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Sublethal toxicity of commercial insecticide formulations and their active ingredients to larval fathead minnow (*Pimephales promelas*)

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ABSTRACT

Toxic effect concentrations of insecticides are generally determined using the technical grade or pure active ingredient. Commercial insecticide formulations, however, contain a significant proportion (>90%) of so-called inert ingredients, which may alter the toxicity of the active ingredient(s). This study compares the sublethal toxicity of two insecticides, the pyrethroid bifenthrin, and the phenylpyrazole fipronil, to their commercial formulations, Talstar® and Termidor®. Both insecticides are used for landscape treatment and structural pest control, and can be transported into surface water bodies via stormwater and irrigation runoff. We used larval fathead minnow (*Pimephales promelas*), to determine effects on growth and swimming performance after short-term (24 h) exposure to sublethal concentrations of pure insecticides and the respective formulations. Significantly enhanced 7 d growth was observed at 10% of the 24 h LC_{10} (53 μg L^{-1}) fipronil. Swimming performance was significantly impaired at 20% of the 24 h LC_{10} (0.14 μg L^{-1}) of bifenthrin and 10% of the 24 h LC_{10} of Talstar® (0.03 μg L^{-1}). Fipronil and Termidor® led to a significant impairment of swimming performance at 142 μg L^{-1} and 148 μg L^{-1} respectively, with more pronounced effects for the formulation. Our data shows that based on dissolved concentrations both formulations were more toxic than the pure active ingredients, suggesting that increased toxicity due to inert ingredients should be considered in risk assessments and regulation of insecticides.

1. Introduction

Insecticides are contaminating surface water bodies of agricultural areas in California, USA, and elsewhere (Schulz, 2004; TDC-Environmental, 2008; Werner et al., 2004). It is, however, a misconception that attributes insecticide use to agricultural activities alone.

Insecticides are also heavily used in urban areas where application by homeowners and professionals for mosquito control, landscape treatment and structural pest control results in an extensive source of contamination (Budd et al., 2007; Sandahl et al., 2007). Even if not applied in the vicinity of surface water bodies, insecticides can be transported via irrigation runoff and stormwater into urban streams and waterways (Brady et al., 2006; Weston et al., 2005). Aquatic invertebrates and fish thus become targets of toxic substances at potentially hazardous concentrations. This is of special concern if sensitive larval and developmental stages are affected.

Toxicity of insecticides to fish and other aquatic species is generally determined via threshold concentrations such as LC/EC_{50} for the pure active ingredient (A.I.) of commercial products (Cox and Surgan, 2006; USEPA, 2007a). However, commercial products contain the A.I. mixed with non-insecticidal ingredients, so-called “inert” or “other” ingredients, which in some cases comprise more than 90% in volume of insecticide formulations (Cox and Surgan, 2006). They need not be identified on the product label, unless classified as highly toxic (USEPA, 2007b), and act as adjuvants, solvents, emulsifiers, surfactants and/or preservatives. Numerous commercial formulations often exist for each A.I., and it is known that availability and toxicity of the A.I. may be substantially altered by inert ingredients (Schmuck et al., 1994). Studies have shown that in many cases the toxicity of commercial formulations is higher than that of the active ingredient, but this is not always the case. Mayer and Ellersieck (1986) compared the toxicity of 161 technical grade pesticides to their formulations and showed that overall toxicity was not affected in 57%, decreased in 11% and increased in 32% of the cases. In a more recent study (Schmuck et al., 1994), 95% of 273 herbicide, fungicide and insecticide formulations were more toxic to fish than the respective pure A.I. The study presented here aims to contribute information about the comparative toxicity of pure bifenthrin and fipronil and two of their formulation products focusing on sublethal endpoints in larval fish. To our knowledge no such information is currently available for these substances.

Both bifenthrin and fipronil are widely used in structural pest control and other urban and agricultural applications (Oros and Werner, 2005; TDC-Environmental, 2008). The pyrethroid, bifenthrin, is one of the most frequently detected contaminants in surface water

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bodies of areas with urban and agricultural land use (Budd et al., 2007). Similarly, the phenylpyrazole, fipronil, was found to be present in runoff from metropolitan areas throughout the United States (Sprague and Nowell, 2008). Both insecticides are commercially available in a large number of formulated products, generally containing <10% A.I. The bifenthrin formulation; Talstar®, contains 7.9% A.I. as microcapsules (as indicated on product label, 2008), where the insecticide is encased in a coat of "inert" ingredients to ensure its slow release and stabilization (Tsuji, 2001). Termidor®, a fipronil formulation, contains 9.1% A.I. in the form of crystalline particles forming a liquid suspension concentrate (as indicated on product label, 2008). Like all pyrethroids, bifenthrin is highly toxic to fish, interfering with Na^+ channel gating in the nerve cell endings, but other ion-channels such as Cl^- and Ca^{2+} channels can be targeted as well (Burr and Ray, 2004). This leads to continuous neurotransmission, causing hyperexcitability, tremors, convulsions and ultimately death (Bradbury and Coats, 1989; Haya, 1989). Reported LC_{50} values of bifenthrin for fish range from 0.15 μg L^{-1} (rainbow trout, 96 h LC_{50}) to 17.5 μg L^{-1} (sheepshead minnow, 96 h LC_{50}) (Kegley et al., 2008; Werner and Moran, 2008). Runoff from residential areas contained bifenthrin at concentrations of 0.12 μg L^{-1} to 6.12 μg L^{-1}, measured at storm water drainage outflows (L. Oki, UC Davis, personal communication). Fipronil is a "new generation" phenylpyrazole insecticide, whose mode of action differs from organophosphates and pyrethroids, to which numerous insects have developed resistance (Bloomquist, 2003; Soderlund, 2008). Phenylpyrazoles interfere with the function of γ-aminobutyric acid (GABA)-gated Cl^- channels (Cole et al., 1993). In insects and mammals, the behavioral effects of GABA antagonists include hyperactivity, hyperexcitability, and convulsions, which are correlated with increased spontaneous nerve activity (Gunasekara et al., 2007). Fish LC_{50} values have been reported for sheepshead minnow (130 μg L^{-1}, 96 h LC_{50}), bluegill sunfish (83 μg L^{-1}, 96 h LC_{50}) and rainbow trout (100 μg L^{-1}, 96 h LC_{50}) (Gunasekara et al., 2007; Kegley et al., 2008). Concentrations measured in irrigation runoff from residential areas ranged from 0.122 to 10.0 μg L^{-1} (L. Oki, UC Davis, personal communication), and ≤9 μg L^{-1} in surface waters downstream of treated rice fields (Schlenk et al., 2001).

Here we tested the hypothesis that the toxicity of the pure active ingredients, bifenthrin and fipronil, differs from the toxicity of their respective formulations, Talstar® and Termidor®. We used swimming performance and growth as toxicological endpoints in larval fathead minnow (*Pimephales promelas* Rafinesque), and a short exposure period (24 h), to mimic runoff-related pulse exposures (Pick et al., 1984; Werner et al., 2004). Sublethal exposure concentrations were based on previously determined acute LC_{10} values.

2. Materials and methods

2.1. Fish source, acclimation and quality assurance

Fathead minnow larvae were obtained from Aquatox Inc. (Hot Springs, AR, USA) at 7 d post-hatch on the day of arrival. Control water consisted of deionized water, modified with salts to meet USEPA specifications (electric conductivity (EC): 265–293 μS cm^{-1}; hardness: 80–100 as mg $CaCO_3$ L^{-1}; alkalinity: 57–64 as mg $CaCO_3$ L^{-1} (USEPA, 2002a)). Fish were acclimated for a minimum period of 4 h in control water at a temperature of 25 °C. During the acclimation period <1% mortality was observed, and the fish fed and swam normally.

During the project period, routine monthly reference toxicant tests were performed using NaCl to ascertain whether organism response fell within the acceptable range according to USEPA requirements (USEPA, 2002a). Each test consists of a dilution series (5 test concentrations) and a control. All test organisms responded normally (within 95% confidence interval of running mean) and sensitivity was considered typical.

2.2. Insecticide exposure

Reference standard grade bifenthrin [[1α3α(2)]-(±)(2-methyl [1,1′-biphenyl]-3-yl)methyl 3-(2-chloro-3,3,3, trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate], 99% purity (CAS number 82657-04-3), and fipronil (5-amino-1 [2,6-dichloro-4-(triflouromethyl) phenyl]-4 [(triflouromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile), 98.5% purity (CAS number 120068-37-3) were obtained from ChemService inc. (West Chester, PA, USA). Commercial insecticide formulations Talstar® (US EPA Reg. No. 279-3155; 7.9% bifenthrin per volume; FMC Corporation, Philadelphia, PA, USA) and Termidor® (US EPA Reg. No. 7969-210; 9.1% fipronil per volume; BASF Corporation, Research Triangle Park, NC, USA) were purchased commercially. Bifenthrin consists of 97% *cis*-isomer both in the pure compound and the formulated product. Pure fipronil is a 50:50 racemic mixture, just like its formulation product. All insecticide exposure experiments were conducted at the Aquatic Toxicology Laboratory, School of Veterinary Medicine, University of California Davis.

To determine acute toxic effects on survival, 7 d old larval fish were exposed for 24 h to the following nominal concentrations: 0.75, 1.0, 1.5, 2.0, 3.0 and 4.0 μg L^{-1} bifenthrin, 3.0, 4.0, 4.5, 5.0 and 6.0 μg L^{-1} bifenthrin as amount A.I. in Talstar®, 150, 200, 250, 300, 350 and 400 μg L^{-1} fipronil, and 150, 200, 350, 400 and 450 μg L^{-1} of fipronil as amount A.I. in Termidor®. The exposure concentrations used to determine acute toxicity refer to A.I. concentrations (pure chemical or respective formulation) to ensure direct comparability. For the pure substances we used 1 ml L^{-1} methanol (MeOH) as the solvent carrier and one treatment group containing the same MeOH concentration in control water was added as a solvent control. No solvent carrier was required for the formulations as they are designed to mix with water.

Stock solutions were prepared in MeOH for pure insecticides (2000 mg L^{-1}) and used for both, 24 h LC_{50} determination and sublethal exposure experiments.

Exposure concentrations used for the swimming performance and growth test series were calculated as percentages of the nominal LC_{10} values derived from the acute toxicity tests. For each chemical, treatments consisted of a control, solvent control (pure chemicals only), and 10%, 20%, 33% and 50% of the nominal LC_{10}. Each treatment consisted of 13 replicate 600 ml Pyrex beakers containing 250 ml test solution and 10 fish larvae. Subsequently, we used 9 replicates to determine swimming performance at three different time points and 4 replicates to determine growth.

At test initiation, 10 larvae were transferred from the acclimatization tank to each beaker and exposed for 24 h at a water temperature of 25 °C and a 16:8 light–dark ratio. Test vessels were then manually distributed in a random manner, within the exposure water bath. Fish were not fed during the exposure period.

For the sublethal concentrations, sub-samples of each test solution (1 L) were preserved with dichloromethane (Fisher Scientific, USA) at test initiation, shipped overnight to the California Department of Fish and Game Water Pollution Laboratory (Rancho Cordova, CA, USA), extracted within 24 h of arrival, and analyzed using gas chromatography with mass spectrometry and ion-trap detection. Reporting limits for detection of bifenthrin and fipronil were 0.002 μg L^{-1} (recovery 88.3%) and 0.2 μg L^{-1} (recovery 83.1%), respectively. Talstar® samples were filtered through 0.45 μm glass fiber filter to separate microcapsules from the water phase, and "particulate" and dissolved bifenthrin concentrations were determined. Concentrations for Talstar® are presented as the dissolved fraction. Measured and nominal insecticide concentrations are shown in Table 2.

2.3. 7 d growth

Following the 24 h insecticide exposure, fish were transferred to control water and maintained for 6 days at 25 °C and a 16:8 light:dark photoperiod. During transfer, fish were gently rinsed in control water,

using a fine-meshed sieve and moved to vessels containing control water. From days 2 to 7, approximately 80% of the water was exchanged daily and the number of surviving fish was recorded. Physicochemical variables (pH, dissolved oxygen, temperature, EC) were measured per treatment before and after each water exchange and at test termination. Measurements were conducted on pooled replicates of each treatment. After each water renewal the test beakers were manually distributed in a random manner, throughout the exposure waterbath. Fish were fed *ad libitum* twice a day with newly hatched *Artemia nauplii* (ranging from 30 to 50 individuals). At test termination, surviving fish were euthanized with MS-222 (Tricaine Methanesulfonate, Sigma, St. Louis, MO, USA), then transferred to pre-weighed aluminium weigh boats and dried for 24 h at 100 °C. Dry weight per fish (±0.001 mg) was calculated by measuring whole dry weight divided by the number of fish in each replicate.

2.4. Swimming performance ("one minute racetrack")

Swimming performance was measured at three different time points: (1) Immediately after the 24 h insecticide exposure; (2) after a total of 48 h (24 h recovery in control water), and (3) after a total of 7 days (6 d recovery in control water). At each time point, seven fish per replicate from three replicate beakers per treatment were tested using a circular "racetrack" method (Heath et al., 1993a). This racetrack consisted of a 13 cm diameter Petri dish with an upside-down 8 cm diameter Petri dish centrally placed, divided into 8 sectors by radiating lines drawn on the bottom of the testing dish, and filled with control water to a depth of 1 cm. Fish from pre-selected beakers were transferred individually into the testing device and allowed to acclimate for 1 min. A plastic rod was then used to trigger the fish's escape response by gently touching the tail fin every time the fish stopped moving. Due to possible bias in experimental technique, groups of fish were tested in a random manner, without the experimenter's knowledge of exposure concentration following Heath et al. (1993b). The number of lines or sectors crossed by the fish within 1 min was recorded and used as a measure of swimming performance. Water in the testing device was renewed after testing 7 fish from individual replicates.

2.5. Statistical analysis

We used the Comprehensive Environmental Toxicity Information System (CETIS) by Tidepool Scientific Software (McKinleyville, CA, USA) to calculate nominal effect concentrations for 24 h survival (NOEC, LC50, LC_{10}) based on A.I. Statistical analyses of sublethal endpoints utilized the measured dissolved A.I. concentrations. The Shapiro–Wilk normality test was used to evaluate whether quantitative data met the assumptions of the parametric ANOVA. For multiple comparisons the JMP 7.0 Software by SAS Institute Inc. was used. To evaluate differences between treatments in swimming performance and growth data we used one-way ANOVA and Dunett's multiple comparison post hoc test to compare insecticide treatments to controls and solvent controls. Assumptions of normality and homogeneity of variances were met, except for the highest concentrations, but due to the large differences in swimming performance, the ANOVA is considered to be robust (Underwood, 1997), particularly since the distribution of residuals was unimodal.

3. Results

3.1. Water chemistry

Physicochemical parameters measured at the start and end of the 24 h exposure period were within the acceptable range for the test organism (USEPA, 2002a,b) for all experiments and treatments. The measured mean values (±standard deviation) were pH: 7.51 (±0.19), dissolved oxygen 7. 2 (±0.5) mg L^{-1}, temperature: 23.1 (±0.3) °C, and EC: 278 (±6) µS cm^{-1}.

3.2. Sublethal effects

Individual effects were observed for each substance at concentrations below 50% of the LC_{10}. Concentration levels in the following sections refer to the measured dissolved fractions of A.I., or to percentages of the nominal LC_{10} values determined by initial acute toxicity tests (Table 1).

3.2.1. Swimming performance

3.2.1.1. Bifenthrin. Immediately following the 24 h exposure to pure bifenthrin, the swimming performance of fish from the lowest concentration treatment (0.07 µg L^{-1} or 10% LC_{10}) showed no statistical difference to control or solvent control treatments (Fig. 1). Swimming performance of fish exposed to concentrations ≥0.14 µg L^{-1} (20% LC_{10}, $p<0.001$) was significantly decreased compared to solvent controls. In comparison, exposure to the commercial formulation Talstar® led to decreased swimming performance at ≥0.03 µg L^{-1} bifenthrin (10% LC_{10}, $p<0.001$). After transfer to control water for a 24 h recovery period, swimming performance of exposed fish improved in most insecticide treatments. Fish exposed to bifenthrin concentrations of 0.07–0.14 µg L^{-1} as pure chemical (Fig. 1A), and 0.03–0.05 µg L^{-1} as Talstar® (Fig. 1B) recovered completely. After a recovery period of 6 days, no statistically significant differences between treatments were observed. When comparing dissolved bifenthrin concentrations between pure bifenthrin and Talstar®, the formulation was approximately 5 times more toxic than the pure active ingredient.

3.2.1.2. Fipronil. Swimming performance after 24 h was significantly decreased in fish exposed to concentrations ≥142 µg L^{-1} pure fipronil (20% LC_{10}, $p<0.001$) and ≥148 µg L^{-1} Termidor® (33% LC_{10}, $p<0.01$) (Fig. 2). Although the measured concentrations at this time point are in a similar range, the formulation had a stronger negative impact on swimming at higher concentrations. Fish exposed to 192 µg L^{-1} Termidor® (50% LC_{10}) exhibited statistically significant lower swimming activity than fish exposed to 333 µg L^{-1} fipronil treatment (33% LC_{10}). After 24 h recovery in control water no significant effects on swimming performance were observed in fish exposed to pure fipronil, but after the 6 d recovery period, there was a statistically significant negative effect ($p<0.01$, Fig. 2A). In contrast to the pure fipronil treatments, swimming performance of fish exposed to

Table 1
Acute nominal effect concentrations (mortality) for 7 d old fathead minnow after 24 h exposure to bifenthrin, fipronil and their formulations, Talstar® and Termidor®. Effective concentrations, LC_{50} and LC_{10}. Values in parenthesis represent 95% confidence intervals determined via probit analysis.

Substance	NOEC [µg L^{-1}]	LOEC [µg L^{-1}]	24 h LC_{50} [µg L^{-1}]	24 h LC_{10} [µg L^{-1}]
Fipronil, pure	300	350	398.29 (376.27–438.79)	305.57 (275.56–324.12)
Fipronil formulation	200	350	379.47 (355.13–405.48)	233.01 (201.99–307.94)
Bifenthrin, pure	0.5	1	1.90 (1.69–2.12)	0.92 (0.72–1.09)
Bifenthrin formulation	<3	3	4.85 (4.47–5.34)	2.99 (2.36–3.39)

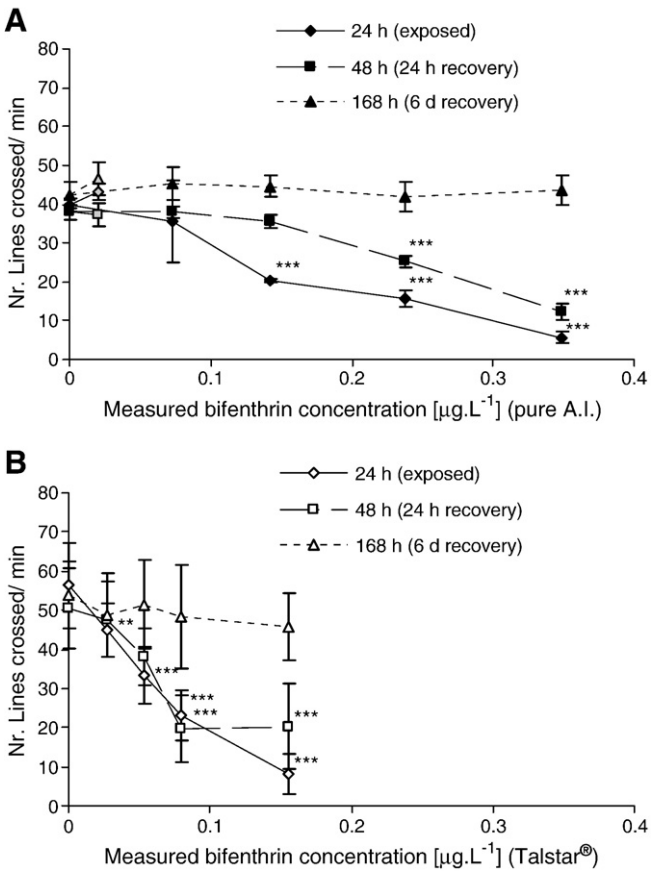

Fig. 1. Swimming performance of larval fathead minnow after 24 h exposure bifenthrin and Talstar®, 24 h recovery and 6 d recovery. Asterisks indicate significant differences in treatments compared to control/solvent control (*: $p<0.05$. **: $p<0.01$. ***: $p<0.001$). Data shown as arithmetic mean ± SD; $n=7$. A: pure bifenthrin, control group shifted to $x=0.02$ for visibility (grey); B: Talstar®.

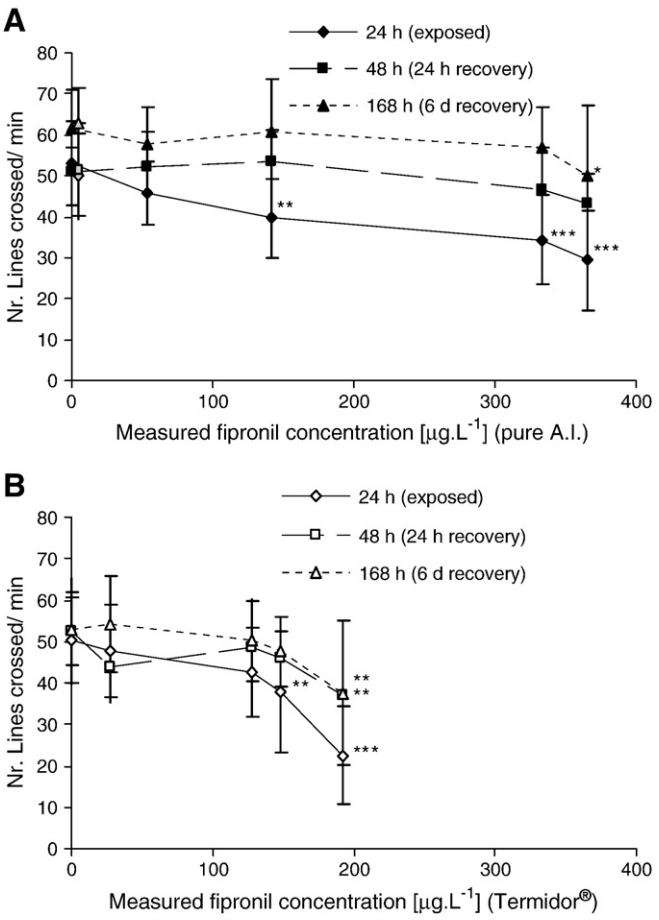

Fig. 2. Swimming performance of larval fathead minnow after 24 h exposure, 24 h recovery and 6 d recovery. Asterisks indicate significant differences in treatments compared to control/solvent control (*: $p<0.05$. **: $p<0.01$. ***: $p<0.001$). Data shown as arithmetic mean ± SD; $n=7$. A: pure fipronil, control group shifted to $x=5$ for visibility (grey); B: Termidor®.

192 µg L^{-1} Termidor® (50% LC_{10}) remained suppressed after the 24 h recovery period. This effect persisted throughout the test, and no recovery of swimming performance was observed after 6 days (Fig. 2B).

3.2.2. 7 d growth

3.2.2.1. Bifenthrin. Neither pure bifenthrin (maximum test concentrations: 0.35 µg L^{-1}, 50% LC_{10},) nor Talstar® (maximum test concentration 0.16 µg L^{-1} A.I., 50% LC10) caused any growth effects in larval fathead minnow (Fig. 3).

3.2.2.2. Fipronil. Fish exposed to pure fipronil at all concentrations tested grew significantly more than fish exposed to the solvent alone (53 µg L^{-1}, $p<0.05$; 333 µg L^{-1}, $p<0.01$; 365 µg L^{-1}, $p<0.01$, Fig. 4). Exposure to Termidor® did not result in negative or positive effects on growth.

In addition to the observed effects on 7 d growth, fish exposed to both pure fipronil and Termidor® showed deformities of the spine (data not presented). Four to five days after the 24 h insecticide exposure, several fish showed scoliosis and in some cases both scoliosis and lordosis. At test termination 5 of the fish exposed to 365 µg L^{-1} and 1 of the fish exposed to 333 µg L^{-1} pure fipronil had developmental abnormalities. The same effect was visible for 4 of the fish exposed to 192 µg L^{-1} and 1 of the fish exposed to 148 µg L^{-1} Termidor®. No such effects were observed in any of the other treatments.

4. Discussion

This study provides new information on the sublethal toxicity of two pure insecticides and two of their commercial formulations to larval stage fathead minnow. Results demonstrate that short-term (24 h) exposures to sublethal concentrations of pure and formulated bifenthrin and fipronil significantly impaired swimming performance

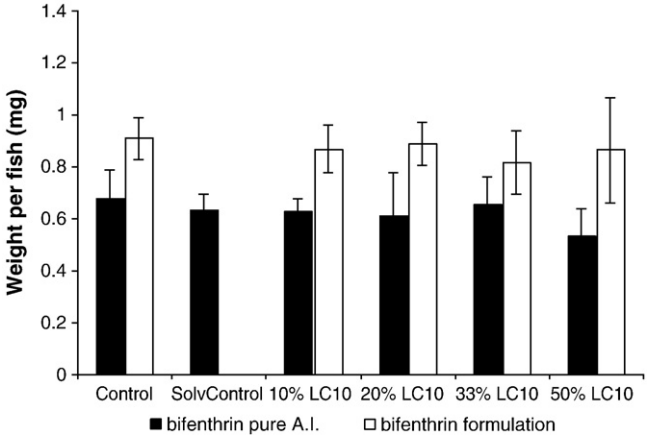

Fig. 3. Average dry weight per fish after 24 h exposure to bifenthrin and Talstar® and 6 d recovery.

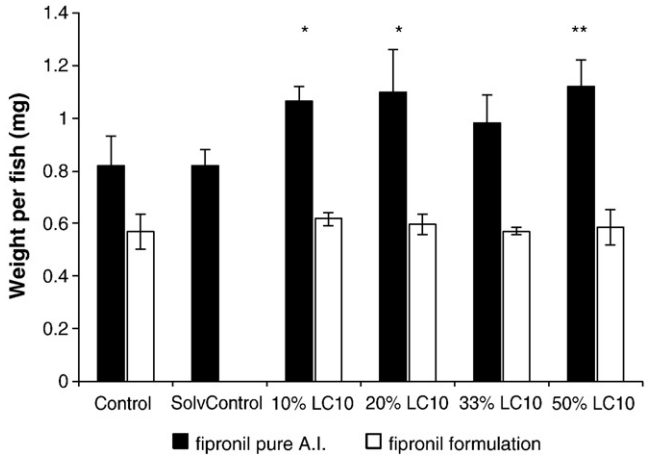

Fig. 4. Average dry weight per fish after 24 h exposure to fipronil and Termidor® and 6 d recovery. Fish exposed to pure fipronil had significantly higher average weight than fish in control treatments (*: $p<0.05$. **: $p<0.01$).

of larval fathead minnows at concentrations as low as 10% of the LC_{10} for the bifenthrin formulation Talstar® and 20% of the LC_{10} for pure bifenthrin.

Bifenthrin and Talstar® concentrations that affected swimming performance (0.14 μg L^{-1} and 0.03 μg L^{-1}, respectively) were in the range of environmental relevance, however, environmental factors such as particulate or dissolved organic matter can reduce bioavailability (Yang et al., 2006) and complicate an ecotoxicological assessment. Sublethal effect concentrations of fipronil and Termidor® (≥142 μg L^{-1} and 148 μg L^{-1}, respectively) were higher than known environmental levels.

Swimming performance is a highly suitable endpoint for estimating individual level effects of environmental contaminants on fish, as it integrates biochemical and physiological processes (Geist et al., 2007; Kane et al., 2005). Especially insecticides with neurotoxic modes of action have been shown to negatively affect swimming ability and predator avoidance (Floyd et al., 2008; Heath et al., 1993b; Little and Finger, 1990). We used a simple and easy to perform test to assess swimming behavior. It simulates predatory chase and integrates both neural and metabolic aspects of fish, since swimming involves nerve cell transmissions and muscle activity (Heath et al., 1993b) which is particularly affected by neurotoxicants (Jin et al., 2009). This is of special ecological importance during early life stages when fish are highly vulnerable to predation. Inability to swim properly after a brief exposure to insecticides therefore negatively affects individual fitness and survival, with potential consequences at the population level (Little and Finger, 1990). As demonstrated in this study fish can recover, but in a field situation, not being able to feed or evade predators for a certain period of time, will likely lead to negative impacts on individual survival and population dynamics.

In this study, growth was not a sensitive endpoint for measuring the effects of bifenthrin. While other pyrethroids have been shown to cause a reduction in growth of fathead minnow and other fish species (Haya, 1989; Jarvinen and Tanner, 1982), we did not observe this effect after bifenthrin and Talstar® exposures. This may be due to the low concentrations used in our experiments (≤50% of the LC_{10}). Floyd et al. (2008) reported significantly reduced 7 d growth in larval fathead minnow after short-term (4 h) exposure to the pyrethroid esfenvalerate, however, effect concentrations were ≥22% of the LC_{50}. The relatively long recovery period (6 d after 24 h exposure) from pyrethroid poisoning may have enabled the fish to compensate for any initial impairment. We did not rigorously quantify food uptake in this study, but during daily water renewal, remaining food quantity was observed to be greater in treatments with decreased swimming performance than in control treatments up to 2 d after insecticide exposure.

Exposure to pure fipronil enhanced growth of larval fathead minnow, while its formulated form, Termidor® did not produce this effect. Enhanced growth following exposure to fipronil has not been previously reported and causative factors should be investigated in more detail, but were beyond the scope of this investigation. A limited number of studies found fipronil to be altering normal thyroid function and thyroid hormone levels in rats (Hurley et al., 1998; Leghait et al., 2009) and chicken (Russ, 2005). As thyroid hormones also play a role in larval and juvenile development of fish (Power et al., 2001) the observed growth abnormalities may be related to this effect.

Developmental effects such as those observed in this study for a small number of the fish exposed to ≥148 μg L^{-1} Termidor® and ≥333 μg L^{-1} pure fipronil, were also reported by Stehr et al. (2006), in particular notochord degeneration and shortening along the rostral-caudal body axis in zebrafish (*Danio rerio*) embryos after continuous exposure to fipronil at nominal concentrations at or above 0.7 mM (333 mg L^{-1}). These authors also observed ineffective tail flips and uncoordinated muscle contractions in response to touch. Although most concentrations used in our study were below that range, similar behavioral abnormalities were observed and resulted in a measurable decrease of swimming performance.

We found strong differences in toxicity between pure and formulated insecticides. Both formulated products were more toxic than the respective A.I., based on measured dissolved concentrations. Talstar® impaired fathead minnow swimming performance at approximately one fifth of the effect concentration of pure bifenthrin. However, when adding the concentration of bifenthrin measured in the particulate fraction of Talstar®, the total concentration that caused negative effects on swimming was approximately 2 times higher for Talstar® than for pure bifenthrin (Table 2). Microcapsules may have been ingested by the larval fish, thus adding a dietary

Table 2
Nominal and measured concentrations for 24 h exposure of 7 d old fathead minnow to bifenthrin, Talstar®, fipronil and Termidor®. Treatment concentrations used for swimming performance and growth tests, calculated as percentages of the LC_{10} value (10%, 20%, 33% and 50% LC_{10}).

Substance	Concentration [μg L^{-1}]	10% LC_{10}	20% LC_{10}	33% LC_{10}	50% LC_{10}
Bifenthrin, pure A.I.	*Measured*	*0.07*	*0.14*	*0.24*	*0.35*
	Nominal	0.09	0.18	0.31	0.46
Bifenthrin, Talstar®	*Measured–dissolved*	*0.03*	*0.05*	*0.08*	*0.16*
	Measured particulate	0.19	0.39	0.57	0.81
	Nominal	0.29	0.59	0.99	1.49
Fipronil, pure A.I.	*Measured*	*53*	*142*	*333*	*365*
	Nominal	31	61	102	153
Fipronil, Termidor®	*Measured*	*28*	*128*	*148*	*192*
	Nominal	23	47	78	117

exposure route to the aqueous exposure to dissolved bifenthrin, which could account for the higher toxicity of the formulated product based on dissolved concentrations. In addition, it is possible that the presence of 0.1 methanol added as a carrier increased bioavailability and toxicity of the pure insecticides, however, we found no difference in swimming performance or growth between control and solvent control treatments. For pure fipronil and Termidor®, effect concentrations for swimming performance were similar, but impairment was more persistent in fish exposed to the formulated product.

Insecticide formulations can act as mixtures and environmental risks cannot be determined by assessing the toxicity of the A.I. alone. The relevance of these findings is obvious as pure insecticides are never applied in the environment. Extrapolating our laboratory results to a field exposure scenario is, however, beyond the scope of this study. For determination of toxicity under environmental conditions many additional factors have to be taken into account. Sediment particles, dissolved organic carbon, water pH and temperature can change the bioavailability and therefore toxicity of pesticides (Maul et al., 2008; Yang et al., 2007). Despite that, the consideration of short-term exposures, delayed effects and sublethal toxicity is of importance as exposure of aquatic organisms to insecticides is most likely to be of short duration and below lethal levels. For example, Brady et al. (2006) demonstrated that the majority of insecticide runoff of two insecticides, diazinon and esfenvalerate, occurred within the first hour of a simulated rain event.

Information on inert ingredients is largely treated as trade secret, but these chemicals have been shown to exert additive or synergistic toxicity, due to either their mechanism of action or through increasing the bioavailability of the A.I. Emulsifiable formulations of pyrethroids were found to be 2.2 to 8.5 times more lethal to fish than the pure substance (Haya, 1989) as a consequence of enhanced uptake via the gill epithelium. In other products, enzyme altering synergists like piperonyl butoxide (PBO) are added (Amweg and Weston, 2007) to enhance toxicity of the A.I. The solvent propylene glycol is part of the Talstar® formulation, but its toxicity to fish is low (fathead minnow 48 h LC_{50}: 790,000 μg L^{-1} (Kegley et al., 2008; TDC-Environmental, 2008)), and it was found to not significantly modify the toxicity of bifenthrin to cultured human cells (Skandrani et al., 2006). Chemicals used in pesticide formulations may also increase mobility of the A.I. thus facilitating pesticide movement into aquatic environments. Suspension liquids such as Termidor® or microencapsulated products like Talstar® are designed to not immediately bind to porous surfaces, and are therefore more susceptible to runoff or leaching. For example, Armbrust and Peeler (2002) reported that the concentration of the insecticide imidacloprid was higher in runoff from turf that was treated with granules than turf treated with wettable powder. Similar formulation effects were observed for herbicide runoff from container plant nurseries (Briggs et al., 2002). Kenimer et al. (1997) reported higher surface runoff of alachlor microencapsulated formulation compared to alachlor emulsifiable concentrate formulation, as microcapsule movement was similar to that of eroded sediment.

Talstar® is formulated as a so-called microencapsulation of bifenthrin, resulting in μm-sized particles, where the A.I. forms a core that is coated by an outer wall consisting of “inert” ingredients (Scher et al., 1998; Tsuji, 2001). The toxicity of this formulation is therefore dependent on how fast and how much of the active ingredient is released through the capsule (Jarvinen and Tanner, 1982). As this formulation is designed to be more persistent at the site of application, the release is probably slow. This explains why measured concentrations of dissolved bifenthrin were lower in the Talstar® experiment than in the exposures to pure bifenthrin. The use of such controlled-release insecticides may lead to lower exposure concentrations but increased exposure time of non-target organisms. Future investigations on these types of products should therefore consider a long-term exposure scenario to lower concentrations.

5. Conclusions

Our study demonstrated that formulated products of two widely used insecticides, the pyrethroid bifenthrin and the phenylpyrazole, fipronil, were approximately 5 and 2 times more toxic to larval fathead minnow than the active ingredients alone. Growth was not a sensitive toxicity endpoint, but the fish's ability to swim normally was impaired at Talstar® (bifenthrin) and Termidor® (fipronil) concentrations 10 and 3 times lower, respectively, than the 24 h LC_{10}. Results suggest that these neurotoxic insecticides can decrease ecological fitness of sensitive aquatic species at concentrations far below the lethal level. We have demonstrated that behavioral endpoints such as swimming are valuable tools to detect sublethal effects of neurotoxic chemicals. Future risk assessments should include information on sublethal endpoints such as swimming behavior, and additional safety factors to account for the greater toxicity of formulated pesticide products.

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