2-9.1	23	72 h	NOEC	growth rate	10000	3	5	[6,14]
Scenedesmus subspicatus		N	S	imidacloprid	tg			8.2-9.1	23	72 h	NOEC	biomass	10000	3	5	[6,14]
Scenedesmus subspicatus		N	S	imidacloprid	92.8			8.1-9.2	23	96 h	NOEC	growth rate	> 10000	3	6	[7]
Diatomea																
Navicula pelliculosa		Y	S	NTN 33893 2F	21.6					96 h	NOEC		6690	4	7	[3]
Crustacea																
Asellus aquaticus	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	EC10	immobility	1.35	3	8	[9]
Asellus aquaticus	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	LC10	mortality	1.71	3	9	[9]
Ceriodaphnia dubia	< 24 h	Y	S	Admire Pro	42.8%	dw	80-100		25	8 d	EC10	population growth rate	0.3	3	10	[10]
Ceriodaphnia dubia	< 24 h	Y	S	Admire Pro	42.8%	dw	80-100		25	8 d	EC15	survival founders	0.3	3	11	[10]
Ceriodaphnia dubia	< 24 h	Y	S	Admire Pro	42.8%	dw	80-100		25	8 d	EC14	offspring/female	0.3	3	12	[10]
Ceriodaphnia dubia	< 24 h	Y	S	Admire Pro	42.8%	dw	80-100		25	8 d	EC27	nr. of indiviuals	0.3	3	13	[10]
Daphnia magna	< 24 h	Y	S	imidacloprid	95.4		140-164	7.7-8.3	20	21 d	NOEC	adult length	1800	2	14	[6,14]
Daphnia magna	< 24 h	Y	R	imidacloprid	≥ 99%	am			21	21 d	NOEC	neonates per adult	1250	2	15	[38]
Daphnia magna	< 24 h	Y	R	Confidor	200 g/L	am			21	21 d	NOEC	neonates per adult	2500	2	15	[38]
Daphnia magna	< 24 h	Y	R	imidacloprid	≥ 99%	am			21	21 d	NOEC	brood size, time to 1st brood	2500	2	15	[38]
Daphnia magna	< 24 h	Y	R	Confidor	200 g/L	am			21	21 d	NOEC	brood size, time to 1st brood	2500	2	15	[38]
Daphnia magna	< 24 h	Y	R	imidacloprid	≥ 99%	am			21	21 d	NOEC	broods per adult	5000	2	15	[38]
Daphnia magna	< 24 h	Y	R	Confidor	200 g/L	am			21	21 d	NOEC	broods per adult	5000	2	15	[38]
Daphnia magna	< 24 h	Y	R	imidacloprid	≥ 99%	am			21	21 d	NOEC	mortality	20000	2	15	[38]
Daphnia magna	< 24 h	Y	R	Confidor	200 g/L	am			21	21 d	NOEC	mortality	5000	2	15	[38]
Daphnia magna	< 24 h	Y	R	imidacloprid					20	21 d	NOEC	reproduction	2000	2	16	[39]
Daphnia magna	< 24 h	Y	R	imidacloprid					20	21 d	EC50	reproduction	5500	2	17	[39]
Daphnia magna	< 24 h	Y	R	imidacloprid					20	21 d	NOEC	growth	4000	2	17	[39]
Daphnia magna	< 24 h	Y	R	imidacloprid					20	21 d	NOEC	mortality	10000	2	17	[39]
Gammarus pulex	different ages	N	S	imidacloprid	tg					28 d	NOEC	swimming behaviour	64	3	18	[6]
Gammarus pulex	different ages	N	S	imidacloprid	tg					28 d	NOEC	mortality	128	3	18	[6]
Gammarus pulex	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	EC10	immobility	2.95	2	19	[9]
Gammarus pulex	field collected	N	S	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	LC10	mortality	5.77	2	19	[9]
Gammarus pulex	field collected	Y	R	imidacloprid/ ^{14}C-imidacloprid	99.9%	am		7	13	14-21 d	NOEC	feeding rate	< 15	3	20	[40]
Gammarus pulex	field collected	Y	R	imidacloprid/ ^{14}C-imidacloprid	99.9%	am		7	13	14-21 d	NOEC	mortality	< 15	3	20	[40]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	10 d	LC50	mortality	7.05	2	21	[21]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	10 d	NOEC	mortality	3.53	2	22	[21]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	10 d	LC10	mortality	1.67	2	23	[21]

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	10 d	EC50	growth	10.31	2	24	[21]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	10 d	EC10	growth	10.7	2	25	[21]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	10 d	NOEC	growth	≥ 11.95	3	26	[21]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	28 d	LC50	mortality	6.98	2	27	[21]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	28 d	LC10	mortality	0.47	2	28	[21]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	28 d	NOEC	mortality	3.44	2	29	[21]
Hyalella azteca	juveniles, 2-9 d	Y	R	Admire	240 g/L	ftw	133	8.2	24	28 d	NOEC	growth	≥ 11.46	2	30	[21]
Insecta																
Caenis horaria	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	EC10	immobility	0.024	2	31	[9]
Caenis horaria	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	LC10	mortality	0.235	2	32	[9]
Chaoborus obscuripes	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	LC10	mortality	1.99	2	33	[9]
Chaoborus obscuripes	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	EC10	immobility	4.57	2	33	[9]
Chironomus riparius	larvae, <2-3 d old, 1st instar	N	S	Confidor SL 200	194 g/L					28 d	EC10	emergence	2.56	3	34	[6]
Chironomus riparius	larvae, <2-3 d old, 1st instar	N	S	imidacloprid	98.4					28 d	EC10	emergence	2.09	3	35	[6]
Chironomus riparius	larvae, <2-3 d old, 1st instar	N	S	imidacloprid	98.4					28 d	EC10	emergence	0.87	2	36	[41]
Chironomus riparius	larvae, <2-3 d old, 1st instar	N	S	Imidacloprid OD 200	196 g/L					28 d	NOEC	emergence	3.2	3	37	[42]
Chironomus riparius	larvae, 4 d, 2nd instar	Y	R	Confidor	200 g/L	rw				10 d	NOEC	growth	0.74	3	38	[29]
Chironomus riparius	larvae, 4 d, 2nd instar	Y	R	Confidor	200 g/L	rw				10 d	NOEC	locomotion, ventilation	0.74	3	39	[29]
Chironomus riparius	larvae, 3 d, 1st instar	Y	R	Confidor	200 g/L	am			20	10 d	NOEC	growth	0.4	2	40	[28]
Chironomus riparius	larvae, 3 d, 1st instar	Y	R	Confidor	200 g/L	am			20	10 d	NOEC	emergence ratio	0.4	2	40	[28]
Chironomus riparius	larvae, 3 d	Y	R	Confidor	200 g/L	am			20	10 d	NOEC	development rate	< 0.4	2	40	[28]
Chironomus riparius	larvae, 3 d	Y	R	Confidor	200 g/L	am			20	6 d	NOEC	burrowing activity	0.768	2	41	[28]
Chironomus tentans	2nd instar	Y	R	imidacloprid	95					10 d	LC50	mortality	3.17	2	42	[3]
Chironomus tentans	2nd instar	Y	R	imidacloprid	95					10 d	NOEC	growth	0.67	2	43	[3]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	10 d	NOEC	mortality	≥ 3.57	2	44	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	10 d	LC10	mortality	1.33	2	45	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	10 d	EC50	growth	3.14	2	46	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	10 d	EC10	growth	1.64	2	47	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	10 d	NOEC	growth	1.17	2	48	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	28 d	LC50	mortality	0.91	2	49	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	28 d	NOEC	mortality	1.14	3	50	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	28 d	LC10	mortality	0.42	2	51	[21]
Chironomus tentans	larvae, 7 d	Y	R	Admire	240 g/L	ftw	140	8.2	24	28 d	NOEC	growth	1.14	2	52	[21]
Cloeon dipterum	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	EC10	immobility	0.033	2	53	[9]
Cloeon dipterum	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	LC10	mortality	0.041	2	53	[9]
Copera annulata	larvae, head width 1.92 mm			Avermectin/ Imidacloprid	1.8% EC	tw	30			15 d	NOEC	mortality	≥ 0.00018	3	54	[43]
Plea minutissima	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	EC10	immobility	2.03	2	55	[9]
Plea minutissima	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	LC10	mortality	4.35	2	55	[9]
Pteronarcys dorsata	field collected	Y	S	Confidor 200 SL	200 g/L	nw			20 ± 3	14 d	LC10	mortality	15.8	2	56	[44]
Pteronarcys dorsata	field collected	Y	S	Confidor 200 SL	200 g/L	nw			20 ± 3	14 d	LC50	mortality	41	2	56	[44]
Pteronarcys dorsata	field collected	Y	S	EcoPrid	50 g/L	nw			20 ± 3	14 d	LC10	mortality	13.3	2	57	[45]
Sericostoma vittatum	larvae, field collected	Y	R	Confidor	200 g/L	am			20	6 d	NOEC	mortality	≥ 5.0	2	58	[28]

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Sericostoma vittatum	larvae, field collected	Y	R	Confidor	200 g/L	am			20	6 d	NOEC	feeding rate	1.23	2	59	[28]
Sialis lutaria	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	EC10	immobility	1.28	2	60	[9]
Sialis lutaria	field collected	Y	R	SL product	200 g/L	rw		7.4-8.3	17.7-19.7	28 d	LC10	mortality	25.1	2	60	[9]
Tipula sp.	field collected	Y	S	Confidor 200 SL	200 g/L	nw			20 ± 3	14 d	LC10	mortality	34	2	61	[44]
Tipula sp.	field collected	Y	S	Confidor 200 SL	200 g/L	nw			20 ± 3	14 d	LC50	mortality	> 63	2	62	[44
Tipula sp.	field collected	Y	S	EcoPrid	50 g/L	nw			20 ± 3	14 d	LC10	mortality	50	3	63	[45]
Amphibia																
Rana pipiens	egg masses, 70-100 eggs										NOEC	hatching success	88000-110000	4	64	[3]
Pseudacris triseriata	egg masses, 70-100 eggs										NOEC	deformities	17500-20000	4	64	[3]
Ambystoma jeffersonianum	egg masses, 70-100 eggs										NOEC	hatching success	88000-110000	4	64	[3]
Bufo americanus	egg masses, 70-100 eggs										NOEC	hatching success	88000-110000	4	64	[3]
Pisces																
Danio rerio	fertilised eggs	N	Rc	imidacloprid		am	367		26	96 h	NOEC	development	≥ 50000	3	65	[46]
Danio rerio	fertilised eggs	N	Rc	imidacloprid		am	367		28	96 h	NOEC	development	≥ 30000	3	66	[46]
Danio rerio	fertilised eggs	N	Rc	imidacloprid		am	367		30	72 h	NOEC	development	≥ 25000	3	66	[46]
Danio rerio	fertilised eggs	N	Rc	imidacloprid		am	367		33.5	72 h	NOEC	development	≥ 25000	3	66	[46]
Danio rerio	fertilised eggs	Y	S	imidacloprid	ag	am			26	48 h	NOEC	development	≥ 320000	2	67	[4]
Danio rerio	fertilised eggs	Y	S	Confidor	200 g/L	am			26	48 h	LC10	mortality	300000	2	68	[4]
Oncorhynchus mykiss	length 7.2 cm, bw 3.9 g	Y	R	imidacloprid			40-60	7.2-8.0	15	21 d	NOEC	length, weight	28500	3	69	[14]
Oncorhynchus mykiss	fertilised eggs	Y	F	imidacloprid	98.2				9-12	91 d	NOEC	development	9020	2	70	[6]
Oncorhynchus mykiss	fertilized eggs, < 4 h	Y	F	imidacloprid	tg					98 d	NOEC	growth	1200	2	71	[3]
Mollusca																
Marisa cornuarietis	fertilised eggs	N	S	imidacloprid		rtw			26	9 d	NOEC	heart rate	10000	3	72	[47]
Marisa cornuarietis	fertilised eggs	N	S	imidacloprid		rtw			26	9 d	NOEC	mortality	≥ 50000	3	73	[47]
Marisa cornuarietis	fertilised eggs	N	S	imidacloprid		rtw			26	9 d	NOEC	hatching	≥ 50000	3	73	[47]
Marisa cornuarietis	fertilised eggs	N	S	imidacloprid		rtw			26	9 d	NOEC	weight	≥ 50000	3	73	[47]

Notes

1 Based on mean measured concentrations. Study previously assigned Ri2, but information on test conditions and test parameter not available.
2 Test probably performed according to ISO guideline. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively. Cells counted only at start and 72 h, initial cell density not reported.
3 Test probably performed according to ISO guideline. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively. Toxicity of formulation is more than 3 times higher than that of active substance, preference is given to test with active. Solvent of formulation included in control tests. Cells counted only at start and 72 h, initial cell density not reported..
4 Test according to OECD 201. Limit test. Concentrations measured, recovery 100-102% of nominal, endpoint based on nominal concentrations.
5 Test according to OECD 201. Concentrations not measured, test performed under light.
6 Test according to OECD 201. Concentrations not measured, test performed under light. Refers to same test as above.
7 Based on mean measured concentrations. Study previously assigned Ri2, but information on test conditions and test parameter not available.
8 Concentration in dosing solution 95.5%, time weighted average concentration 95.3% of nominal, results expressed as nominal; control immobility too high (20%)
9 Concentration in dosing solution 95.5%, time weighted average concentration 95.3% of nominal, results expressed as nominal; control mortality too high (20%)
10 One concentration tested (0.3 µg/L), with 10% decrease as compared to control. Not possible to check concentration-effect relationship. Mean measured concentration identical to nominal. Concurrent study indicated little degradation over 8 d (measured concentrations between 83-106% of nominal).

11 One concentration tested (0.3 µg/L), with 15% decrease in survival as compared to control. No concentration-effect relationship established. Mean measured concentration identical to nominal. Concurrent study indicated little degradation over 8 d (measured concentrations between 83-106% of nominal).
12 One concentration tested (0.3 µg/L), with 14% decrease as compared to control. No concentration-effect relationship established. Mean measured concentration identical to nominal. Concurrent study indicated little degradation over 8 d (measured concentrations between 83-106% of nominal).
13 One concentration tested (0.3 µg/L), with 27% decrease as compared to control. No concentration-effect relationship established. Mean measured concentration identical to nominal. Concurrent study indicated little degradation over 8 d (measured concentrations between 83-106% of nominal).
14 Test according to OECD 202. DMF 01. mL/L, solvent control included. Endpoint based on mean measured concentrations.
15 Test conditions according to ISO 17025 (acute toxicity for D. magna). Renewal every 2 d. Stability between renewals confirmed, <20% deviation from nominal. Endpoint expressed as nominal concentration. Results presented as LOEC, next lower concentration taken as NOEC.
16 Test according to OECD 211. Measured concentrations in highest and lowest test concentration and stock within 5% of nominal. Endpoint expressed as nominal concentration. NOEC read from bar-graph in which significant differences from control are presented. Water quality parameters within accepted range.
17 Test according to OECD 211. Measured concentrations in highest and lowest test concentration and stock within 5% of nominal. Endpoint expressed as nominal concentration. Water quality parameters within accepted range.
18 Test according to OECD 219 (draft). Water/sediment system. Concentrations not measured, endpoints based on nominal initial concentrations.
19 Concentration in dosing solution 95.5%, time weighted average concentration 97% of nominal, results expressed as nominal
20 Feeding; renewal every 5 d; 12:12 h L:D, wavelength 380-730 nm; measured concentration constant at level of nominal, but analysis for total radioactivity only; not clear if increased wavelength has prevented degradation
21 Mean measured concentration 118-130% of nominal, results based on mean measured concentrations; results for first 10 d of 28-d test; number of test concentrations (4) low, but considered acceptable for LC50 calculation.
22 Mean measured concentration 118-130% of nominal, results based on mean measured concentrations; results for first 10 d of 28-d test.
23 Endpoint recalculated using TechDig analysis of graph; LC50 estimated using TechDig is 7.1 µg/L, which is similar to author's value; mean measured concentration 118-130% of nominal, results based on mean measured concentrations; 122% recovery assumed for 0.3 µg/L nominal (not analysed); results for first 10 d of 28-d test; NOEC is higher than LC25, and LOEC higher than LC50, but concentration-response relationship present; number of test concentrations (4) low, but considered acceptable for LC10 calculation.
24 Mean measured concentration 118-130% of nominal, results based on mean measured concentrations; results for first 10 d of 28-d test; NOEC and LOEC are higher than EC50, but clear concentration-response relationship present; number of test concentrations (4) low, but considered acceptable for EC50 calculation.
25 Endpoint recalculated using TechDig analysis of graph; EC50 estimated using TechDig is 12.4 µg/L, which is slightly higher than author's value; mean measured concentration 118-130% of nominal, results based on mean measured concentrations; 122% recovery assumed for 0.3 µg/L nominal (not analysed); results for first 10 d of 28-d test; NOEC and LOEC are higher than EC50, but clear concentration-response relationship present; number of test concentrations (4) low, but considered acceptable for EC50 calculation.
26 Mean measured concentration 118-130% of nominal, results based on mean measured concentrations. Results for first 10 d of 28-d test. NOEC reported as 11.95 µg/L, but since LOEC is reported as >11.95 µg/L, NOEC should read ≥ 11.95 µg/L. NOEC and LOEC are higher than EC50, probably reduced power because of variation between replicates and/or applied statistical test. Clear concentration-response relationship, preference is given to EC10.
27 Mean measured concentration 115-146% of nominal, results based on mean measured concentrations.
28 Endpoint recalculated using TechDig analysis of graph; mean measured concentration 115-146% of nominal, results based on mean measured concentrations; EC10 marginally lower than lowest test concentration/2, result considered acceptable.
29 Mean measured concentration 115-146% of nominal, results based on mean measured concentrations. LOEC is higher than LC50. Clear concentration-response relationship, preference is given to LC10.
30 Mean measured concentration 115-146% of nominal, results based on mean measured concentrations; no clear concentration-response relationship; NOEC reported as 11.46 µg/L, but since LOEC is reported as >11.46 µg/L, NOEC should read ≥ 11.46 µg/L.
31 Concentration in dosing solution 95.5%, time weighted average concentration 84.9% of nominal, results expressed as nominal; control immobility relatively high (17%), but lower than validity criterion of OECD 211 (chronic Daphnia)
32 Concentration in dosing solution 95.5%, time weighted average concentration 84.9% of nominal, results expressed as nominal
33 Concentration in dosing solution 95.5%, time weighted average concentration 91.7% of nominal, results expressed as nominal
34 Test according to OECD 219 (draft); water/sediment system; endpoints based on nominal initial concentrations; endpoint previously reported as 0.0132 mg/L, but this value refers to the formulation; recalculated to active content, the NOEC is 2.56 µg/L; DAR gives EC15 of 2.7 µg/L as surrogate for NOEC.
35 Test according to OECD 219 (draft); water/sediment system; endpoints based on nominal initial concentrations
36 Test according to OECD 219 (draft); water/sediment system; endpoint 2.09 µg/L based on nominal initial concentrations in water/phase (see above) recalculated using geometric mean concentration in water phase on days 0, 7 and 28.
37 Test according to OECD 219 (draft); water/sediment system; endpoint based on nominal initial concentrations, actual concentrations in water declined from 100% at the start to 25-26% at the end.
38 Measured concentrations are reported as 0.39, 0.74 and 2.15 µg/L (78, 49 and 48% of nominal) at the end of the constant exposure test. Not clear which time period is meant, probably the end of the 10-days exposure period in which half of the test solutions was renewed every 48 h. Exposure over the actual test period not known. Acid-washed inorganic fine sediment present. 34% reduction in growth as compared to control at next higher concentration. Doubtful whether 10-d growth is to be considered as a true chronic endpoint when starting with 2nd instar larvae.

39 Measured concentrations are reported as 0.39, 0.74 and 2.15 µg/L (78, 49 and 48% of nominal) at the end of the constant exposure test. Not clear which time period is meant, probably the end of the 10-days exposure period in which half of the test solutions was renewed every 48 h. Exposure over the actual test period not known. Acid-washed inorganic fine sediment present. ca. 30% reduction in locomotion as compared to control at next higher concentration, and almost no ventilation.

40 Test according to OECD 219. Partial renewal (100 out of 150 mL) every 48 h. Inorganic fine sediment present. Endpoint based on nominal concentrations, measured concentration in old solutions 96% of nominal at level of NOEC. Test performed under 14:10 h L:D.

41 Test according to OECD 219. Partial renewal (100 out of 150 mL) every 48 h. Inorganic fine sediment present. Endpoint recalculated from nominal concentration in paper (LOEC 3.7 µg/L → NOEC 1.2 µg/L), using reported recovery in old solutions of 64% of nominal. Test performed under 14:10 h L:D.

42 DAR only reports endpoints, but summary available in Anatra-Cordone and Durkin, 2005. Original study also cited in Stoughton et al., 2008. Endpoint based on measured concentrations.

43 DAR only reports endpoints, but summary available in Anatra-Cordone and Durkin, 2005. Original study also cited in Stoughton et al., 2008. Endpoint based on measured concentrations. Doubtful whether 10-d growth is to be considered as a true chronic endpoint when starting with 2nd instar larvae.

44 Mean measured concentration 117-160% of nominal, results based on mean measured concentrations. Results for first 10 d of 28-d test. Survival includes emergence. NOEC reported as 3.57 µg/L, but since LOEC is reported as >3.57 µg/L, NOEC should read ≥ 3.57 µg/L.

45 Endpoint recalculated using TechDig analysis of graph. Mean measured concentration 117-160% of nominal, results based on mean measured concentrations. Results for first 10 d of 28-d test. Number of test concentrations (4) low, but considered acceptable for EC10 calculation.

46 Mean measured concentration 117-160% of nominal, results based on mean measured concentrations. Results for first 10 d of 28-d test. LOEC is higher than EC50, but clear concentration-response relationship present. Number of test concentrations (4) low, but considered acceptable for EC50 calculation. Doubtful whether 10-d growth is to be considered as a true chronic endpoint when starting with 7-d old larvae.

47 Endpoint recalculated using TechDig analysis of graph; mean measured concentration 117-160% of nominal, results based on mean measured concentrations; results for first 10 d of 28-d test; number of test concentrations (4) low, but considered acceptable for EC10 calculation; doubtful whether 10-d growth is to be considered as a true chronic endpoint when starting with 7-d old larvae.

48 Mean measured concentration 117-160% of nominal, results based on mean measured concentrations; results for first 10 d of 28-d test; clear concentration-response relationship, preference is given to EC10.

49 Mean measured concentration 114-150% of nominal, results based on mean measured concentrations; survival measured as emergence; NOEC and LOEC are higher than LC50, but clear concentration-response relationship present; number of test concentrations (4) low, but considered acceptable for LC50 calculation.

50 Mean measured concentration 114-150% of nominal, results based on mean measured concentrations. Survival measured as emergence. 55% reduction at 1.14 µg/L, but not significant. LOEC and NOEC are higher than LC50, probably reduced power because of variation between replicates and/or applied statistical test. Clear concentration-response relationship, preference is given to LC10.

51 Endpoint recalculated using TechDig analysis of graph; mean measured concentration 114-150% of nominal, results based on mean measured concentrations; survival measured as emergence; number of test concentrations (4) low, but considered acceptable for LC10 calculation; LC10 marginally lower than lowest test concentration/2, result considered acceptable.

52 Mean measured concentration 114-150% of nominal, results based on mean measured concentrations. No significant difference at 1.14 µg/L, but full mortality at next higher concentration.

53 Concentration in dosing solution 95.5%, time weighted average concentration 86.4% of nominal, results expressed as nominal

54 Renewal after 10 d. Concentrations not measured. Mixture of avermectin and imidacloprid, content of individual compounds not given. Test concentrations presented as insecticide, not clear whether corrected for active content.

55 Concentration in dosing solution 95.5%, time weighted average concentration 92.4% of nominal, results expressed as nominal

56 Results from microcosm experiment with stonefly and cranefly, organic material present, initial concentrations 96-108% of nominal, decline by 53-55% after 14 d, result recalculated based on geometric mean measured concentrations using mortality data from paper.

56 Results from microcosm experiment with stonefly and cranefly, organic material present, initial concentrations 96-108% of nominal, decline by 53-55% after 14 d, result recalculated based on geometric mean measured concentrations using mortality data from paper.

57 Results from microcosm experiment with stonefly and cranefly, organic material present, result recalculated based on twa measured concentrations using mortality data from paper.

58 Partial renewal (100 out of 150 mL) every 48 h. Inorganic fine sediment present. Mortality at all concentrations reported to be <10%, 20% at intermediate concentration 1.9 µg/L nominal, so NOEC is considered to be ≥ 7.8 µg/L nominal. Using reported recovery in old solutions of 66-63% of nominal, this is equal to >5.0 µg/L. Test performed under 14:10 h L:D.

59 Partial renewal (100 out of 150 mL) every 48 h. Inorganic fine sediment present. Endpoint recalculated from nominal concentration in paper (LOEC 3.9 µg/L → NOEC 1.9 µg/L), using reported recovery in old solutions of 66-63% of nominal. Test performed under 14:10 h L:D. Animals acclimated for 14 d. Feeding activity measured as weight loss of alder leaf discs. Feeding rate is not a parameter that is considered for risk limit derivation.

60 Concentration in dosing solution 95.5%, time weighted average concentration 95.3% of nominal, results expressed as nominal

60 Concentration in dosing solution 95.5%, time weighted average concentration 95.3% of nominal, results expressed as nominal

61 Results from microcosm experiment with stonefly and cranefly, organic material present, initial concentrations 96-108% of nominal, decline by 53-55% after 14 d, result recalculated based on geometric mean measured concentrations using mortality data from paper

62 Results from microcosm experiment with stonefly and cranefly, organic material present, initial concentrations 96-108% of nominal, decline by 53-55% after 14 d, result recalculated based on geometric mean measured concentrations, <50% mortality at highest concentration

63 Results from microcosm experiment with stonefly and cranefly, organic material present, result recalculated based on twa measured concentrations using mortality data from paper, ambiguous fit

64 Not clear if based on measured concentrations, test duration and conditions not reported. Original study not available.

65 In view of life stage, test is considered as chronic. Purity of test compound not reported. Stock solutions kept in dark. Renewal every 48 h. Test performed under 12:12 h L:D, but concentrations not measured. No effects at highest concentration tested. Hardness recalculated from reported concentrations of Ca and Mg.

66 In view of life stage, test is considered as chronic. Purity of test compound not reported. Test performed under 12:12 h L:D, but concentrations not measured. Stock solutions kept in dark. Renewal every 48 h. No effects at highest concentration tested. Hardness recalculated from reported concentrations of Ca and Mg.

67 In view of life stage, test is considered as chronic. No effect on series of parameters tested. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively.

68 In view of life stage, test is considered as chronic. Endpoint is most sensitive parameter (heart beat) from series of developmental parameters tested. Test with solvents alone shows contribution of solvent to effect. Stablity of test concentrations in distilled water checked for 21 d at 21ºC, room light. At 70 mg/L and lower stable for 21 d, at 105 and 140 mg/L stable for 17 d, thereafter decline to 84 and 76% of nominal, respectively.

69 Test according to OECD 204. Endpoint based on mean measured concentrations (95-105% of nominal), but precipitation and turbidity was noted at all test concentrations.

70 Test according to OECD 210. Previously assigned Ri3 because endpoint was based on nominal. However, additional information shows that endpoint is based on mean measured concentrations.

71 Endpoint based on mean measured concentrations. Most sensitive endpoint growth after 36 days.

72 Endpoint expressed as nominal concentration. Concentrations not measured, test performed under 12:12 L:D. Test water is tap water with added seasalt, up to conductivity of 820 µS/cm. Significant effect on heart rate.

73 Endpoint expressed as nominal concentration. Concentrations not measured, test performed under 12:12 L:D. Test water is tap water with added seasalt, up to conductivity of 820 µS/cm.

Table S1.3 Acute toxicity of imidacloprid for marine species

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Bacteria																
Vibrio fischeri		N	S	Confidor			20	2		30 min	EC50	bioluminescence	226000	3	1	[5]
Vibrio fischeri		Y	S	imidacloprid		am				15 min	EC50	bioluminescence	101000	3	2	[48]
Crustacea																
Artemia sp.	4th instar nauplii	N	S	imidacloprid	tg	am	38	8	27	48 h	LC50	mortality	361230	3	3	[49,50]
Artemia sp.	4th instar nauplii	N	S	imidacloprid	tg	am	9.5	8	27	48 h	LC50	mortality	> 300000	3	4	[49]
Americamysis bahia	< 24 h old	Y	F	240 S Formulation	22.7%	nw	20	8.2-8.5	19.7-25.0	96 h	LC50	mortality	36	2	5	[3]
Americamysis bahia	< 24 h old	Y	F	imidacloprid	96.2%					96 h	LC50	mortality	37.7	2	6	[3,6]
Americamysis bahia	< 24 h old	Y	F	imidacloprid	96.2%					96 h	LC50	mortality	34.1	2	7	[3,6]
Artemia parthenogenetica	2nd-3rd instar nauplii	N	S	imidacloprid		asw			28	24 h	LC50	mortality	1170	3	10	[51]
Palaemonetes pugio	larvae, 1-2 d, F1 from field collected animals	N	R	imidacloprid	99.5%		20		25	96 h	LC50	mortality	309	3	8	[52]
Palaemonetes pugio	adult, field collected, acclimated 2 wk	N	R	imidacloprid	99.5%		20		25	96 h	LC50	mortality	564	3	8	[52]
Callinectes sapidus	larvae, megalopa stage	N	S	imidacloprid	99.5%	nw	35		25	24 h	LC50	mortality	10	3	9	[53]
Callinectes sapidus	larvae, megalopa stage	N	S	TrimaxPro	40.8%	nw	35		25	24 h	LC50	mortality	313	3	9	[53]
Callinectes sapidus	juveniles	N	S	imidacloprid	99.5%	nw	35		25	24 h	LC50	mortality	1112	3	9	[53]
Callinectes sapidus	juveniles	N	S	TrimaxPro	40.8%	nw	35		25	24 h	LC50	mortality	817	3	9	[53]
Mollusca																
Crassostrea virginica		Y	F	imidacloprid	96.2					96 h	EC50	shell growth	> 23300	2	11	[3,6]
Crassostrea virginica		Y	F	imidacloprid	95.8					96 h	EC50	shell growth	> 145000	2	12	[3,6]
Insecta																
Aedes taeniorhynchus	1st instar	N	S	imidacloprid	tg	am	38	8	27	48 h	LC50	mortality	13	3	13	[49,50]
Aedes taeniorhynchus	1st instar	N	S	imidacloprid	tg	am	12.7	8	27	72 h	LC50	mortality	21	3	4	[49]
Pisces																
Cyprinodon variegatus	29 mm, 0.77 g	Y	S	imidacloprid	96.2					96 h	LC50	mortality	161000	2	14	[3,6]

Notes

1 Concentrations not measured; no details on test water; no details on test compound; Microtox test.
2 Measured concentrations not reported
3 Actual concentrations not measured, test performed under light. Hyperosmotic conditions. Solvent control included.
4 Actual concentrations not measured, test performed under light. Isosmotic conditions. Solvent control included.
5 DO below protocol requirements. Based on measured concentrations.
6 DAR only reports endpoints, but summary available in Anatra-Cordone and Durkin, 2005. Endpoint based on mean measured concentrations.
7 DAR only reports endpoints, but summary available in Anatra-Cordone and Durkin, 2005.
8 Concentrations measured in stock solutions only (103% of nominal), test performed under 16:8 h L:D. Acetone used as solvent in max. 0.1%. Test water parameters measured, but not reported.
9 Concentrations not measured; author confirmed that ambient overhead fluorescent light was present, app. 10:14 h L:D
10 Test compound added as solution in methanol, dried under vacuum before addition of nauplii suspension; incubation under light; concentrations not measured; no details on test substance
11 Test reported in table in the DAR. Not considered valid in the DAR because control performance was less than required. According to information in Anatra-Cordone and Durkin, 2005, results are based on measured concentrations.
12 DAR only reports EC50 >145 mg/L. Limit test, inhibition 22%. According to information in Anatra-Cordone and Durkin, 2005, results are based on measured concentrations.
13 Actual concentrations not measured, test performed under light. Hyperosmotic conditions. Solvent control included. Endpoint refers to most relevant test duration and lowest endpoint.
14 DAR only reports endpoints. According to information in Anatra-Cordone and Durkin, 2005, results are based on measured concentrations.

Table S1.4 Chronic toxicity of imidacloprid for marine species

Species	Species properties	A	Test type	Test compound	Purity [%]	Test water	Hardness $CaCO_3$ [mg/L]	pH	T [°C]	Exp. time	Crit.	Test endpoint	Value [µg/L]	Ri	Note	Ref.
Crustacea																
Americamysis bahia	<24 h old	Y	F	imidacloprid	96.2%					28 d	NOEC	reproduction	0.56	3	1	[6]
Americamysis bahia	<24 h old	Y	F	imidacloprid	96.2%					28 d	NOEC	growth	0.163	3	2	[6]
Callinectes sapidus	juveniles	N	S	imidacloprid	99.5%	nw					NOEC	time to metamorphosis	≥ 3.8	3	3	[53]
Mollusca																
Crassostrea virginica		Y	F	imidacloprid	96.2					96 h	NOEC	shell growth	≥ 23300	2	4	[3,6]
Crassostrea virginica		Y	F	imidacloprid	95.8					96 h	NOEC	shell growth	< 145000	3	5	[3,6]

Notes

1 No further details on test conditions provided in DAR, information available from Anatra-Cordone and Durkin, 2005. Endpoint based on measured concentrations. Study rejected in DAR because reproduction rate of controls was too low, and information on individual females is missing.

2 No further details on test conditions provided in DAR, information available from Anatra-Cordone and Durkin, 2005. Study rejected in DAR because reproduction rate of controls was too low, and information on individual females is missing. NOEC for growth was 3.8 µg/L in first test, reason for difference is not clear.

3 Concentrations not measured; static test performed under ambient light

4 Short-term test, but in view of endpoint considered as chronic. DAR only reports EC50 >23.3 mg/L. Information available from Anatra-Cordone and Durkin, 2005. Based on measured concentrations.

5 Short-term test, but in view of endpoint considered as chronic. DAR only reports EC50 >145 mg/L. Information available from Anatra-Cordone and Durkin, 2005. Based on measured concentrations. Decrease by 22% observed. Limit test, not possible to check concentration response relationship.

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Supporting information 2. Evaluation of micro- and mesocosmstudies.

Study 1	
Reference	[1,2]
Species; Population; Community	Phytoplankton, periphyton, invertebrates, zooplankton
Test Method	Mesocosm
System properties	Outdoor ponds, 2.0-2.2 m diameter, 1.0 m deep, 3100-3800 L
Formulation	Imidacloprid SL 200
Exposure regime	0, 0.6, 1.5, 3.8, 9.4 and 23.5 µg/L; 2 applications (May 2 and May 23)
Analysed	Y
Temperature [°C]	Not reported in summary
pH range	Not reported in summary
Hardness [mg $CaCO_3$/L]	Not reported in summary
Exposure time	182 d
Criterion	NOEC
Test endpoint	Population response of benthic invertebrates and zooplankton
Value [µg/L]	0.6 (nominal)
GLP	Y
Guideline	OECD, SETAC
Notes	Original reports not available, based on summary and evaluation in DAR
Ri	2

Description

Test system

Thirteen mesocosms of 2.0-2.2 m diameter, 10 cm natural sediment and 1.0 m water, total 3100-3800 L, sediment not specified. Organisms were added with the sediment and phytoplankton and zooplankton were obtained from natural ponds. Ponds were left to establish during 6 months. Application took place on May 2 and 23, 2001, Treatments, 0, 0.6, 1.5, 3.8, 9.4 and 23.5 a.s. µg/L in duplicate, untreated in triplicate. The substance was sprayed on the pond surface.

Analytical sampling

Concentration was measured in the application solutions, and in initial concentrations in pond water samplings, and regularly during the experiment in water and sediment.

Effect sampling

Effect parameters zooplankton, phytoplankton, chlorophyll-a, emerging insects and macrozoobenthos (by artificial substrate and sediment) were regularly monitored.

Statistical analysis

Univariate and multivariate analyses, PRC.

Results

Chemical analysis

Before the 2nd application, 12-20% of the nominal concentrations was present in the waterphase. The DT_{50} ranged from 5.8 to 13.0 days at all test concentrations after both applications, average DT_{50} 8.2 d. Initial measured concentrations are not reported, but it was concluded that nominal concentrations could be used to express initial exposure. Imidacloprid was found in the sediment, with the highest concentrations one week after second application. Thereafter, the concentration decreased to below LOQ of 7 µg/kg in the highest concentrations after 56-70 d. In the lower treatments, a similar pattern was seen, however the concentrations were close to the LOQ. DT_{50} for imidacloprid in the whole system (determined in the two highest dosages only) is 14.8 d.

Biological observations

Insects (caught by the emergence traps) were the most significantly affected organisms, from 1.5 µg/L upwards. Effects were found on community parameters such as taxa richness, diversity, similarity and principal response. Chironomidae and Baetidae were the most sensitive taxa. No effects were found at 0.6 µg/L, which can be seen as the NOEC. Indirect effects were found on algae, but only the NOEAEC (defined as recovery within 8 weeks

after last application) of 23.5 µg/L is reported. For zooplankton, a NOEC of 9.4 µg/L is reported for copepods and cladocerans, for macrozoobenthos the NOEC for the most sensitive species (*Chaoborus* spp.) is 9.4 µg/L.

Evaluation of the scientific reliability of the field study
Criteria for a suitable (semi)field study

- Does the test system represent a realistic freshwater community? Yes, natural populations of algae, zooplankton and macroinvertebrates were present. Macrophytes and fish were not present.
- Is the description of the experimental set-up adequate and unambiguous? Unclear, not all details are reported in the available summary.
- Is the exposure regime adequately described? Yes.
- Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, but potentially sensitive taxa such (Ostracoda, Amphipoda, Ephemeroptera) were not or not well represented.
- Is it possible to evaluate the observed effects statistically? No, no details concerning measurement endpoint are given for concentrations and effect data. The data are analysed according to up-to-date methods, however.

The study is considered less reliable (Ri 2) mainly because potentially sensitive taxa such as Ostracoda and Amphipoda are not or not well represented, and numbers of Ephemeroptera were too low for statistical analysis. In the DAR, the 0.6 µg/L-treatment is considered as the NOEC (equivalent to 0.51 µg/L expressed as 48-h TWA concentration). No agreement was reached on the level of the NOEAEC [3,4], mainly because doubts were raised on the representativeness of the recovery potential of chironomids for univoltine species. This however, is not relevant since recovery is not taken into account for EQS-derivation.

Conclusion
The NOEC of 0.6 µg/L nominal will be considered for EQS-derivation.

Study 2	
Reference	[5]
Species; Population; Community	Larvae of two frog species (*Acris crepitans* and *Rana clamitans*), periphyton, phytoplankton, zooplankton
Test Method	Mesocosm
System properties	Outdoor ponds, 1.85 m in diameter, ca. 900 L of water and 1 kg of litter
Formulation	Merit
Exposure regime	0 and 9000 µg/L
Analysed	N
Temperature [°C]	Not reported
pH range	Not reported
Hardness [mg $CaCO_3$/L]	Not reported
Exposure time	55 d
Criterion	NOEC
Test endpoint	mortality of amphibians
Value [µg/L]	9000
GLP	No
Guideline	No
Notes	Two experiments were performed, (1) leaves systemically treated with imidacloprid and (2) exposure via water. Experiment 2 is summarized here.
Ri	3 (no measurements of test concentration)

Description
Test system
Aquatic communities in ponds, 1.85 m in diameter, ca. 900 L of water and 1 kg of litter, plankton introduced. Ponds were established ca. 1 month before application. Start experiment: 3 July 2008. Treatments: 0 and 9000 µg a.s./L, four replicates. Other treatments were exposure to predators (fish, crayfish) and a combination of imidacloprid and predators. These treatments are left out of consideration here.

Analytical sampling
Concentration was not measured.

Effect sampling
Survival larvae of frog species *Acris crepitans* and *Ranaclamitans*, periphyton, phytoplankton, zooplankton.

Statistical analysis
Univariate analysis.

Results
Chemical analysis
No chemicals analysis reported.

Biological observations
Tadpoles of *A. crepitans* were significantly affected (mortality) at 9000 µg/L. No effects for *R. clamitans*. Increased oxygen levels by the end of the study (55 days).

Evaluation of the scientific reliability of the field study
Criteria for a suitable (semi)field study

- Does the test system represent a realistic freshwater community? Yes, but the study only focussed on survival of amphibian larvae.
- Is the description of the experimental set-up adequate and unambiguous? Yes.
- Is the exposure regime adequately described? No. Intended concentration is reported only.
- Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? No, representatives of arthropods are 3 to 4 orders of magnitude more sensitive.
- Is it possible to evaluate the observed effects statistically? Yes (univariate only). However, one test concentration studied only. The effect class system is not designed for this type of studies.

The study is considered to be unreliable (Ri 3), due to the fact that the intended test concentration is not analytically verified. Furthermore, relatively insensitive species were tested.

Conclusion
This study will not be used for EQS-derivation.

Study 3	
Reference	[6]
Species; Population; Community	caged *Gammarus roeseli*
Test Method	Mesocosm
System properties	Indoor stream mesocosm, 73 m, 16.1 m^3, depth 0.2 m, stream velocity 10 cm/s
Formulation	not specified
Exposure regime	Pulse (3 x 12 h) – 7 d interval (application on day 1, 8, 15 and 50, 57, 64); 0 and 12 µg/L
Analysed	Y
Temperature [°C]	16.4
pH range	7.9
Hardness [mg $CaCO_3$/L]	176 (calculated from reported Ca^{2+} and Mg^{2+})
Exposure time	70
Criterion	
Test endpoint	abundance, size distribution, reproductive status, litter degradation
Value [µg/L]	
GLP	No
Guideline	No
Notes	Single species test, no effect class evaluation possible
Ri	2

Description

Test system

Experimental stream indoor mesocosms (length 73 m, volume 16.1 m^3, depth 0.2 m; stream velocity 10 cm/s). Treatment with two series of three 12 µg/L pulses each, weekly interval, first series on day 1, 8 and 15, second series on day 50, 57 and 64. Application overnight to prevent photolysis. Four pairs of treatment and control, treated on four consecutive days.

Field collected *Gammarus roeseli* were exposed in cages with alder or straw as food source, 32 cages per stream with 10 adults each and four additional cages with food but without animals per stream.

Analytical sampling

Homogeneity of application recorded using fluorescent tracer, exchange of water between stream and cages checked. Water samples every 4 days, analysis of imidacloprid, nutrients and ion compounds; pH, temperature, oxygen and conductivity were monitored permanently.

Effect sampling

Duplicate cages sampled weekly 1 h prior to imidacloprid application, between the two pulse series on day 21 and 28, and after the last pulse on day 70. Gammarids were counted, size distribution was recorded. Females carrying eggs or early instars were counted. Litter material was sieved out and separated into size classes, and analysed for lignin, cellulose and phenols, carbon and nitrogen.

Results

Chemical analysis

Longitudinal homogeneity reached within 10 flow cycles (135 min.) after application. Exchange of stream water with the cages reached within 15 min. Mean measured concentration was 11.9 µg/L after reaching homogeneity, and dropped to 0.08 µg/L when total water renewal was achieved. No significant differences between controls and treatments with respect to water characteristics.

Biological observations

No effects on total abundance, population development, litter decomposition, and size classes. Trend towards lower number of brood carrying females in imidacloprid treatment in presence with alder. At the end, number was 19.8 in control and 13 in treatment (34% difference). Difference was significant on day 49 and 70 when control and treatment were tested in pairs, but not when controls and treatments were tested against each other. Authors conclude that imidacloprid has a delayed effect on brood carrying females.

Evaluation of the scientific reliability of the field study

Criteria for a suitable (semi)field study

- Does the test system represent a realistic freshwater community? No, study is single species test in mesocosm.
- Is the description of the experimental set-up adequate and unambiguous? Yes.
- Is the exposure regime adequately described? Yes.
- Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, *G. roeseli* belongs to the relatively sensitive species on the basis of acute laboratory data
- Is it possible to evaluate the observed effects statistically? Yes/No. One test concentration studied only, difference in outcome of statistical analysis (testing in pairs/testing all treatments) indicates influence of experimental set-up. The effect class system is not designed for this type of studies.

In view of these criteria, the study is considered to be less reliable (Ri 2), mainly due to the unclear statistical evaluation and the fact that exposure was shorter than the time window considered for derivation of the MAC-$QS_{fw, eco}$. derivation. It is not fully clear what the observed reduction of 34% in brood carrying females means in terms of population development and how the food source interacts with the observed effect. The study can be used as an indication that repeated short-term pulses of 12 µg/L may induce long-term or delayed effects, but it is not possible to establish a statistically underpinned NOEC.

Conclusion

This study is not used for EQS-derivation.

Study 4	
Reference	[7]
Species; Population; Community	Leaf-shredding insects (stonefly: *Pteronarcis dorsata* and crane fly: *Tipula* sp.), microbial decomposers.
Test Method	Microcosm
System properties	Aquaria: 13 X 30 x 21 cm, 6 L, indoor
Formulation	Ecoprid
Exposure regime	0, 1.2, 12, 120, 1200, 12000 µg/L (0, 1.0, 12.0, 135,1550, 15400 µg/L measured 1 h after treatment).
Analysed	Y
Temperature [°C]	18.9-20.4
pH range	6.1-7.1
Hardness [mg $CaCO_3$/L]	Not reported
Exposure time	14 d
Criterion	LC10
Test endpoint	Population response of leaf-shredding insects and microbial decomposers
Value [µg/L]	13.3 (*P. dorsata*)
GLP	No
Guideline	No
Notes	Multi-species test (2 insect species), short study (14 d), no effect class evaluation possible
Ri	2

Description

Test system

Indoor microcosms (glass aquaria, LxWxH 30x13x21 cm), 6 L natural stream water (Sault Ste. Marie, Ontario, Canada), 300 mL stream detritus (1-5 mm sieved; organisms killed by freezing), 10 twigs from speckled alder (*Alnus incana*) trees. Stonefly nymphs (*Pteronarcys dorsata* Say) and cranefly larvae (*Tipula* sp. L.) sampled from local stream. Microcosms were operated for 1 week prior to treatment, organisms (n=9) introduced 2 days before treatment. Treatments 0, 1.0, 12.0, 135, 1550 and 15400 µg a.s./L, four replicates plus two additional replicates for fate assessment. The substance was added to the water surface, while the water was gently stirred.

Analytical sampling

Concentration was in initial concentrations in water samples, and regularly during the experiment in water and leaf material introduced.

Effect sampling

Effect parameters: Stonefly and cranefly were counted after 14 days, microbial decomposition was monitored after 7 and 14 days.

Statistical analysis

Univariate analysis.

Results

Chemical analysis

Initial measured concentrations were 1.0, 12.0, 135, 1550 and 15400 µg a.s./L. Half-lives not reported. Concentrations, were ca. 50% (mean) after 14 days. Average actual concentrations calculated as ≈0.2, 6.1, 73, 902 and 9664 µg a.s./L based on reported measured concentrations in fate replicates. Imidacloprid was found in the introduced leaf material taken in samples of 2 days and later.

Biological observations

Both insect species were significantly affected (mortality) from 135 µg/L and higher. No effects (mortality; including mordibundancy) were found at 12 µg/L, which can be seen as the NOEC. There were no significant differences from controls in oxygen uptake at any test concentration. Microbial decomposition activity was significantly increased at the highest test concentration.

Evaluation of the scientific reliability of the field study
Criteria for a suitable (semi)field study

- Does the test system represent a realistic freshwater community? No, this study may be considered as a multi-species test (two insect species tested).
- Is the description of the experimental set-up adequate and unambiguous? Yes, but number of test organisms is low.
- Is the exposure regime adequately described? Yes.
- Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, in case of the insects.
- Is it possible to evaluate the observed effects statistically? Yes (univariate only). However, no realistic invertebrate community was tested. Duration of test was 14 days, recovery and community interaction cannot be evaluated. The effect class system cannot be applied.

The study is considered to be less reliable (Ri 2) for evaluation of effects on realistic freshwater communitie. Using the reported measured concentrations and data on mortality, the 14-days LC10 was estimated as 13.3 µg a.s./L for *P. dorsata* and 50 µg/L for *Tipula* sp. The latter value is not considered reliable due to an ambiguous fit.

Conclusion
The LC10 of 13.3 µg/L for *P. dorsata* is included in the chronic dataset.

Study 5	
Reference	[8]
Species; Population; Community	Leaf-shredding insects (stonefly: *Pteronarcis dorsata* and crane fly: *Tipula* sp.), microbial decomposers.
Test Method	Microcosm
System properties	Aquaria: 13 X 30 x 21 cm, 6 L, indoor
Formulation	Confidor 200SL
Exposure regime	Single application of 0, 12, 24, 48, 96 µg/L
Analysed	Y
Temperature [°C]	20 ± 3
pH range	Not reported
Hardness [mg $CaCO_3$/L]	Not reported
Exposure time	14 d
Criterion	LC10, LC50, NOEC
Test endpoint	Mortality, feeding
Value [µg/L]	LC10 15.8, LC50 41 (*P. dorsata*), LC10 34, LC50 > 63 (*Tipulia sp.*) NOEC feeding < 8.8
GLP	No
Guideline	No
Notes	Multi-species test (2 insect species), short study (14 d), no effect class evaluation possible
Ri	2

Description
Test system
Indoor microcosms (glass aquaria, LxWxH 30x13x21 cm), 6 L natural stream water, 300 mL stream detritus (1-5 mm sieved; organisms killed by freezing), 10 twigs from speckled alder (*Alnus incana*) trees. Stonefly nymphs (*Pteronarcys dorsata* Say) and cranefly larvae (*Tipula* sp. L.) sampled from local stream. Microcosms were set up 1 week prior to treatment, organisms (n=9) introduced 2 days before treatment. Treatments, 0, 12, 24, 48 and 96 µg a.s./L, in triplicate. The substance was added to the water surface, mixing by gently stirring.

Analytical sampling
Initial concentrations in water samples were measured, and by the end of the study (14 d).

Effect sampling
Effect parameters: Stonefly and cranefly were counted after 14 days, microbial decomposition was monitored.

Statistical analysis
Univariate analysis.

Results
Chemical analysis
Average initial within 96%–108% of nominal (CV < 10%), final concentrations 53%–55%. Geometric mean concentrations were 8.8, 16, 32 and 63 µg/L.

Effects
Mortality of *P. dorsata* was 3.7% in the control, 3.7 and 7.3% at 12 and 24 µg/L, and 40.7 and 70.4% at 48 and 96 µg/L, latter significant. 14-days LC10 was reported as 20.8 µg/L, 14-days LC50 70.1 µg/L. Mortality of the cranefly, *Tipula* sp., was 11.1% in the control, 7.4, 7.4, 18.5 and 33.3% at the respective test concentrations, differences were not significant. 14-days LC10 was reported as 16.2 µg/L, 14-days LC50 139 µg/L. Live tipulids were sluggish, authors conclude that if those had been quantified and counted as dead, the effects on *Tipula* were similar to those on *P. dorsata*.
Mass loss of leaf material in the imidacloprid treatments was significantly lower than in the control, no visible signs of shredding at 48 and 96 µg/L. Signs of insect feeding at lower concentrations, but at lower rates than the control. No indications of inhibition of microbial decomposition. Authors conclude that concentrations of 12 µg/L are likely to cause significant feeding inhibition in leaf-shredding insects which has the potential to interfere with ecosystem processes.

Evaluation of the scientific reliability of the field study
Criteria for a suitable (semi)field study

- Does the test system represent a realistic freshwater community? No, this study may be considered a multi-species test (two insect species tested).
- Is the description of the experimental set-up adequate and unambiguous? Yes, but number of replicates and organisms is low.
- Is the exposure regime adequately described? Yes, but no analytical measurements in between
- Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, in case of the insects.
- Is it possible to evaluate the observed effects statistically? Yes (univariate only). However, no realistic invertebrate community was tested. Duration of test was 14 days, recovery and community interaction cannot be evaluated. The effect class system cannot be applied.

The study is considered to be less reliable (Ri 2) for evaluation of effects on realistic freshwater communities. Endpoints for *P. dorsata* were recalculated using geometric mean concentrations, LC10 15.8, LC50 41 µg/L. LC10 for *Tipula* sp. is estimated as 34 µg/L, LC50 is > 63 µg/L.

Conclusion
LC10 15.8 µg/L and LC50 41 µg/L for *P. dorsata* and LC10 34 µg/L and LC50 > 63 µg/L for *Tipula* sp. are included in the chronic dataset.

Study 6	
Reference	[9]
Species; Population; Community	Benthic macroinvertebrate assemblage, periphyton
Test Method	Mesocosm
System properties	Outdoor stream mesocosms; planar area: 0.065 m^2, 10 L volume, flow-through with water velocity of 11-12 cm/s, coarse and fine substratum
Formulation	Admire (240 g a.s./L)
Exposure regime	Pulse (3 x 24-h) – 7d interval: 0, 1.63, 17.60 µg/L. Average measured peak concentrations
Analysed	Y
Temperature [°C]	14.5 – 14.9
pH range	Not reported
Hardness [mg $CaCO_3$/L]	Not reported
Exposure time	20 d
Criterion	NOEC (Class 1-2)
Test endpoint	Benthic invertebrates: abundance, emergence; microbial decomposition leaf material
Value [µg/L]	1.63 (average measured peak concentration)
GLP	No
Guideline	No
Notes	Short study (20 d), one sampling date, no effect class evaluation possible
Ri	2

Description

Test system

Artificial streams, flow-through, 10 L volume. Inoculated with a benthic invertebrate stream community. The sediment consisted of substratum obtained from gravel beds adjacent to the invertebrate sampling site (Nashwaak River, Canada). Test specimens were introduced 1 day before application. Treatment with three 24-hour pulses at a 7 days interval, concentrations 0, 2 and 20 µg a.s./L. Number of replicates probably 16 (not fully clear from paper). Test performed in August 2005.

Analytical sampling

Samples for imidacloprid analyses were taken at the onset, during and at the end of the pulse.

Effect sampling

Abundance and emergence of benthic invertebrates, one sampling at the end of the experiment (20 days). Microbial decomposition leaf material.

Statistical analysis

Univariate analysis and biotic indices for community response

Results

Chemical analysis

Average measured concentrations over the 24-hours pulse were 1.63 and 17.60 µg/L.

Biological observations

High densities of insects were observed in the control by day 20, dominant taxa were Heptageniidae (Ephemeroptera), Lepidostomatidae, Hydropsychidae and Helicopsychidae (Trichoptera), chironomids, dipteran pupae and elmidae beetles. No differences between both treatments and controls on microbial decomposition rates. Imidacloprid had an adverse effect on benthic communities, ca. 5% reduction at the low pulse (not significant) and 42% at the high pulse (significant). In the high pulse treatment a significant reduction (69%) was observed in combined Ephemeroptera, Plecoptera and Tricoptera taxa (EPT-taxa). Coleoptera were less affected (ca. 29 % reduction). No significant effects were observed for chironomids. Oligochaetes showed a high sensitivity (75% reduction, significant).

Evaluation of the scientific reliability of the field study
Criteria for a suitable (semi)field study

- Does the test system represent a realistic freshwater community? Yes.
- Is the description of the experimental set-up adequate and unambiguous? Yes.
- Is the exposure regime adequately described? Yes.
- Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, sensitive insect taxa included.
- Is it possible to evaluate the observed effects statistically? Yes. However, effect observations were made only shortly (7 days) after the last of the three 24-hour pulses and recovery and community interactions cannot be evaluated. The effect class system cannot be applied by its full merits, since it involved one sampling date only.

The study is considered less reliable (Ri 2) for the evaluation of effects of short-term exposure peaks on realistic freshwater communities, because longer-term effects were not evaluated. However, Effect class 1 and 2 could be derived for the endpoints reported:

	Treatment level [µg/L]	
	1.63	**17.60**
EPT*	1-2↓	4↓
Diptera (chironomids)	1	1
Coleoptera	1	1-2↓
Oligochaeta	1	4↓
Microbial decomposition	1	1
Most sensitive endpoint	1-2	4

*Ephemeroptera, Plecoptera, Trichoptera

Conclusion
The NOEC is 1.63 µg a.s./L, this value is considered for EQS-derivation.

Study 7	
Reference	[10]
Species; Population; Community	Benthic steam community; effects on two mayfly species reported
Test Method	Microcosm
System properties	Artificial streams; planar area: 0.065 m^2, 10 L volume, flow-through with water velocity of 11-12 cm/s, coarse and fine substratum; outdoor
Formulation	Admire
Exposure regime	Pulse (12-h): 0, 0.1, 0.3, 3.9, 9.1 µg/L Continuous (20 d): 0, 0.1, 0.3, 0.8 µg/L (actual measured)
Analysed	Y
Temperature [°C]	Not reported
pH range	Not reported
Hardness [mg $CaCO_3$/L]	Not reported
Exposure time	20 d
Criterion	NOEC
Test endpoint	Abundance, emergence, adult body size
Value [µg/L]	Pulse (12-h): 3.9 (abundance); 3.9 (emergence); < 0.1 (size); Continuous(20 d): 0.3 (abundance); 0.1 (emergence); < 0.1 (size)
GLP	No
Guideline	No
Notes	Effects on 2 mayfly species reported, being part of a benthic invertebrate stream community. Short study (20 d), no effect class evaluation possible
Ri	2

Description

Test system

Artificial streams, flow-through, 10 L volume. Inoculated with a benthic invertebrate stream community. Sediment consisted of substratum obtained from gravel beds adjacent to the invertebrate sampling site (Nashwaak River, Canada). Test location: Agri-foods Canada facility, New Brunswick, Canada.
Test organisms: mayfly species *Epeorus* spp. (Heptageniidae) and *Baetis* spp. (Baetidae), introduced 1 day before application. Intended treatments; pulse (12h): 0, 0.1, 0.5, 1, 5 and 10 µg a.s./L and continuous: 0, 0.1, 0.5, 1 µg a.s./L, n= 8 in both regimes.

Analytical sampling

Samples for imidacloprid analyses were taken at the onset, during and at the end of the pulse and every 5 days for the continuous exposures.

Effect sampling

Abundance, emergence, adult body size.

Statistical analysis

Univariate analysis

Results

Chemical analysis

Actual measured concentrations 0, 0.1, 0.3, 3.9, 9.1 µg a.s./L for pulse treatment and 0, 0.1, 0.3, 0.8 µg a.s./L for continuous exposure.

Biological observations

No differences between both treatment types and controls in algal biomass (chlorofyll-a, ash free biomass). NOECs for abundance, emergence and thorax or head length are presented in the table.

Exposure type		Endpoint	NOEC [µg/L]
Continuous	*Epeorus* spp.	abundance	0.3
		emergence	0.1
		adult male thorax length	0.1
		adult female thorax/head length	≥ 0.8
	Baetis spp.	abundance	0.3
		emergence	≥ 0.8
		adult male head length	< 0.1
		adult female thorax/head length	≥ 0.8
Pulse	*Epeorus* spp.	abundance	3.9
		emergence	3.9
		adult male thorax length	< 0.1
		adult female thorax/head length	≥ 9.1
	Baetis spp.	abundance	≥ 9.1
		emergence	≥ 9.1
		adult male head length	< 0.1
		adult female thorax/head length	≥ 9.1

Evaluation of the scientific reliability of the field study

Criteria for a suitable (semi)field study

- Does the test system represent a realistic freshwater community? Yes, but the study focussed on effects on two mayfly genera. Effects on other species are not reported.
- Is the description of the experimental set-up adequate and unambiguous? Yes.
- Is the exposure regime adequately described? Yes.
- Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, mayflies belong to the most sensitive taxa from the laboratory dataset.

- Is it possible to evaluate the observed effects statistically? Yes (univariate only). Duration of test was 20 days, recovery and community interactions cannot be/were not evaluated. The effect class system cannot be applied by its full merits.

In view of these criteria, the study is considered less reliable (Ri 2), mainly because species of only two genera were reported, and longer-term effects cannot be evaluated. However, NOECs (Class 1 effects) could be derived for species reported.

Conclusion
The 12-hours NOECs of 3.9 µg/L and the 20-days NOEC of 0.1 µg/L are considered for EQS-derivation. Effect on head and thorax length is taken into account.

Study 8	
Reference	[11,12]
Species; Population; Community	Macrophytes, plankton, macroinvertebrates
Test Method	Mesocosm
System properties	Indoor streams, 75 m long, 1 m wide, 0.2 m water, flow-through with water velocity of 10 cm/s, sand / fine sediment substratum, pool sections
Formulation	Imidacloprid, 99.9% pure
Exposure regime	Pulse (3 x 12 h) – 7 d interval; two series, 2nd series about 50 d after 1st pulse; 0 and 12 µg/L
Analysed	Y
Temperature [°C]	15.7 - 16.3 (1st series), 17.5 - 19.3 (2nd series)
pH range	7.5-8.2
Hardness [mg $CaCO_3$/L]	Not reported
Exposure time	11 w
Criterion	NOEC
Test endpoint	community, drift
Value [µg/L]	< 12
GLP	No
Guideline	No
Notes	Only one concentration tested
Ri	2

Description
Test system
Experimental stream indoor mesocosms (length 753 m, 1 m wide, depth 0.2 m; stream velocity 10 cm/s), sand substratum and equipped with 4 pool sections (3 m long, 1.2 m wide), stocked with macrophyte *Sparganium erectum*. Treatment with two series of three 12 µg/L pulses each, weekly interval, simulating spring and autumn treatment, 2nd series started about 50 days after 1st pulse. Application overnight to prevent photolysis. Four pairs of treatment and control, treated on four consecutive days. Streams were stocked with straw litterbags that had been kept for 2 weeks in a reference stream in spring and were then transported to the mesocosm site and emptied in the streams. Re-stocking with summer communities about two weeks before the 2nd pulse series.

Analytical sampling
Homogeneity of application recorded using fluorescent tracer. Water samples were taken 11.5 h after starting the pulses.

Effect sampling
Quantitative emergence and benthos sampling on 10 occasions, 5 weekly samples during each pulse series. Emergence with 1 m^2 traps, benthos sampling at walls, sand and straw, total abundance estimated using sand to straw area. Live counts of large gammarids were made repreatedly in designated sand areas, *Neureclipsis* sp. (Trichoptera, caddisfly) were quantified by counting filtration nets prior to the 2nd application series.

Drift before, during, and after the pulses was measured using two drift nets that were placed in the middle of the stream bottom above the sediment surface in front of the 2nd and the 4th pool section (distance between nets = 20 m) with opening in flow direction. Additional drift nets were placed in each stream behind pool sections 1 and 3 on three. In the week prior to dosing, cathes were made during day and night as a references, after dosing, each drift net was checked at the end of each pulse (1st night), at the end of the following day (1st day), and on the second morning (2nd night). Specimens of *G. roeseli* ≤ 3.8 mm total length were counted separately, the 3 large size classes were pooled to one class > 3.8 mm).

Statistical analysis

Univariate and multivariate analysis (PRC), effects of imidacloprid on macroinvertebrate drift were calculated as quotient of all driftnet catches in the treatments and all driftnet catches in the corresponding control stream. Significant differences ($p < 0.05$) between treatment and control catches of driftnets, which were synchronously exposed in the same stream mesocosms, and between replicates were tested pulse by pulse with the Wilcoxon signed-rank test.

Results

Chemical analysis

Longitudinal homogeneity confirmed, measured concentrations during pulse 11.1 to 12.1 µg a.s./L.

Biological observations

Abundance, emergence [12]

Colonisation in spring resulted in mean abundance of 2432 individual per litter bag, dipterans were dominant followed by crustaceans. Latter group was dominant in the summer stock. Coefficient of variation between bags in spring and summer was ≈ 30 and 40% for crustaceans and ephemerids, ≈30 and 55% for trichopterans and 14 and 30% for dipterans. Higher variation was found for rare taxa. All functional groups were present, percentage of predators was ca. 10%. Initial abundance in the streams was ca. 1000 ind/m^2. Overall, 48 taxa were identified, with dipterans being most species rich. Gammarids increased after introduction, insects decreased.

Number of taxa declined over time in control and treatments, mainly due to emergence of dipterans. PRC on abundance of taxa was not significant and showed weak effects of treatment. Species weights indicated that Tanypodinae (Chironomidae) and *Baetis* (Ephemeroptera) were among the potentially affected taxa. Numbers of Tanypodinae were significantly lower in the treated streams on 2 successive occasions during the 2nd pulse series, non-significant decreases were observed for Diptera, Trichoptera and Ephemeroptera during the 2nd pulse series.

Non-emerging arthropods such as gammarids increased during the study. Based on population count data alone, no effects were observed. Live counts revealed significantly lower numbers of larger gammaridson sediment immediately after the 5th pulse. Numbers increased to control values but were significantly lower after the 6th pulse and remained significantly lower on three consecutive samplings for about 10 days. Authors conclude that gammarids have sought shelter in the straw after the pulses and returned to the sand after exposure.

Neureclipsis sp. showed a steady decrease in the control during the 2nd pulse series. In the treatment, numbers remained fairly constant but declined to almost 0 after the 4th pulse and were significantly different from the control on four consecutive samplings during ca. 10 days. Unlike for gammarids, no recovery was observed.

PRC for emergent insects was significant on three sampling occasions after the 4th pulse. A similar but not significant pattern was observed after the 1st pulse series. Significantly lower emergence was observed for

- Tanypodinae: 1 sampling after pulse 3, 2 samplings after pulse 5, no emergence on last sampling (day 70)
- Tanytarsini: 1 sampling after pulse 4
- Orthocladiinae: 1 sampling after pulse 4
- Ephemeroptera: no emergence during 1st pulse series, significant reduction from 4th pulse on, no emergence on last sampling day.

Drift [11]

Pre-exposure catches revealed significantly higher night-time drift in *Baetis* sp., chironomids (except for some species), higher night drift became more apparent during 2nd series in summer. Only few catches for *Caenis* sp. (Ephemeroptera). Significantly higher drift during and after imidacloprid pulses was observed for *Baetis* sp.,

Corynoneura sp. and Orthocladiinae (Chironomidae) and *G. roeseli* (< 3.8 mm). No significant effect on *G. roeseli* (> 3.8 mm) and Tanypodinae.

Evaluation of the scientific reliability of the field study
Criteria for a suitable (semi)field study

- Does the test system represent a realistic freshwater community? Yes.
- Is the description of the experimental set-up adequate and unambiguous? Yes.
- Is the exposure regime adequately described? Yes.
- Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes.
- Is it possible to evaluate the observed effects statistically? Yes. Last observations were 70 days (emergence; taxa abundance) or 95 days (gammarids, Neureclipsis) after 1st pulse, but because of restocking 2nd series should be considered separately and duration is 3 - 6 weeks. The effect class system cannot be applied by its full merits.

The study is considered to be less reliable (Ri 2), mainly because only one concentration was tested and duration was too short to consider recovery. Re-stocking can be considered as a kind of re-colonisation, which under natural conditions would only be possible from uncontaminated upstream water. Pulses were shorter than the time window considered for derivation of the MAC-$QS_{fw, eco}$, but repetition represents a worst case. The effects are summarised below according to the Effect class methodology.

	Effect class
abundance	
all taxa	1
Gammarus sp.	1
Diptera	1-2↓
Tanypodinae	3A
Trichoptera	4#
Ephemeroptera	3A#
PRC	1
life counts	
gammarids	3A
Neureclipsis sp.	3A
emergence	
Tanypodinae	4
Tanytarsini	2
Orthocladiinae	2
Ephemeroptera	4
PRC	4

not indicated as significant, but figure suggests otherwise

Conclusion
The study shows that repeated 12-hour pulses of 12 µg a.s./L lead to effects on abundance and emergence of several taxa, with Ephemeroptera (affected after single pulse), Trichoptera (id.), Chironomidae and Gammaridae being most sensitive. Increased drift was observed for *Baetis*, chironomids and *G. roeseli*. Since only one, relatively high, concentration was tested, the relevance for EQS-derivation is limited, but the study will be considered for EQS-derivation.

Study 9	
Reference	[13]
Species; Population; Community	Macroinvertebrates
Test Method	Outdoor microcosm
System properties	Cosms: 45.5 cm x 30 cm x 21 cm
Formulation	Not specified
Exposure regime	Y
Analysed	3 weekly applications
Temperature [°C]	
pH range	
Hardness [mg $CaCO_3$/L]	
Exposure time	10 weeks
Criterion	NOEC
Test endpoint	Abundance, emergence
Value [µg/L]	1.4 µg/L nominal
GLP	No
Guideline	No
Notes	
Ri	2

Description

Test system.

56 outdoor microcosms (20 L, lxwxh = 45.5 cm x 30 cm x 21 cm) in a reservoir pond in Berlin, Germany. Microcosms were filled with 750 mL fine homogenized sediment (silt and clay loam with 3% o.m.), from an uncontaminated lake, and with 15 L water from the reservoir pond. The microcosm were left floating, covered with a 2 cm mesh net for colonization for three weeks (late May to June). During this period every week an application with imidacloprid took place. After this colonization period, microcosm were covered with a fine nylon mesh and sampling lasted for seven weeks after third application.

In the control microcosms an average number of 680 individuals/microcosm was collected during the entire experiment. The macroinvertebrate assemblage was dominated by Chironomidae (Diptera) (65 %) from the subfamilies Chironominae, Tanypodinae, and Orthocladiinae. The second most abundant and frequent family was Gastropoda (18 %), represented by the pulmonate snail *Radix* sp., which probably entered the microcosms at the planktonic stage with the water. Other relatively abundant insect families were Ephemeroptera (*Caenis* sp. and *Cloeon* sp.), whereas Ceratopogonidae. Chaoboridae, Culicidae, other Diptera, and Nematoda were present in only a small number of microcosms.

Systems were exposed to 0.6, 1.4, 3.2, 7.5, 17.3, and 40 µg/L imidacloprid. 7 replicates for treatments, 14 replicates for untreated control. Exact dates (and year) not specified in the paper. Test item not specified else than imidacloprid.

Analytical sampling.

Concentrations were measured 6 h, 1 week and 6 weeks after each treatment and at the end of the experiment. Furthermore sacrificial tanks were set up for the 17.3 µg/L treatment. Here water was additionally sampled 1, 2, 3 and 7 days after each pulse. Whole sediment was taken from the sacrificial microcosm for chemical analyses.

Effect sampling.

Abiotic parameters (O_2, pH, temperature, turbidity, conductivity) were measured weekly. UV radiation was also recorded. Emerging insects were collected weekly after the third pulse. At the end of the experiment the content of each microcosm was filtered through a 500 µm sieve to collect remaining insect larvae. Total abundance, number of species and number of adults of nommon taxa were monitored as endpoints for the experiment.

Statistical analysis

For comparison of abundance, Kruskal-Wallis and Mann-Whitney U tests were performed. Jonckheere-Terpstra trens test was used to detect trend of gradually decrease of endpoints with increasing imidacloprid concentrations. Power analysis was performed to determine the power of the study design.

Results

Chemical analysis.

The DT50 for dissipation in water was determined as 20-36 h in the 17.3 µg/L treated cosms. At the end of the experiment concentrations were < 6% of nominal. TWA values were calculated for all treatments. Although not specified in the manuscript, it is assumed from the context that the TWA is calculated for 1 week, the results are then consistent with the reported DT50. Table below shows the nominal concentrations and the corresponding mean TWA concentrations (mean for three pulse dosages).

Imidacloprid concentrations, nominal and TWA concentrations.

Nominal concentration (µg/L)	Mean TWA (µg/L)	Water concentration at end of experiment (µg/L)	Sediment concentration at end of experiment (µg/kg)
0.6	0.2	0.0	0.0
1.4	0.4	0.06	0.0
3.2	1.0	0.13	0.0
7.5	2.3	0.37	0.02
17.3	5.2	0.99	0.04
40	12	1.72	0.13

The authors discuss that due to the rapid degradation in the water column (partly due to high radiation, and unhindered transmission in water), concentrations in sediment are low as well, and the study might represent a best-case scenario.

Abiotic parameters

pH 8-9, water temperature 16-22°C, conductivity decreased from 835 µS/cm at the start to 615 µS/cm at the end. Air temperature 10-24°C, radiation 6-11 µW/cm^2. Conductivity decreased in cosms with the highest growth. Differences were present till the end of the experiment.

Biological observations.

Macroinvertebrates

Total # of species and abundance of Chironimidae were significantly decreased in the two highest treatment levels. Effects were caused mainly by three species belonging to the subfamily Orhocladiinae. For Tanypodinae, effects were seen from 7.5 µg/L, significant in the highest treatment.

Number of *Radix* sp. increased significantly at the highest concentration. Ephemeroptera decreased significantly in the two highest concentrations. Since not all control cosms were colonised, it was not possible to run a powerful statistical test.

Effects on emergence appeared to be related to the mortality in the cosms rather than to effects on emergence itself. Ephemeroptera were sensitive, at concentrations >1.4 µg/L nominal no emerging *Caenis* sp. adults were found.

Evaluation of the scientific reliability of the field study

Criteria for a suitable (semi)field study

1. Does the test system represent a realistic freshwater community? Partly, macro-invertebrates that can colonize the cosm or were introduced with the sediment were studied and reported. Other organisms were not reported.
2. Is the description of the experimental set-up adequate and unambiguous? Yes.
3. Is the exposure regime adequately described? No, Test item not described in detail, application method not specified.
4. Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, imidacloprid is an insecticide, and insects are included in the study.
5. Is it possible to evaluate the observed effects statistically? Data are not presented, it is indicated that the power was estimated, data are not presented however. Re-evaluating is not possible with the available data.

In view of these criteria, the study is considered less reliable (Ri 2). Clear effects occur at the two highest concentrations of 17.3 and 40 µg/L nominal. However, for some groups (Ephemeroptera) emergence effects

were found in the 3.2 µg/L treatment. At 1.4 µg/L no significant effects were found. Considering the DT50 of 28 hours, the 48-hours TWA for this treatment is 0.82 µg/L.

Conclusion
The study shows that repeated applications of 1.4 µg a.s./L do not lead to effects on abundance and emergence of macroinvertebrates. Due to the fast dissipation of the compound, the study cannot be used for derivation of the $QS_{fw, eco}$, but the 48-hours TWA NOEC of 0.82 µg/L is considered for the MAC-$QS_{fw, eco}$

Study 10	
Reference	[14]
Species; Population; Community	Cloeon dipterum, macrophytes; large predators actively removed
Test Method	Outdoor enclosure
System properties	Enclosures in outdoor experimental ditch, fine sandy clay sediment
Formulation	Imidacloprid SL 200
Exposure regime	two applications, 21 d interval; concentrations 0, 0.097, 0.243, 0.608, 1.520, 3.800 µg a.s./L.
Analysed	Y
Temperature [°C]	5,5 – 14,8
pH range	7.62-10.16
Hardness [mg $CaCO_3$/L]	Not specified
Exposure time	Application on day 0 and 21, test duration until 37 d
Criterion	NOEC
Test endpoint	Abundance
Value [µg/L]	1.52 (nominal)
GLP	Y
Guideline	
Notes	Single species test
Ri	2

Description
Test system
Enclosures of a polycarbonate, translucent cylinder (diameter: 1.05 m; height: 0.9 m; water volume: ca. 0.45 m^3), placed in experimental ditches. Total of 21 enclosures (four controls, 15 treated at five different concentrations (n=3), two shaded fate enclosures). Fine sandy clay sediment. Water from a water supply basin at the test facility. Macrophytes were present (developing *Elodea* vegetation). Light aeration during experiment.
Aquatic larvae of the mayfly *Cloeon dipterum* were inserted on three occasions (September 16th, 19th and 23rd, 2013). Larvae were collected from previously unused and therefore uncontaminated experimental ditches at the test facility and equally divided over the test systems. In total approximately 900 individuals per enclosure were introduced. Larger predators such as backswimmers (Notonecta) and dragonfly larvae (Anisoptera) and were actively removed.
Test substance was applied twice on October 7th and October 28st, 2013. Treatment levels: 0 µg/L (control), 0.097, 0.243, 0.608, 1.520, 3.800 µg a.s./L. Application by pouring dosing solutions and gently stirring.

Analytical sampling
In all enclosures, water samples were taken (day 0: 2 h before application; day 21: 1 h before application), and 4 hours after the application. Additional samples in the (1.520 and 3.800 µg a.s./L, both shaded and unshaded) test systems at 2, 4, 7, 11, 14, 23, 25, 28, 32, and 37 days post first application and sediment samples at day -5, 14, 28 and 37 post first application. Macrophytes were sampled for fate analysis on day 37 in the control systems and fate enclosures (1.520 and 3.800 µg a.s./L, shaded and unshaded).

Effect sampling
Nymphal stages of the mayfly *Cloeon dipterum* were captured by using net samples combined with an artificial substrate (pebble basket). Sampling took place -5, 2, 9, 16, 23, 30 and 37 after the first application. *Cloeon dipterum* nymphs were counted alive and returned to their respective test system. No emergence due to low temperatures.

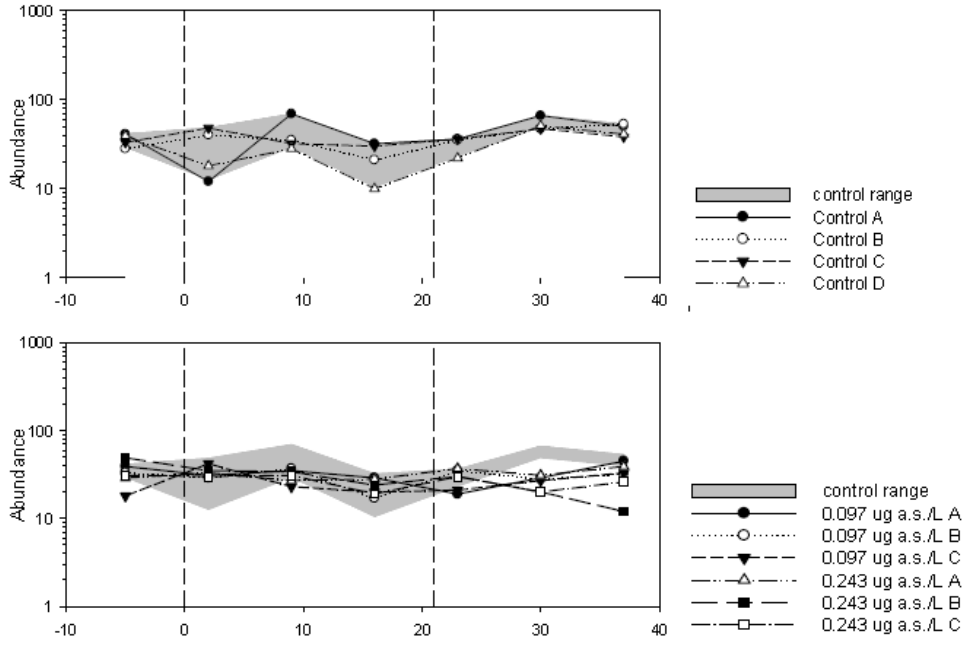
Temperature, pH, dissolved oxygen (DO) and electrical conductivity (EC) were measured in the morning on days 2, 9, 16, 23, 30 and 37 after first application.

Statistical analysis
Univariate analyses of abundance of *Cloeon dipterum* and community metabolism endpoints.

Results
Chemical analysis
Concentration in dosing solutions were 93-101% of nominal. Measured concentrations 4 h after 1st application were < LOD for control and 0.097 µg a.s./L, 260% of nominal at 0.024 µg a.s./L, and 82-109% of nominal at the higher concentrations. Concentrations at 0.024 µg a.s./L are considered not reliable according to the authors due to the low level and incomplete mixing.
At 1.52 and 3.8 µg a.s./L, 36 and 40%% of initial was present just before the 2nd application. The DT50 for dissipation from the water phase was estimated by the evaluator by non-linear regression of 1st order exponential decay using GraphPad Prism 6.03 with measured concentrations at 1.52 and 3.8 µg a.s./L. DT50 in the respective treatment levels was 10.8 and 13.0 days after the first application, and 14 and 14.5 days after the second.

Statistical power
Authors calculated the Minimum Detectable Difference (MDD), which is the percentage change relative to the control that is needed to detect a change as significant. MDD was 33% before application, and ranged from 49% to 63% after application.

Biological observations
Abundance in the respective treatments is presented in the figures below (copied from report). No statistically significant effects were observed at concentrations up to and including 1.52 µg a.s./L nominal. At 3.8 µg a.s./L, a clear decline was observed in one replicate on three last sampling dates (days 23, 30 and 37). Authors conclude that 1.52 µg a.s./L is the NOEC.



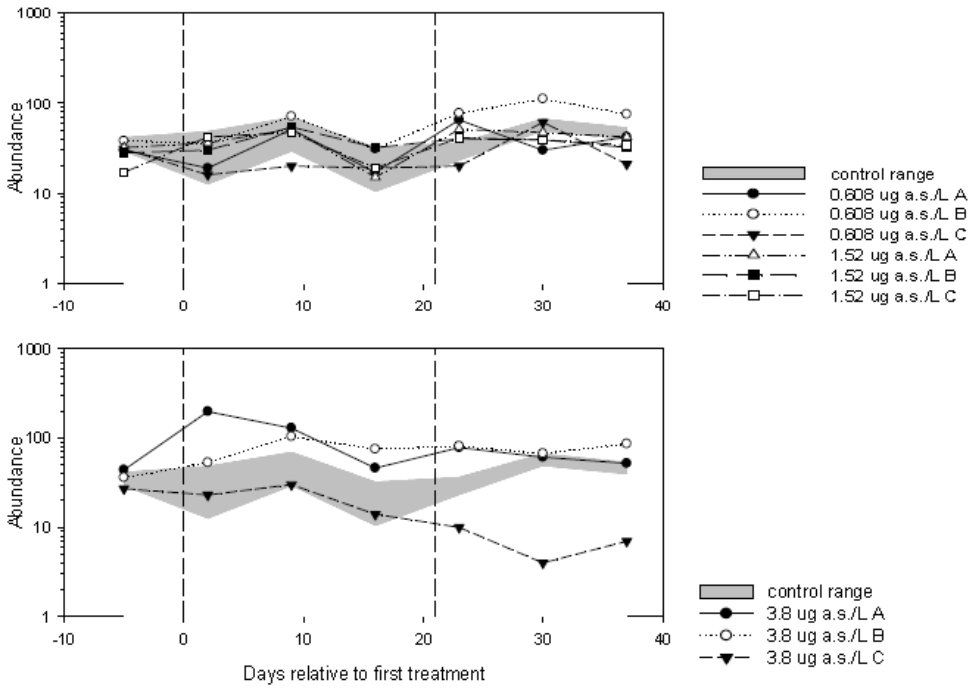


Figure 1. Abundance of *Cloeon dipterum* over time

Evaluation of the scientific reliability of the field study

Criteria for a suitable (semi)field study

- Does the test system represent a realistic freshwater community? Partly, species composition not described, large predators removed
- Is the description of the experimental set-up adequate and unambiguous? Partly, efficiency of sampling method not specified.
- Is the exposure regime adequately described? Yes.
- Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound? Yes, test was aimed at a specific sensitive organism.
- Is it possible to evaluate the observed effects statistically? Yes.

It is recognised that the MDD achieved in this study is considered acceptable by EFSA [15]. However, it is noted that EFSA considers an MDD of 70-90% acceptable, whereas for field studies with other organism groups (earthworms, non-target arthropods) a lower percentage of 50% is used [16,17]. Since the MDD is only recently introduced as a reporting requirement for mesocosm studies, experience has to be gained as to how the MDD should be used as a criterion for assigning the reliability index.

Specimens are nymphal stages, sampled with a net and from pebble baskets. In a number of cases (e.g. cosm A in lowest part of Figure 1), considerable increases in abundance are found. Since nymphal stages do not reproduce, this increase can only be caused be introduction of new larvae (by adults, laying eggs), or it is an artefact of the sampling method. Given the time of the season, it is not very likely that new larvae are introduced. Upon request, the authors confirmed that the differences are caused by variability of the sampling method. They state that the current variation observed in the *Cloeon* abundances is rather normal for macrofauna endpoints in model ecosystem studies, indicating the variation caused by the sampling method reflects the normal technical limitations of such a study. The authors consider the response observed in the replicate systems of 3.8 µg a.s./L as an exception to the normal variation. Although not statistically significantly different from controls, they consider the decline in replicate C as a potential effect of imidacloprid and consequently did not designate this treatment level as a possible NOEC value (pers. comm. I. Roessink, Alterra). Given the time course of abundance (see figure above), it seems reasonable to assume that the oberved decline at 3.8 µg a.s./L was not caused by the 2nd application, but already started as a result of the 1st.

The variation which is caused by the sampling method might have influenced the results, which is a reason to consider the study less reliable. On the other hand, this variation is likely to be present in the control too, and is then accounted for in the MDD. The statement of the authors that the variation is similar to what is normally

seen in mesocosm studies is accepted, but it should be noted that full mesocosm studies consider endpoints for multiple species. Moreover, emergence is usually included as an additional parameter to further underpin the sampling methods used here. Therefore, while accepting that the NOEC in this study is the 1.52 µg a.s./L treatment, the representativeness of this NOEC for other systems and other application periods remains to be seen.

Conclusion
The NOEC of 1.52 µg a.s./L nominal is considered for EQS-derivation.

Studies not further evaluated
Mesocosm study in rice paddies [18,19]. Mesocosms were dosed by transplanting nursery boxes with rice seedlings that were treated with imidacloprid in a granular formulation. Treatment was performed in 2010 and repeated in 2011, paddies were drained and left dry in between. Due to the way of dosing and emission, ecosystem characteristics, and agricultural practice, the study might be relevant for risk assessment of imidacloprid in rice cultivation. However, the relevance for standard derivation of surface water in general is limited. Therefore, the studies are not further discussed here.

Mesocosm studies in rice paddies [20,21]. Mesocosms were dosed by transplanting nursery boxes with rice seedlings that were treated with imidacloprid in a granular formulation. Moreover, fish were introducted in the systems. Similar to the study above, the study design is not considered relevant for standard derivation for surface waters in general.

Study in which eggs of *Sympetrum infuscatum* were placed on the surface of a micro-paddy lysimeter (small lysimeters with soil and rice seedlings) that was treated with imidacloprid in a granular formulation [22]. The study might be relevant for risk assessment of imidacloprid in rice cultivation, but the dosing and exposure is not considered relevant for derivation of standards for surface water. The study is not further discussed here.

Study in which the fate of imidacloprid was assessed after application to a rice plot in Portugal [23]. Measured concentrations in paddy water were compared with modelled concentrations. Water from the plots was sampled and used for laboratory bioassays with *Daphnia magna*, *Heterocypris incongruens*, *Pseudokirchneriella subcapitata* and *Lemna minor* (only results presented, no further details given). Results were used for a risk assessment on the basis of SSDs with literature data.

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