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Assessing Toxicity of the Insecticide Thiacloprid on *Chironomus riparius* (Insecta: Diptera) Using Multiple End Points

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Abstract Since data documentation on neonicotinic toxicity to nontarget organisms should be enhanced, we investigate the effects of thiacloprid, a novel neonicotinoid insecticide, on the sediment-dwelling nontarget insect *Chironomus riparius*. Further, we wanted to validate the sensitivity of end points on different biological levels and obtain the greatest amount of information regarding the effects of this compound by using a battery of several end points such as larval mortality, behavior, body weight gain, emergence rate, time of development, gender ratio, Hsp70 stress protein level, and larval mouthpart deformities after exposure at a concentration range of 0.1 to 1000 μg/L thiacloprid. *C. riparius* was impacted starting at concentrations of 0.5 μg/L, a concentration that can be considered environmentally relevant. Larval mortality, behavior, emergence, and Hsp70 protein level were sensitive indicators for the toxic effect of thiacloprid, whereas gender ratio and mouthpart morphology were not affected. In our case life-cycle end points like survival rate (LC_{50}: 1.57 μg/L) and emergence rate (EC_{50}: 0.54 μg/L) proved to be more sensitive than tested physiological end points for the neurotoxic insecticide.

The use of pesticides can affect nontarget species of organisms, since they can be at risk from pesticide leaching, spray drift, or surface runoff into aquatic ecosystems (Kreuger 1998; Palma et al. 2004). To evaluate this risk for aquatic organisms, a number of test systems have been developed. However, these methods focus primarily on pelagic organisms, such as fish and daphnids. Communities of benthic organisms play a key role in energy, nutrient, and contaminant fluxes and they play a key role in transferring environmental contaminants to higher trophic levels (Burton 1991; Reynoldson 1987). For this reason, the consequences of toxic substance exposure to benthic communities should be studied more closely.

Thiacloprid, a neurotoxic insecticide, belongs to the new and commercially very successful family of the neonicotinoids. Both its structure and its mode of action are related to imidacloprid—one of the biggest-selling insecticides worldwide (Jeschke et al. 2001). In 2007 it was registered in more than 50 countries (Bayer Crop Science 2008). Thiacloprid, as all neonicotinoids, acts on the insect nervous system as an agonist of the nicotinic acetylcholine receptor (nAChR) (Jeschke et al. 2001). It exhibits high water solubility (184–186 mg/L) and a relatively low log-K_{ow} (1.26) at 20°C. This insecticide has a half-life (DT_{50}) in water of 6–11 days and a DT_{50} in water sediment systems lasting between 11 and 27 days (European Commission 2004). Thiacloprid may therefore contaminate surface waters following rainstorm events (Beketov and Liess 2008b). The currently predicted worst-case environmental concentration for thiacloprid via spray drift in surface water has been predicted to be 1.99 μg/L (ornamentals) and 17.52 μg/L (orchards), respectively (Schmuck 2001). For runoff events this scenario is not yet available. Studies on measured thiacloprid concentrations in the environment are rare due to its brief time of availability on the market. A single study conducted in apple orchards in the area surrounding Hamburg, Germany, detected thiacloprid concentrations of 4.5 μg/L in a nearby water system (Süß et al. 2006).

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Information about the toxicity of thiacloprid to nontarget freshwater invertebrates and about its potential effects on freshwater ecosystems is limited (Beketov and Liess 2008b). However, in previous experiments insects showed a higher sensitivity to this insecticide than other freshwater arthropods (Beketov and Liess 2008a, b; Beketov et al. 2008). This strongly suggests the inclusion of insects in further additional ecotoxicological testing.

The worldwide-distributed family of Chironomidae are suitable test species due to their aquatic and sediment-bound larval stages. Chironomids are frequently the most abundant group of insects in freshwater environments and their function as prey for other species makes them environmentally relevant (Armitage et al. 1995; Pinder 1986). The nonbiting midge, *Chironomus riparius,* is widely used for toxicity testing (OECD 2004a, b; US EPA 2000) due to its easy cultivation, short generation time, and relative sensitivity to pollutants.

Beketov and Liess (2008b) pointed out that the knowledge of sublethal effects of thiacloprid on life cycle traits of insect species is limited. The aim of this study was to evaluate the effects of thiacloprid on the insect *C. riparius* using established end points such as emergence rate, time needed for development, and larval body weight gain (OECD 2004a, b). In addition, these main parameters were complemented with other end points including hatching rate, behavior changes, larval mortality (L3 and L4), stress protein response (70-kD heat shock protein family; Hsp70), mouthpart deformation, and gender ratio.

Materials and Methods

Maintenance of Parent Animals

Stock cultures of *Chironomus riparius,* from different genetic sources, in order to avoid genetic impoverishment (LimCo International, Germany; University of Joensuu, Finland; and Universidade de Coimbra, Portugal), were kept as larvae in fine quartz sand and dechlorinated tap water under constant aeration. Every day the chironomid larvae were fed with finely ground fish flakes (50% Tetramin, 50% Tetraphyll; Tetra, Germany). Declorinated tap water was exchanged one or two times per week. Before emergence occurred, a breeding cage (55 × 65 × 120 cm) was installed over the stock containers, in which the adults were allowed to fly, swarm, and breed. The egg masses which were attached to the vessel wall were collected every morning and used subsequently for experiments. Stock breeding and all experiments were conducted in a climatized chamber at 21.0 ± 0.5°C, with a light–dark cycle of 16:8 h artificial daylight (Philips standard daylight 54765; 2500 lumen; Germany).

Preparation of Insecticide Stock Solutions

At room temperature (RT) a 5 mg/L thiacloprid (Riedel-de Haën; CAS 111988499 analytical standard; Germany) stock solution was prepared every third day with declorinated tap water (pH 7.8 ± 0.2) and stirred for 14 h in the dark. The following nominal test concentrations were directly prepared before use with aerated declorinated tap water—0.1, 0.5, 1, 5, 10, and 1000 µg/L thiacloprid—as well as one negative control containing only pure dechlorinated tap water. Concentrations of insecticide were chosen on the basis of a preliminary range of tests with L3 larvae and also from measured environmental concentrations (Süß et al. 2006). Each treatment was replicated four times.

Spiking of Sediment

To simulate natural conditions we first spiked the sediments with an aqueous solution assuming that the partitioning between sediments and the water phase takes 24 h to reach equilibrium. The day before being used in the experiment 50 g of quartz sediment (particle size, 0.1–0.3 mm; burned for 3 h at 500°C to remove organic matter; Dehner, Germany) was filled into a 250-ml glass beaker. For spiking, the sediment was covered with 200 ml of the respective test solution and subsequently shaken for 24 h in the dark. Subsequently we removed the water phase and added fresh test solution representing the environmental conditions under which thiacloprid is repeatedly introduced. The aim of this was to stabilize the concentration of insecticide in both the water and the sediment.

Egg Preparation and Exposure

Different egg clutches from the breeding stock were collected at 8 a.m., separated into smaller clusters of visually the same size, and mixed randomly. A preliminary experiment was designed to test whether egg clutch disassembly with and without thiacloprid treatment had an effect on hatching rate.

After counting the eggs, between 100 and 120 eggs per replicate were exposed to the appropriate thiacloprid concentration. The number of hatched larvae was counted under a stereomicroscope daily at the same time of day for a period of 6 days after oviposition. Since larvae exposed to the highest thiacloprid concentration of 1000 µg/L did not hatch, but died immediately after hatching (see results), only concentrations up to 10 µg/L thiacloprid were tested in the procedures that follow.

Larval Exposure and Maintenance

After 3 days 33 first-instar larvae from each replicate were transferred with a glass pipette to glass beakers (providing a

density of 1.34 individuals/cm^2). The beakers were covered with parafilm to reduce evaporation (Parafilm 'M;' American National Can, Chicago, IL, USA). Larvae were fed daily with 12 ± 1 mg finely ground fish food (50% Tetramin, 50% Tetraphyll; Tetra), corresponding to ≥0.36 mg/day/larva. From the second day after transfer the beakers were aerated through a glass Pasteur pipette. Water was exchanged every third day with new insecticide-spiked water. Temperature, pH, conductivity, dissolved oxygen saturation, and nitrite content were regularly measured in the newly spiked and old exchanged water. Every week larvae were transferred into new beakers with freshly spiked sand and test solution. Beakers were gently swayed until the larvae came to the sediment surface. Larvae were then aspirated gently with a cut plastic pipette, transferred, and counted.

Larval Survival

The survival rate of third-instar (L3) and fourth-instar (L4) larvae was monitored 10 and 17 days after oviposition. This made it possible to distinguish between early and later larval mortality and, furthermore, to differentiate between mortality and disturbances of the emergence process, both resulting in a reduced emergence rate.

Behavior Measurement

During survival monitoring the behavior of 12 larvae per test insecticide concentration (3 randomly selected animals from each of the four replicates) was measured for 2 h with the Multispecies Freshwater Biomonitor (MFB; LimCo International) in dechlorinated tap water. The MFB is an online biomonitor which continuously and quantitatively records the behavior pattern of animals (Gerhardt et al. 1994). Behavior signals of the chironomids were analyzed with a fast Fourier transformation resulting in a histogram of different signal frequencies, hence enabling distinction among different types of behavior such as locomotion and ventilation (Gerhardt et al. 1998). For each individual, mean locomotor (0.5–2.5 Hz; band 1) and ventilatory (3–8 Hz; band 2) activity (percentage of time spent on locomotion and ventilation, respectively) was calculated for a time period of 2 h. MFB chambers, which differed for the different larvae stages (L3, 4 cm long and 1 cm in diameter; L4, 4.5 cm long and 2 cm in diameter), allowed free movements of the chironomid larvae and were sealed with a porus lid (mesh size, 0.25 mm). Larvae were put back into their respective beakers after the behavior measurements.

Emergence

When emergence started, the number and the gender of emerged midges were determined daily. To detect changes in development time, for each concentration the developmental rate was calculated using the formula given in the OECD Guideline (OECD 2004a, b). The gender ratio of emerged chironomids was calculated in each replicate as the number of male-to-female organisms.

Mouthpart Deformities

The remaining head capsule exuviae of the emerged midges were collected and stored in 100% alcohol. One day before morphological preparation, the head capsules were separated from the body or exuvia rests and stored overnight in Rotihistol (Carl Roth GmbH, Germany). The next day, the head capsules were placed on a glass slide with the ventral side facing upward, then covered with Roti-Histokit (Carl Roth GmbH) and squeezed gently with a coverslip. The strongly scleroticized mentum and mandibles were evaluated under a microscope at 40× magnification. Missing teeth, extra teeth, and mentum split medial teeth were counted as deformities (Bird 1994; Gerhardt and Bisthoven 1995; Servia et al. 1998). The ratio of the number individuals with deformed mouthparts to the number of examined individuals was calculated (Hämäläinen 1999) using two different approaches. First, the total deformity rate was calculated using the number of individuals with deformed mouthparts to the total number of examined individuals. Second, the number of different deformity types to the number of examined individuals was calculated.

Body Weight Gain

To investigate body weight gain, the same experiments were conducted as described above. But as recommended in OECD Guideline 218/219, 10 days after oviposition the surviving animals were collected and dried at 100°C for 24 h in aluminum foil and weighed (Sartorius LE 324S; Germany). Subsequently, the average dry weight of the larvae in each replicate was calculated.

Hsp70 Protein Analysis

Ten fourth-instar larvae (17 days after oviposition) per treatment were shock-frozen in liquid nitrogen and stored at −20°C for later Hsp70 analysis. The Hsp70 content was investigated at day 17, because at this stage (L4) the chironomid larvae had a 'total protein content' that was sufficient to analyze the Hsp70 in individual larvae. For standard Hsp70 analysis see also Köhler et al. (1992, 2007). Frozen larvae were homogenized individually with a plastic pestle in 45 µl extraction buffer (80 mM potassium acetate, 4 mM magnesium acetate, 20 mM Hepes, 2% protease inhibitor Sigma P8340, pH 7.5) and centrifuged for 10 min

at 20,000*g* and 4°C. The supernatant was used for further analysis. The total protein concentration was determined according to the method of Bradford (1976). The supernatant was mixed with 12.5 µl sodium dodecyl sulfate (SDS) and cooked for 5 min at 95–100°C. Constant amounts of total protein (40 µg) from each sample were subjected to SDS–PAGE (SDS–polyacrylamide gel electrophoresis; 12% acrylamide-bisacrylamide) for 15 min at 80 V and then 90 min at 120 V. The protein was transferred to a nitrocellulose membrane in a semidry blotting chamber (360 mA, 2 h; Panther Hep-1; Owl). Subsequently, the nitrocellulose membranes were blocked for 2 h in Tris-buffered saline (TBS) solution containing horse serum. After washing for 5 min in TBS, the membranes were incubated overnight with a monoclonal antibody at 21°C (mouse anti-human Hsp70 [Dianova, Hamburg, Germany]; dilution, 1:5000, in 10% horse serum/TBS). After washing again for 5 min with TBS, filters were incubated with a secondary antibody for 2 h at 21°C (peroxidase-conjugated goat anti-mouse IgG [Dianova]; dilution, 1:1000, in 10% horse serum/TBS). Following a repeated wash with TBS for 5 min, the antibody complex was detected by adding 1 mM 4-chloro(1)naphthol and 0.015% H_2O_2 in 30 mM Tris, pH 8.5, containing 6% methanol. The gray scale values of the protein bands were quantified using a densiometric image analysis system (Herolab E.A.S.Y., Germany) and set in relation to an internal snail Hsp70 standard (*Xeropicta derbentina*) run in parallel on each gel.

Statistics

Because some data were not normally distributed, nonparametric tests were used. All data were analyzed using Friedmann's ANOVA (Statistica 5.0; Statsoft, USA), followed by a Wilcoxon two-group test (JMP 4.0; SAS Systems; USA) to examine differences between control and exposure treatments. The Excel macro REGTOX (based on the Marquardt algorithm; http://eric.vindimian.9online.fr) was used to compute lethal concentration (LC) and effect concentrations (ECs) and to estimate the confidence intervals of the parameters by a bootstrap nonparametric simulation.

Results

Abiotic Parameters

Water quality parameters measured during the experiment were comparable among the treatments (pH 7.8–8.2; temperature, 21.2–21.5°C; conductivity, 400–500 µS/cm; dissolved oxygen level, >60% of saturation; nitrite content, 0–0.5 mg/L).

Hatching Rate

In the preliminary experiment no difference was found between disassembled egg clutches and nonmanipulated egg clutches in the two additional treatments investigated, the control and the treatment with 1000 µg/L thiacloprid (Wilcoxon test, n.s.).

Hatching of first-instar larvae started 2 days after oviposition but peaked at 3 and 4 days after oviposition. No impact on hatching rate could be observed in the presence of 10 µg/L thiacloprid compared to the control treatment (overall mean hatching rate, 92.44 ± 6.77 [SD] after oviposition; Wilcoxon, n.s.). Only at 1000 µg/L was the hatching rate significantly reduced (mean hatching rate, 5.57 ± 8.47; Wilcoxon, $p < 0.0209$) compared to the control and other treatments with lower insecticide concentrations. Even the small number of hatched larvae in the 1000 µg/L treatment died immediately after hatching; for this reason thiacloprid only up to 10 µg/L was used in further tests.

Larval Survival

Ten and seventeen days after oviposition the larval survival rate was significantly decreased at 0.5 µg/L and higher concentrations of insecticide compared to the control treatment. With increasing exposure time fewer larvae survived at the respective exposure concentrations (Fig. 1). The calculated LC_{50} was 5.18 µg/L (10 days) and 1.5 µg/L (17 days).

Emergence

The first emergence of the organisms was observed on day 19 after oviposition. At the highest test concentrations (5 and 10 µg/L thiacloprid), larvae died before achieving

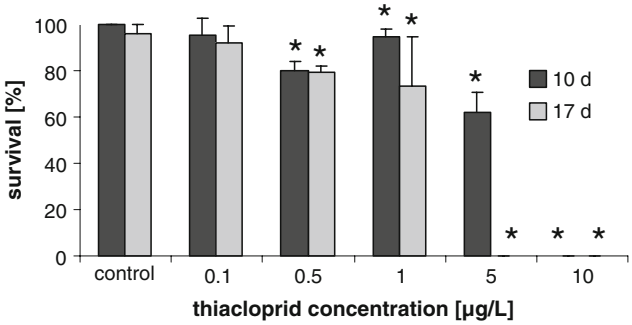

Fig. 1 Mean survival rate of *Chironomus riparius* larvae exposed to different thiacloprid treatments at the ages of 10 and 17 days after oviposition. Mean ± SD (four replicates per treatment, with 33 larvae each). Significant difference from the control, at * $p \leq 0.05$ (Wilcoxon test)

the pupal stage, therefore no emergence could be observed (Figs. 1, 5). At 1 µg/L only two animals emerged. At 0.5 µg/L the total emergence was significantly reduced compared to the control. At 0.1 µg/L the number of emerged animals did not differ significantly from the control, although the emergence rate was higher. The calculated EC_{50} for the end point 'total emergence' was 0.54 µg/L thiacloprid.

The developmental rate in the treatments with emerging *C. riparius* (0.1, 0.5, and 1 µg/L) did not differ significantly among the different thiacloprid treatments (Friedmann's ANOVA, n.s.). The gender ratio of emerged chironomids varied but did not differ significantly (n.s.).

Behavior

In both larval stages (L3 and L4) the locomotor activity was significantly reduced at the highest insecticide concentrations, 5 µg/L after 10 days and 1 µg/L at 17 days (Figs. 2a, b), respectively, which still allowed survival as shown in Fig. 1. L3 larvae also showed reduced ventilatory activity at 5 µg/L thiacloprid (Fig. 2a).

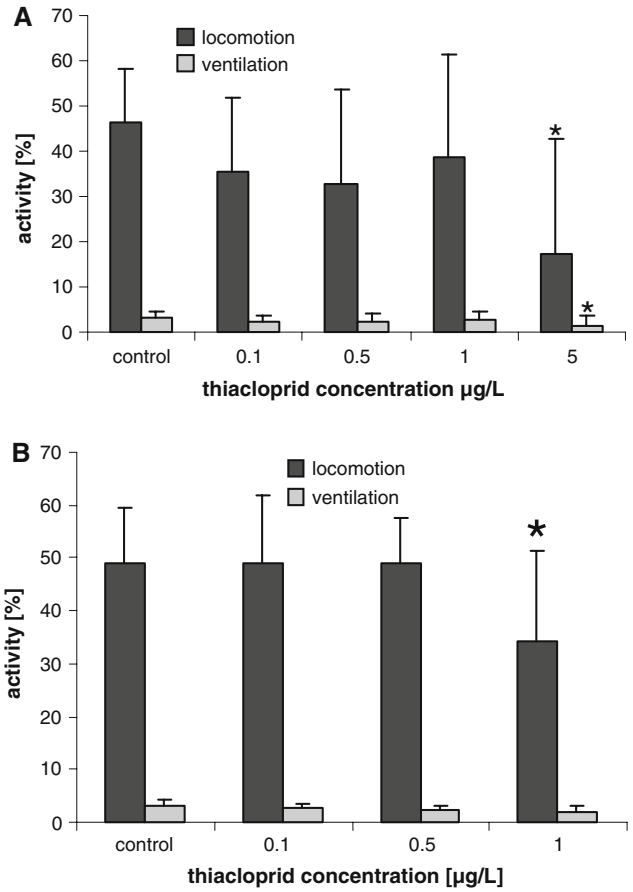

Fig. 2 Mean activity (%) of *Chironomus riparius* larvae exposed to different thiacloprid concentrations at an age of 10 days (**a**) and 17 days (**b**) after oviposition. Mean ± SD; $n = 12$. Significant differences from the respective control treatment, at * $p \leq 0.05$ (Wilcoxon test)

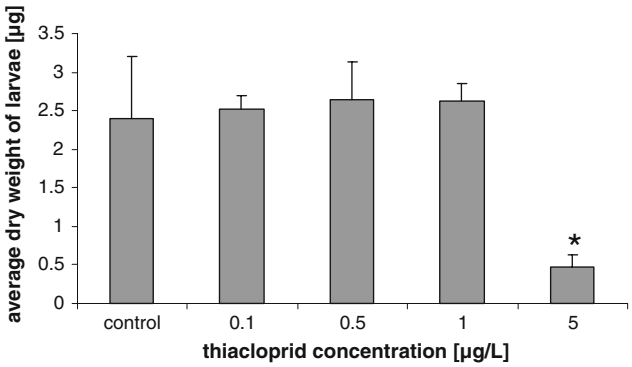

Fig. 3 Average dry weight per larva (mean ± SD) of *Chironomus riparius* exposed to thiacloprid 10 days after oviposition ($n = 4$). Significant difference from the respective control treatment, at * $p \leq 0.05$ (Wilcoxon test)

Body Weight Gain

The average mean dry weight of *Chironomus riparius* larvae exposed to 5 µg/L thiacloprid for 10 days was significantly reduced compared to the control (Fig. 3).

Hsp70

The relative level of the stress protein Hsp70 significantly increased at 1 µg/L thiacloprid compared to the control (Fig. 4). Higher concentrations of insecticide exposure could not be investigated because the animals did not survive until day 17.

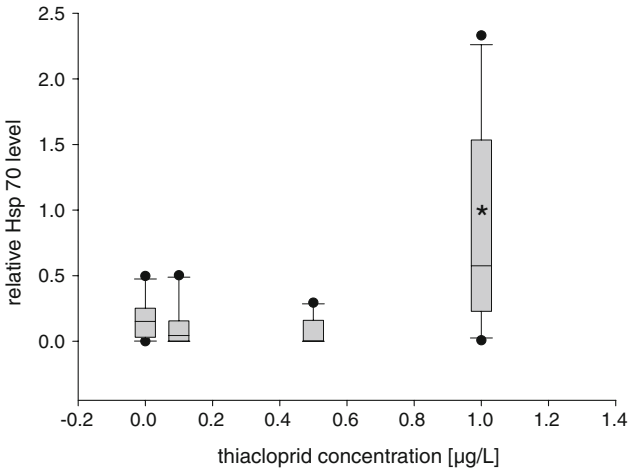

Fig. 4 Hsp70 levels (relative to standard) of L4 larvae of *Chironomus riparius* exposed to different thiacloprid concentrations for 17 days ($n = 10$). Box plots display the median, lower (25%) and higher percentile (75%), and outliers. Significant difference from the control, at * $p \leq 0.05$ (Wilcoxon test)

Mouthpart Deformities

In all treatments some chironomids showed deformities in the mentum and the mandibles. Slight deformities were observed such as missing teeth, extra teeth, and mentum split medial teeth. No severe deformities such as the Köhn gap were found (Köhn and Frank 1980). The control treatment showed a mean total deformity rate of 17%. No significant differences in deformity rate between the thiacloprid treatments and the control were observed.

Discussion

The aim of the present study was to quantify the effects of thiacloprid on different life-cycle traits of *Chironomus riparius*, as well as to compare the sensitivity of those end points from different biological levels which have rarely been addressed in this combination in previous studies. To achieve these goals, a number of modifications to the OECD Guideline (OECD 2004a, b) were made.

In the present study, chironomid eggs were exposed to thiacloprid directly after oviposition, and therefore, effects on embryonic development were likely to be detected. The hatching rate was affected above 10 µg/L, and thus it was less sensitive compared to other tested end points, which reacted at considerably lower concentrations. It was shown that dissection of the egg clutches, along with partial removal of the gelatinous matrix, had no negative effects on hatching rate. Therefore, in our study, dissection of egg clutches was found to be a suitable tool to determine the exact number of exposed eggs, allowing reliable calculation of the hatching rate.

The aim of this study was to test the toxicity of the hydrophilic insecticide thiacloprid ($\log K_{ow}$, 1.26) on *C. riparius* and not to focus on its binding potential to natural sediments. Therefore pure inert quartz sand was used, in accordance with other chironomids studies (Hahn et al. 2001; Nowak et al. 2008; Vogt et al. 2007a, b). Since thiacloprid is stable to hydrolysis at pH values of 5–9 at 25°C, and has a reported half-life in aerobic natural water sediment systems varying between 12 and 20 days (Public Release Summary 2001), and since water was exchanged twice a week and replaced by fresh solutions, nominal thiacloprid concentrations can be assumed to closely represent actual concentrations.

In this study, an intermediate larval density of 1.34 larvae/cm^2, ranging between low densities of 0.2 larvae/cm^2 (Forbes and Cold 2005) and high densities of 4 larvae/cm^2 (Hooper et al. 2003), was used. However, this larval density value was lower than the recorded field densities of *C. riparius* of 10 individuals/cm^2 (Groenendijk et al. 1998). At thiacloprid concentrations >1 µg/L all larvae died within 17 days of exposure. Reduced survival (LC_{50}: 1.57 µg/L after 17 days) and total emergence rate (EC_{50}: 0.54 µg/L) were the most sensitive end points.

As the second test end point behavioral changes were used, as behavior integrates biochemical and physiological processes (Dell'Omo 2002). Consequently, it is sensible to use this end point, especially when testing substances like the neurotoxic insecticide thiacloprid. The detected behavioral changes occurred regularly at 5 µg/L (L3 larvae) and 1 µg/L (L4 larvae) thiacloprid and caused high mortality rates after a period of 7 days. In the *C. riparius* life cycle, behavioral parameters can therefore be regarded as early-response sublethal end points. Thiacloprid is also known to induce behavioral changes in the aquatic insect *Simulium latigonium* at concentrations several times lower than the LC_{50} as soon as 2 h after contamination (Beketov and Liess 2008a).

To investigate general stress, the expression of Hsp70 was included as an end point. This protein family is ubiquitous, and its isoforms can act as chaperones for correct protein folding (Berg et al. 2003). When proteins start to unfold, for example, due to heat stress, the presence of toxic metals, or other proteotoxic conditions, the Hsp70 level is increased to compensate for these effects via feedback coupling (Beckmann et al. 1992; Gething and Sambrook 1992; Morimoto 1993). Therefore, a change in the Hsp70 level has been proposed as a general biomarker for proteotoxicity in environmental monitoring (Yoshimi et al. 2002). In our study, the Hsp70 level was significantly higher in L4 *C. riparius* larvae at 1 µg/L thiacoprid exposure compared to the control. Considering the neurotoxic mode of action, a direct proteotoxic effect of thiacloprid is unlikely, although the upregulation of Hsp70 might indicate a reaction of the animal to the harmful stress situation. Up to now only a few studies have used Hsp70 as a biomarker for toxic stress in chironomids. An increase in *hsp70* gene expression was found in *C. riparius* exposed to 10 mM cadmium (Martínez-Guitarte et al. 2007) or to 1 µg/L nonylphenol, a potential endocrine disruptor (Lee and Choi 2006). In addition, an increase in *hsp70* expression was reported for *Chironomus yoshimatsui* exposed to 0.4 µg/L fenitrothion, an organophosphorus insecticide, and to 1.1 µg/L ethofenprox, a synthetic pyrethrioid (Yoshimi et al. 2002). Karouna-Renier and Zehr (2003) were able to demonstrate an increase in the Hsp70 protein level in *C. riparius* exposed to 0.25 mg/L copper for 24 h. Considering the results presented in this study and those found in the literature, the Hsp70 response seems to be a quite sensitive sublethal indicator for the toxicity of substances with different modes of action or other unfavorable conditions in *C. riparius*.

The end point body weight gain was only significantly reduced when the survival was also considerably reduced.

Consequently, upon thiacloprid exposure, the end point 'body weight gain' is less sensitive than the others.

Although the developmental rate of the surviving chironomids was not impaired as evidenced by the simultaneously occurring emergence peaks (Fig. 5), the total number of emerged chironomids was significantly reduced at ≥0.5 µg/L thiacloprid (EC_{50}: 0.54 µg/L). It is reasonable to assume that thiacloprid did not affect the emergence process itself, but the survival of larvae, due to the high young larval mortality. The emergence period (between day 19 and day 34) of control *C. riparius* in this study is rather lagged compared to the results of some other chironomid studies (Paumen et al. 2008; Vogt et al. 2007c), but our results are comparable to those of Forbes and Cold (2005) and Watts and Pascoe (2000). Additionally, in contrast to OECD guidelines 218 and 219, days were counted from oviposition, which might explain the seemingly later start of emergence.

A positive spatial relationship between sediment contamination level and larval deformities of midges in the field has been documented (Hämäläinen 1999). Therefore, the use of Chironomidae morphological deformities in bioassessment and biomonitoring of contamination stress in lakes and streams has been suggested. However, few experimental studies have demonstrated an exposure–response relationship between contaminants and deformities (Hämäläinen 1999; Janssens De Bisthoven et al. 1998a, b; Martinez et al. 2001). Also, in the present study, no dose–response dependence was found between the total mouthpart deformity rate or single deformities and increasing thiacloprid concentration. It is still unknown how pollutants may induce deformities. However, there is an ongoing discussion that mouthpart deformities develop at the endocrine-regulated molting stage, and a disruption of this complex process is likely at the base of their ontogeny (Meregalli and Ollevier 2001). DDT, 4-nonylphenol, and heavy metals are known to induce mouthpart deformities. The organics, DDT and 4-nonylphenol, disrupt endocrine processes directly and heavy metals have been described to act indirectly on the endocrine system (Meregalli et al. 2001). It seems that thiacloprid at concentrations which allowed for the survival of some individuals (at 0.1 and 0.5 µg/L) does not interfere with these processes. It was also not possible to find a relation between the deformity rate and any of the other affected end points. Therefore, it must be questioned whether mouthpart deformities really mirror the health conditions of *C. riparius*. A high percentage (17%) of mouthpart deformities in the control seems not to be unusual for *C. riparius*, a chironomid genus which naturally shows a high percentage of deformities (Servia et al. 1998). Similarly, in other studies the deformity rate ranged between 7 and 19% (Janssens de Bisthoven et al. 1998b; Meregalli and Ollevier 2001; Meregalli et al. 2001).

The use of a complete set of end points at different developmental stages gave detailed insight into the lethal and sublethal effects of thiacloprid on *C. riparius*. Hatching rate, body weight gain, larval mortality, behavior, Hsp70 level, and emergence rate were affected in the tested concentration range, whereas developmental rate, gender ratio, and mouthpart deformations seemed not to be impaired in surviving chironomids. The widely accepted end point 'total emergence rate,' which is established in the OECD guidelines (OECD 2004a, b), seems to be the most sensitive end point in connection with larval survival. Considering the neurotoxic potential of thiacloprid, it was expected that, first, behavior, then survival, and, finally, emergence rate were affected at lower concentrations than

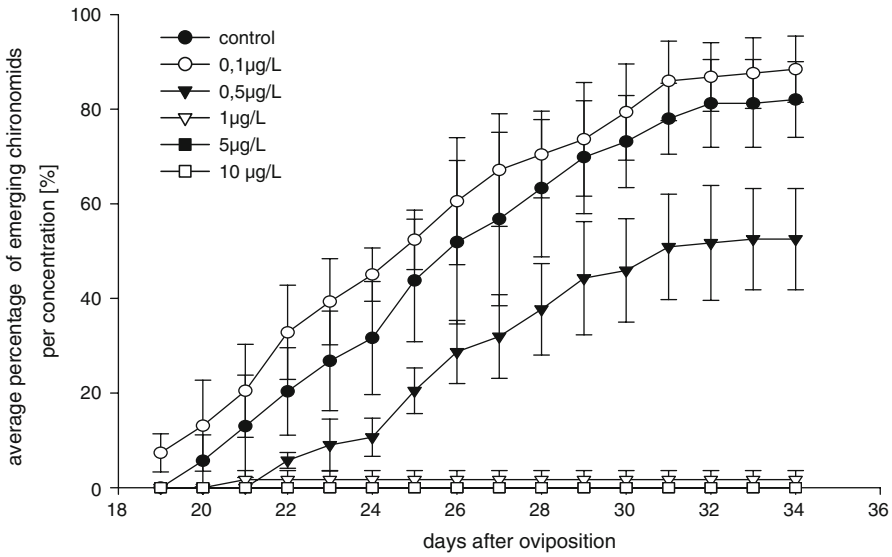

Fig. 5 Mean ± SD cumulative numbers of emerged *Chironomus riparius* imagos exposed to different thiacloprid treatments. $n = 4$ (30 or 31 animals per replicate). The number of emerged *C. riparius* was significantly lower in the 0.5, 1, 5, and 10 µg/L treatments compared to the control, * $p \leq 0.05$ (Wilcoxon test). Data obtained for 5 and 10 µg/L are identical

growth rate or hatching rate. Additionally, this mode of action suggests that the gender ratio and the deformity rate of the surviving chironomids were not affected by chronic exposure to thiacloprid.

The results of this study provided further evidence that thiacloprid is highly toxic to the nontarget insect *C. riparius*. The obtained LOEC level was 0.5 μg/L for larval survival and emergence rate; the calculated EC_{50} ranged between 5.18 μg/L for survival (10 days) and 0.54 μg/L for total emergence rate. In a previous *C. riparius* study (28 days) with thiacloprid an EC_{15} of 1.75 μg/L (range, 1.54–1.99 μg/L) was obtained (Schmuck 2001), however, the measured end point was not clarified. Although not directly comparable, recently published acute studies with thiacloprid also indicated a high toxicity to aquatic nontarget insects. Beketov and Liess (2008b) were able to demonstrate that thiacloprid is toxic to several freshwater arthropods after a 24-h exposure. Especially *Notidobia ciliares* (LC_{50}, 5.47 μg/L) and *Simulium latigonium* (LC_{50}, 5.76 μg/L) were very sensitive to thiacloprid. In another study, *Baetis rhodani* exhibited an LC_{50} of 4.6 μg/L after 96 h of exposure (Beketov and Liess 2008a).

In a stream mesocosm experiment a single thiacloprid pulse contamination (0.1, 3.2, and 100 μg/L) resulted in long-term (7-month) alteration of the overall invertebrate community structure, with an LOEC of 3.2 μg/L (Beketov et al. 2008). This value is slightly below the acute LC_{50} for sensitive invertebrates relevant in this mesocosm study. Therefore this indicates that concentrations of pesticides at which the majority of species are affected can be predicted by acute organism-level toxicity tests with sensitive species (Beketov et al. 2008).

The total insect abundance was recovered in the mesocosm study after 10 weeks, whereas no recovery was observed for insect taxon richness at 3.2 and 100 μg/L during 7 months of observation time. In addition, the monitored abundance and taxon richness of emerged insects were suppressed but had fully recovered 4 and 8 weeks after thiacloprid contamination. As in the present experiment, Chironomidae were severely affected by thiacloprid contamination, but in the mesocosm study they had fully recovered 10 weeks following the exposure. The fast recovery was attributed to the multivoltine lifestyle of the Chironomidae because, in contrast, uni- or semivoltine species did not recover during the same observation time (Beketov et al. 2008). Based on this fact it can be concluded that life-cycle characteristics are an important factor for recovery dynamics of single species after stressor occurrences (Beketov et al. 2008).

The present study, in accordance with the other mentioned results, demonstrates that *Chironomus riparius* can be regarded as a model organism that is very sensitive to thiacloprid. But for evaluation of further long-term risks on the macroinvertebrate community in the field, other information, such as life-cycle characteristics, spatial variation, and additional stressors, must be considered as well (Beketov and Liess 2008c; Beketov et al. 2008).

Since the lowest ecologically acceptable concentration of 1.54 μg/L thiacloprid found in a mesocosm study (Schmuck 2001) suggested a high environmental risk of thiacloprid to aquatic nontarget organisms, appropriate risk mitigation procedures concerning spray drift, also suggested by the manufacturer (Schmuck 2001), might be applied to protect nontarget species in aquatic systems.

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