

Long-term effects of fenoxycarb on two mayfly species in artificial indoor streams

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Received 11 December 2002; received in revised form 19 August 2003; accepted 25 August 2003

Abstract

The effects of the juvenile hormone analog fenoxycarb (CAS No. 72490-01-8) were investigated in artificial indoor streams. The results from aufwuchs and two mayfly species (*Rhithrogena semicolorata* and *Ephemerella ignita*) are presented. Four concentrations ranging from 0.05 to 50 µg/L (with a spacing factor of 10) were tested. Fenoxycarb disappeared rapidly from the water phase ($DT_{50} \approx 5$ days in the highest concentration, less in the other concentrations). Physico-chemical parameters and aufwuchs were not affected by fenoxycarb. The mayfly *R. semicolorata*, introduced at the start of the experiment, was affected by treatments of 5 and 50 µg/L. For the larvae in the streams a LC_{50} of 3.3 µg/L and for the larvae in the enclosures a LC_{50} of 2.5 µg/L were calculated. The second species (*E. ignita*) was introduced 72 days after the application, at which time no fenoxycarb was detectable in the water of the streams (limit of detection of 0.5 ng/L). The emergence of *E. ignita* was affected in the highest treatment (50 µg/L). Ninety percent of the emerged imagoes showed morphological abnormalities at the abdomen.

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Keywords: Artificial indoor streams; Aufwuchs; Ecotoxicology; Fenoxycarb; Mayfly; Microcosm

1. Introduction

Pesticide development has produced some chemicals with specific modes of action for controlling pests, including the group of juvenile hormone analogs (Dhadialla et al., 1998). The juvenile hormone analogs, sometimes called third generation pesticides (Grenier and Grenier, 1993; Kayser et al., 2001), interact with the natural hormones of insect development (Sláma, 1995) to inhibit metamorphosis to the adult stage (Miyamoto et al., 1993).

One of these juvenile hormone analogs is fenoxycarb (IUPAC: ethyl (2-(4-phenoxyphenoxy)ethyl)carbamate, CAS No. 72490-01-8). It is reported to have an ovidical activity (Charmillot et al., 2001; Bortolotti et al., 2000; Kayser et al., 2001). Fenoxycarb is used for flea, mosquito, and cockroach control (Extension Toxicology Network, 1993; Grenier and Grenier, 1993). Further-

more, it is used to control moths and sucking insects in stored products and in integrated pest management (Liu and Chen, 2001; Solomon and Fitzgerald, 1990; Valentine et al., 1996). In Europe, fenoxycarb is mainly used to control Lepidoptera in fruit orchards and vineyards. The ecotoxicological effects of this substance are difficult to assess with the standard aquatic tests (fish, *Daphnia*, algae) because the specific mode of action on insects is not covered by these tests. Some data on aquatic insects (especially Diptera) are available because fenoxycarb is also used in mosquito control (Dorn et al., 1981; Miura and Takahashi, 1987; Mohsen and Zahyia, 1995; Mohsen and Al-Chalabi, 1989; Mulla et al., 1985; Schaefer et al., 1987; Walker and Edman, 1990).

To study the effects of fenoxycarb on a representative, laboratory-assembled lotic community, a microcosm experiment was conducted in artificial indoor streams. The artificial indoor streams were previously tested in an experiment investigating the effects of the herbicide terbutryn (Brust et al., 2001) on aufwuchs (epilithic biocoenosis consisting mainly of algae and microorganisms), oligochaeta (*Lumbriculus variegatus*), and crustaceans (*Gammarus fossarum* and *Asellus aquaticus*). In the

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experiment with the insecticide fenoxycarb mayflies, in addition to these species, were investigated to assess its specific mode of action.

Mayflies are important organisms in stream ecosystems (Allan, 1995); in recent years the mayfly has become more popular as a test organism in ecotoxicology (for example: van der Geest et al., 2000a,b; Schulz and Dabrowski, 2001; Kennedy et al., 2002). The mayflies *Rhithrogena semicolorata* (Curtis, 1834) and *Ephemera ignita* (Poda, 1761) were chosen because they are widespread in Europe (Buffagni et al., 1995; Riano et al., 1997) and represent two different types of grazers. *R. semicolorata* graze mainly on periphyton, whereas *E. ignita* graze on detritus (Rosillon, 1988).

R. semicolorata was introduced before the application of fenoxycarb and *E. ignita* 72 days after the application to simulate a recolonization of mayfly larvae after the substance had disappeared from the water. Our experiment thus tested the effects of fenoxycarb on the mayfly, which is potentially affected during molt, and on its food source, aufwuchs, in a comparatively realistic exposure situation.

2. Materials and methods

2.1. Artificial indoor streams

The design of the artificial indoor streams and their physical characteristics are described in detail by Jungmann et al. (2001). Briefly, five equal streams made of stainless steel were placed in a greenhouse with a South–North alignment. The streams were 4.2 m long. The streambed (length of 3.7×0.5 m) was restricted by an inflow reservoir, upstream and downstream by an outflow reservoir. These two reservoirs were connected with a pump that enabled water to circulate from the outflow reservoir over the streambed to the inflow reservoir. The streambed was divided by a steel plate into two channels (a and b). To prevent the drift of organisms into the outflow and inflow reservoirs, these were separated from the channels by mesh sieves (mesh size of $250 \mu\text{m}$ to the outflow reservoir and $500 \mu\text{m}$ to the inflow reservoir). The streambed was covered to a height of about 3 cm with washed gravel (from a gravel pit) of the following size and composition [mm (%): 16–32 (18), 8–16 (47), and 2–8 (35)]. The stream was then filled with activated carbon-filtered tap water up to a level of 10 cm (resulting in a total water volume of approximately 450 L per stream). The surface water velocity was adjusted to 0.2 m/s and the temperature was maintained at $15 \pm 1^\circ\text{C}$. To imitate shading through vegetation, the roof and walls of the greenhouse were dimmed with white plastic foil and white fabric. The nutrient concentration (4 mg/L nitrogen, 0.05 mg/L phosphorus,

and 2.7 mg/L silicon) was adjusted by the addition of dissolved nutrients to reach the initial concentration.

2.2. Experimental design and timetable

Before the streams were filled with tap water, enclosures were placed on the streambeds to determine the endpoints of the introduced organisms. Fig. 1 gives a view from above of the set-up for the organisms presented in this paper (aufwuchs and mayfly).

The day of application of the chemical to the water is day 0; negative time values represent the preapplication period and positive values the exposure period. The experiment ended on day 98. The designation of each stream signifies its nominal concentration of fenoxycarb (cf. substance characteristics and application).

2.3. Physico-chemical parameters

Physico-chemical parameters like oxygen concentration, pH, temperature, and conductivity were measured every third day using a Multilab P4 with sensors for the different parameters (TriOximatic 300, Tetracon 96, pH electrode E56, TFK 150, all from WTW, Weilheim, Germany). Concentrations of nitrogen in nitrate, nitrite, and ammonia, the phosphorous concentration as orthophosphate, and the silicon concentration as silicate were determined photometrically weekly using NANOCOLOR test kits (MACHEREY–NAGEL GmbH & Co. KG, Düren, Germany). After analysis, nutrients were replaced by the addition of the specific amount of stock solution. At this time we compensated for evaporative loss by adding back the amount of water that had been depleted.

2.4. Aufwuchs

To establish aufwuchs in the artificial streams, five stones ($\varnothing \approx 15\text{--}20$ cm) were taken from the Lockwitzbach (a stream southeast of Dresden, Germany) one day before the preapplication period started (day-22). The Lockwitzbach, a summer-cold and due to the geology of the catchment, silicate (SiO_2)-dominated

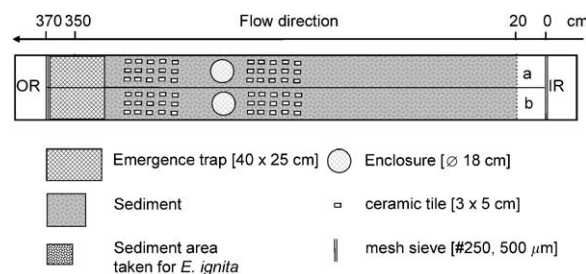


Fig. 1. View from above of an artificial indoor stream. Only the set-up relevant for aufwuchs and mayflies is shown. IR, inflow reservoir; OR, outflow reservoir. See the text for further explanations.

stream, is classified as β -mesosaprob (class II) (Landesamt für Umwelt und Geologie, 1995; Staatliches Umweltfachamt Radebeul, 2000). The aufwuchs on the stones were washed off with a water jet and collected in a plastic aquarium. The suspension was filtered through a mesh (mesh size of 100 μm) to retain other organisms. The filtered aufwuchs sedimented for 8 h, and the supernatant was decanted. The precipitate was mixed with a spatula. The homogenized suspension was divided into five equal subsamples (graduated cylinders, 100 mL) and gently transferred to the appropriate stream to allow a uniform development of aufwuchs during the preapplication period. Species determination in a previous experiment indicated that the community would consist mainly of the diatoms *Achnanthes* sp. and *Nitzschia* sp. and the green algae *Ulothrix* sp. and *Cladophora* sp. (Brust et al., 2001).

Artificial substrates were used for sampling of the aufwuchs during the experiment. Unglazed ceramic tiles (3 \times 5 cm) were placed on top of the gravel before the experiment started, prior to the addition of the aufwuchs' start biomass. One tile was sampled from each channel of the five streams at 12-day intervals. The aufwuchs on the tile were scraped off with a razor blade and transferred to beakers by water jet. The aufwuchs suspension was filtered on preashed, glass fiber filters (\varnothing 55 mm, Schleicher & Schüll No. 62, Dassel, Germany). The filters with the aufwuchs were dried at 75°C for 12 h, weighed (RC 210S, Sartorius, Göttingen, Germany), ashed at 450°C for 1 h, and reweighed. The difference between the two weights was the ash-free dry weight of the aufwuchs.

From these data the area under the curve (AUC) was calculated following the linear trapezoidal rule (Purves, 1992) with the formula provided in OECD Guideline 201 (Organization for Economic Cooperation and Development) (1984):

$$AUC = [(AFDW_{x2} - AFDW_{x1})/2] + \{[(AFDW_{x2} + AFDW_{x3} - 2 * AFDW_{x1})/2] * (t_{x2} - t_{x1})\} + \dots + \{[(AFDW_{xn-1} + AFDW_{xn} - 2 * AFDW_{x1})/2] * (t_{xn} - t_{xn-1})\}. \quad (1)$$

$AFDW_{x1}$ and $AFDW_{x2}$ were the ash-free dry weights at the first and second sampling dates, respectively; t_{x1} and t_{x2} were the dates of the first and second samplings, respectively.

These AUC were set in relation to the control (in percentages).

2.5. Mayfly larvae

In natural streams the two chosen species, *R. semicolorata* (Ephemeroptera: Heptageniidae) and *E. ignita* (Ephemeroptera: Ephemerellidae), appear successively (Hefti and Tomka, 1990) due to the different

life cycles of each species. *R. semicolorata* overwinters as larvae in the streambed and their life cycle finishes very rapidly in spring/early summer, whereas *E. ignita* overwinters in the egg stage, with their larvae occurring in May and emergence in July (Elliot et al., 1988).

2.5.1. *Rhithrogena semicolorata*

The larvae of the mayfly *R. semicolorata* were collected in the Lockwitzbach with a modified Surber sampler (area of 0.25 m², mesh size of 0.3 mm; Metag, 2000). The samples were transported to the laboratory where the larvae of *R. semicolorata* were separated. The larvae were kept in glass aquaria filled with dechlorinated tap water at 15 \pm 2°C until they were transferred to the streams during the preapplication period. On day -1 the larvae were randomly transferred to each channel of the stream (66 larvae per channel) and also to the enclosures (18 per enclosure). The enclosures were made of steel mesh (mesh size of 500 μm) that was placed on a Petri dish (\varnothing 18 cm) and fixed to the streambed with a steel plate. Each Petri dish was filled with sediment of the same composition as that of the respective stream (see Jungmann et al. (2001) and Licht et al. (2003) for more details). The enclosure was covered with a nylon mesh (mesh size of 1 mm). To catch the emerging imagoes, a trap (40 \times 25 cm) was put at the end of each channel. The traps were triangular with a height of 25 cm at the side of the outflow reservoir. The frame was made of wooden bars (\varnothing 1 \times 1 cm) and covered with nylon mesh (mesh size of 1 mm).

During the experiment all emerged imagoes from the channel and the enclosures were preserved in 70% ethanol. They were counted, their sex was determined, and the maximum eye distance (a morphometric character for the size of an imago) was measured using a binocular microscope fitted with an ocular micrometer (Wild M10, Ocular 2.5 \times , Leica, Bensheim, Germany). Dead larvae and exuviae that were found on the mesh at the outflow reservoir in the channels and on the walls of the enclosures were also preserved in 70% ethanol. The head capsule width of these larvae (a morphometric character for the size of larvae) was measured with a binocular microscope as well. The following endpoints were investigated: retrieval of larvae (number of dead and emerged larvae/number of introduced larvae), sex ratio, morphometric character for the size of the organisms (head capsule width and maximum eye distance, Meyer, 1989), emergence characteristics (EmT₅₀ and EmP₉₅), and number of exuviae.

2.5.2. *Ephemerella ignita*

E. ignita was the second mayfly investigated in the experiment. The animals were collected as described above for *R. semicolorata*. Because *E. ignita* can be found at a high density in aquatic vegetation (Elliot et al., 1988), vegetation was sampled and transferred to

the laboratory. The larvae of *E. ignita* were treated as described above for *R. semicolorata*. Twenty-two larvae were placed randomly on day 72 in one enclosure per channel. New enclosures filled with sediment from the streams, taken from the area in front of the outflow reservoir, were used. During the experiment the same endpoints as those described for *R. semicolorata* were determined.

2.6. Fenoxycarb

2.6.1. Substance characteristics and application

Fenoxycarb was kindly provided by Novartis Crop Protection AG (Basel, Switzerland; now Syngenta Crop Protection AG). Fig. 2 shows the chemical structure of fenoxycarb. The physico-chemical properties were taken from Tomlin (2000). The molecular weight of fenoxycarb ($C_{17}H_{19}NO_4$) is 301.30 g/mol. The water solubility is 6 mg/L at 20°C and the melting point 53°C. The substance has a log K_{OW} of 4.3 and a vapor pressure of 7.8×10^{-3} mPa at 20°C. Its Henry's law constant is 4.6×10^{-5} Pa m³/mol and a K_{OC} of 1500 is reported (Sullivan, 2000). A Mackay Level 1 calculation (Mackay Level 1, Ver. 2.11) with standard parameter except for the compartment size (air: 1×10^{14} ; water: 2×10^{11} ; sediment: 9×10^9 ; suspended sediment: 1×10^6 ; soil: 0 m³) leads to the following distribution: water: 2.8%; sediment, 97.2% and air: <0.1%. Technical material (purity of 98%) was used in the experiment.

The nominal concentrations of fenoxycarb were 0.05, 0.5, 5, and 50 µg/L. One stream served as an untreated control. The respective amount of substance was dissolved in stock solution (10 mL methanol, with Nanopure water added to 1000 mL). The solution was applied to the outflow reservoir of the streams in a single dose at day 0. To the control 10 mL methanol, with Nanopure water added to 1000 mL, was also added (resulting in approximately 0.02‰ methanol in the water of the treatments).

2.6.2. Analytics

The concentrations of fenoxycarb were measured 1-hour after application and then at days 5, 12, 19, and 26. Stream water (1 L) was sampled in a 1-L brown glass bottles. The samples were filtered through glass fiber filters (Ø 55 mm, Schleicher & Schüll No. 62) and were concentrated by solid-phase extraction. The

cartridges (LiChrolut EN, 3 mL, Merck KGaA) were activated with 5 mL of ethyl acetate and 5 mL methanol (ULTRA RESI-ANALYZED, J.T. Baker) and conditioned with 5 mL nanopure water. Elution was performed with ethyl acetate (1 mL) three times. The eluate was evaporated under a gentle stream of nitrogen (5.0, purity >99.999 vol%) to a volume of 0.2 mL. An internal standard (0.05 mL EPA mix 524) was added and the volume was filled with ethyl acetate to 0.5 mL.

The samples were analyzed by GC/MS. This method is suitable for the detection of fenoxycarb in water (Climent and Miranda, 1996), even though other methods may be used for specific matrices (Bicchi et al., 1990; Natangelo et al., 1999; Reyzer and Brodbelt, 2001). The GC/MS consists of a Hewlett–Packard (HP) (Avondale, PA, USA) 5860A gas chromatograph equipped with a 70-eV, electron-impact mass spectrometer (HP 5970B). A HP-5MS capillary column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 µm) was used. The samples were injected with a HP 7673A autosampler, (see Brust et al. (2001) for more details). The injection volume was 1 µL. The initial temperature was 130°C, increased by 18°C/min up to 280°C and held for 5 min. The total duration of the analysis was 20 min. Fenoxycarb was detected at 9.32 min with 116 m/z for quantification and 186 m/z for identification (SIM mode, EMV 2000V). Quantification was based on the relative peak area compared to the internal standard 1,2-dichlorobenzene-d4. The limit of detection (LOD) was 0.5 ng/L (recovery: 79.5%; precision: 7.8% R.S.D.).

2.7. Calculation and statistics

For comparisons of treatments the data of the channels were pooled as means (for aufwuchs biomass) and by summation (for mayfly larvae) because the channels received the same water and therefore were pseudoreplicates in the sense of Hurlbert (1984). Correlation analyses were performed for dead larvae of *R. semicolorata* with SPSS 11.0.1 (15 Nov. 2001, SPSS Inc., Chicago, IL, USA) using Spearman's "ρ" (rank correlation coefficient). The LC_{50} was calculated using probit transformation and mortality in the control was compensated with Abbott's formula (Abbott, 1925). These calculations were performed with ToxRat (ToxRat Solution GmbH, Alsdorf, Germany). The EmT_{50} [d] (date, where 50% of the larvae have emerged) and the EmP_{95} [d] (period, where 95% of the larvae have emerged) were calculated from the cumulative emergence/time plot. For the EmT_{50} the median was used and for EmP_{95} the period of 95% emergence was determined by graphic interpolation.

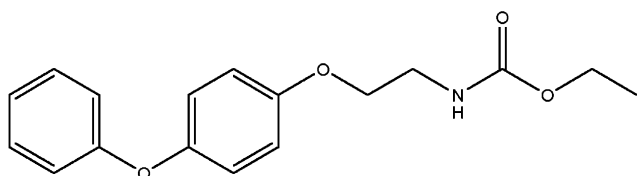


Fig. 2. Chemical structure of fenoxycarb (CAS No. 72490-01-8).

3. Results

3.1. Analytics

Results of the fenoxycarb analyses in the water of the streams are shown in Table 1. The first analyses 1 h after the application of fenoxycarb indicate that the nominal concentrations were well established. Except for the treatment of 0.05 µg/L, for which about half of the nominal concentration was measured, all other analyzed concentrations were within ±15% of the nominal concentration. In the control no fenoxycarb could be detected (LOD: 0.5 ng/L). On day 5 fenoxycarb was detected only in the treatments with the two highest concentrations, 0.45 and 26.22 µg/L. On day 12 the concentration of fenoxycarb in the treatment with the highest concentration was 0.15 µg/L and on day 19 fenoxycarb could not be detected in the water of all streams (LOD: 0.5 ng/L). The dissipation time for 50% of the substance (DT₅₀) was estimated to be 1–2 days, except in the initial 5 days of the highest treatment; when the DT₅₀ was estimated to be about 5 days.

3.2. Chemical and physical parameters

The median, minimum, and maximum values of the chemical and physical parameters in the water of the

Table 1
Fenoxycarb concentrations in the water of the artificial indoor streams

Time (days)	Treatment (µg/L)			
	0.05	0.5	5	50
1 h	0.02	0.56	4.42	53.8
5	ND	ND	0.45	26.2
12	ND	ND	ND	0.15
19	ND	ND	ND	ND
26	ND	ND	ND	ND

ND, not detected; LOD, 0.5 ng/L.

Table 2
Physico-chemical parameters in the water of the streams with different fenoxycarb treatments

Parameters	Treatment (µg/L)				
	Control	0.05	0.5	5	50
<i>Chemical</i>					
Nitrate-N (mg/L)	2.1 (0.3–5.0)	2.2 (0.3–4.8)	3.0 (ND–4.6)	2.6 (ND–5.1)	1.0 (ND–5.0)
Nitrite-N (µg/L)	11 (ND–24)	15 (ND–32)	15 (1–78)	13 (1–80)	7 (ND–37)
Ammonia-N (µg/L)	22 (ND–76)	24 (ND–110)	27 (ND–104)	27 (6–57)	19 (ND–66)
<i>o</i> -Phosphate-P (µg/L)	ND (ND–44)	ND (ND–54)	ND (ND–41)	ND (ND–56)	ND (ND–46)
Silicon-Si (mg/L)	0.5 (ND–2.4)	1.1 (ND–2.5)	0.7 (ND–2.4)	0.7 (ND–2.5)	0.5 (ND–2.8)
<i>Physical</i>					
O ₂ (mg/L)	10.1 (9.0–11.8)	10.1 (8.8–11.1)	10.2 (8.9–11.4)	10.1 (8.9–13.6)	10.2 (9.0–11.4)
Temperature (°C)	14.4 (14.0–14.8)	14.6 (14.3–14.9)	14.3 (14.2–14.6)	14.1 (12.7–14.4)	14.2 (13.9–14.3)
pH	8.6 (8.2–9.1)	8.5 (8.2–9.1)	8.5 (8.3–9.2)	8.5 (8.2–9.1)	8.6 (8.3–9.2)
Conductivity (µS/cm)	270 (217–359)	252 (215–346)	268 (230–360)	262 (222–357)	265 (226–363)

ND, not detected. Values are given as median and range (min/max).

streams are presented in Table 2. The median values of the chemical parameters showed minor differences and no concentration–effect relationship. However, the minimum and maximum values over time are the ones which may cause limitation or toxicity and thus are important. In particular, the median value of the major nutrient *ortho*-phosphate in all streams was below the detection limit; possibly affecting periphyton growth (cf. Section 4). The maxima of all parameters never reached levels that affect the survival of the invertebrates.

3.3. Aufwuchs

The dynamics of the aufwuchs biomass (mean from channels a and b) are shown in Fig. 3. The first samples were taken on day 0, shortly before fenoxycarb was applied. The biomass was very similar in all five streams during the preapplication period and until day 12. The ash-free dry weight ranged between 0.85 and 1.15 mg/cm² on these sampling dates. During the experiment the biomass of aufwuchs reached a maximum in all streams between day 36 and day 47, except in the highest treatment. The maximum biomass in the 0.5 µg/L treatment was greater (3.83 mg/cm²) than that in the

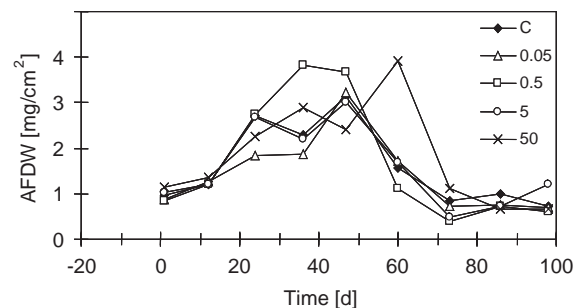


Fig. 3. The development of aufwuchs during the fenoxycarb-experiment in the artificial indoor streams (ash-free dry weight, AFDW). The first sample was taken on day 0 (before fenoxycarb was applied).

other treatments (maximum biomasses ranged from 3.00 to 3.23 mg/cm²). A slightly different dynamic of aufwuchs was found in the 50 µg/L, treatment, in which the maximum biomass reached 3.92 mg/cm² on day 60. At this time in all other treatments a decrease in biomass was observed. At the end of the experiment all treatments showed a comparable level of aufwuchs biomass of between 0.60 and 1.2 mg/cm². The AUC showed no concentration–effect relationship (control: 175.1 = 100%; 0.05 µg/L: 156.7 = 89.5%; 0.5 µg/L: 181.9 = 103.9%; 5 µg/L: 169.4 = 96.8%; 50 µg/L: 202.5 = 115.7%).

3.4. *Rhithrogena semicolorata*

The effects of fenoxycarb on the death and emergence of *R. semicolorata* larvae are shown in Fig. 4. The data are pooled from channels a and b by summation. The percentage of dead larvae found in the streams increased from page 13% (control) to 40% (treatment of 5 µg/L) with increasing fenoxycarb concentration and remained constant in the treatment with 50 µg/L. The correlation is statistically significant (Spearman’s ρ = 1, P < 0.01).

The number of emerged imagoes in the streams (Fig. 4) showed no clear concentration–effect relationship and is not statistically significant (Spearman’s ρ = –0.7, P = 0.094). However, the percentage of emerged imagoes in the treatments of 5 (14%) and 50 µg/L (11%) decreased, compared to the control (39%) and treatment with 0.5 µg/L (39%). In the treatment with 0.05 µg/L, 22% of the introduced larvae emerged. In the enclosures a similar pattern of dead larvae and emerged imagoes was found compared to in the streams. The correlations are statistically significant (dead larvae, Spearman’s ρ = 0.872, P = 0.027; emerged imagoes, Spearman’s ρ = –0.894, P = 0.020).

The retrieval of larvae showed no concentration–effect relationship (Table 3), so data for dead and emerged larvae were related to the number of animals retrieved (dead + emerged). In the control, 25% (17/68) of the larvae retrieved in the stream were dead. In the other treatments the percentages were: 0.05 µg/L: 46.3%

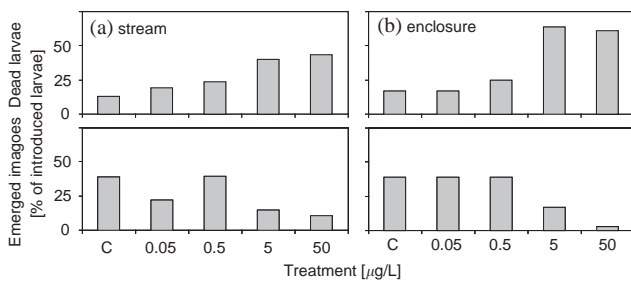


Fig. 4. Effects of fenoxycarb on *R. semicolorata*. Upper, percentages of dead larvae; lower, percentages of emerged imagoes. The differences to 100% (n = 2 × 66 per stream and 2 × 18 per enclosure) could not be retrieved. See the text for further explanations.

Table 3
Effects of fenoxycarb on *R. semicolorata* in the streams (pooled results from channels a and b)

Endpoint	Treatment (µg/L)				
	Control	0.05	0.5	5	50
Retrieval of larvae (%)	52	41	63	55	54
Sex ratio (% male)	49	69	45	54	55
Maximum eye distance (mean) (mm)	2.05	2.14	2.14	2.19	2.24
EmT ₅₀ (d)	13.0	5.5	8.5	9.5	2.5
EmP ₉₅ (d)	25.7	21.2	24.8	24.3	35.5
No Exuvie	174	159	164	157	136
HCW (mean) (mm)	2.12	2.12	2.16	2.16	2.12

HCW, head capsule width.

(24/54); 0.5 µg/L: 37.3% (31/81); 5 µg/L: 73.6% (53/72) and 50 µg/L: 80.3% (57/71). Using these data the LC₅₀ was 3.3 µg/L (95% confidence limit, 1.4–7.5 µg/L). In the enclosures the following data were obtained: control and 0.05 µg/L: 30% (6/20); 0.5 µg/L: 39.1% (9/23); 5 µg/L: 79.3% (23/29) and 50 µg/L: 95.7% (22/23). For the larvae in the enclosures the LC₅₀ was 2.5 µg/L (95% confidence limit; 1.0–6.2 µg/L). In Table 3 only the results from the streams are shown.

The retrieval of larvae was also chosen as an endpoint. The retrieval of larvae of *R. semicolorata* ranged from 40% to 60% and showed no concentration–effect relationship. The sex ratio (% male) showed no concentration–effect relationship and was unaffected by fenoxycarb.

The mean of the maximum eye distance of the imagoes as a parameter for the size of the larvae increased with the concentration of fenoxycarb from 2.05 mm in the control up to 2.24 mm in the highest treatment (50 µg/L). The EmT₅₀ showed no concentration–effect relationship and varied from day 5.5 to 13.0 in the control and the lower treatments. In the highest treatment (50 µg/L) the EmT₅₀ was day 2.5. In the control and the lower treatments of fenoxycarb the EmP₉₅ was between 21.2 and 25.7 days. This period increased to 35.5 days in the highest treatment. The numbers of exuviae as a measure of molts were comparable in the control and the three lower treatments (157–174). Only in the highest treatment was the number of exuviae lower (136). The head capsule width of the exuviae found in the streams varied about 2.3 mm and was not affected by fenoxycarb.

3.5. *Ephemerella ignita*

On day 72 the larvae of *E. ignita* were introduced exclusively in the enclosures. A summary of the effects on *E. ignita* is given in Table 4. The data are pooled from channels a and b by summation. The retrieval of *E. ignita* was high in comparison with that of *R. semicolorata*, varying from 70% to 89%, and showing no concentration–effect relationship.

Table 4
Effects of fenoxycarb on *E. ignita* in the enclosures (pooled results from channels a and b).

Endpoint	Treatment ($\mu\text{g/L}$)				
	Control	0.05	0.5	5	50
Retrieval of larvae (%)	73	80	89	70	86
Sex ratio (% male)	53	51	51	52	45
Max eye distance (mean) (mm)	1.32	1.29	1.32	1.28	1.35
EmT ₅₀ (d)	82	84	83	84	82
EmP ₉₅ (d)	17	16	17	15	15
No Exuvie	39	37 (+2)	39 (+2)	38 (+1)	26 (+9)
HCW (mean) (mm)	1.09	1.09	1.11	1.10	1.13
Emergence incomplete (%)	0	5.1	4.9	2.6	24.3
Abdominal abnormalities (%)	0	0	0	0	92

HCW, head capsule width.

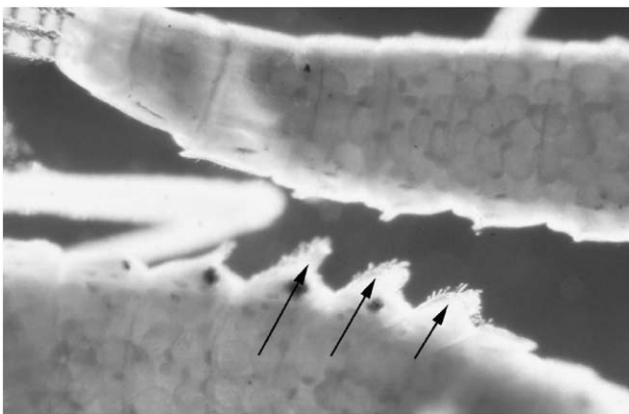


Fig. 5. Abnormalities of the abdomen of *E. ignita*. The photo shows the abdomens of two imagoes (upper, control, lower, treatment with 50 $\mu\text{g/L}$). Arrows indicate the abnormalities at the abdomen.

The sex ratio (% male) was about 50%. Only in the highest treatment it was slightly lower at 45%. The mean of the maximum eye distance of the imagoes was about 1.3 mm. The EmT₅₀ varied from day 81 to day 84 (9–12 days after the introduction of the larvae at day 72) and the EmP₉₅ varied from 13.2 to 19.4 days. These endpoints showed no concentration–effect relationship.

The number of exuviae found on the mesh of the enclosures was reduced only in the highest treatment, with 26 compared to 39 in the control. In this treatment 9 imagoes could not leave the exuviae at the time of emergence. The mean of the head capsule width of the exuviae varied about 1.1 mm and showed no concentration–effect relationship.

Besides the effect of fenoxycarb on the ability to complete emergence, another effect was observed. In the highest treatment over 90% of the emerged imagoes showed abnormalities of the abdomen. Fig. 5 shows a comparison of the abdomens of control imago (upper specimen) and an imago from the 50 $\mu\text{g/L}$ treatment (lower specimen). On 3–4 terga of the abdomen of the imago from the 50 $\mu\text{g/L}$ treatment are structures that can be described as larval “relics”. Quite similar

structures can be found on the sides of the abdominal segments of the larvae. These abnormalities occurred on both males and females.

4. Discussion

The substance disappeared very quickly from the water phase. After 19 days no fenoxycarb could be found even in the highest treatment of 50 $\mu\text{g/L}$. This corresponds with the Mackay calculation (cf. substance characteristics and application). Sullivan (2000) reported a water sediment study with a dissipation time (DT₅₀) from the water of 4 days and an aerobic aquatic half-life of 19 days (water plus sediment). Schaefer et al. (1987) also reported a rapid loss of fenoxycarb from the water phase via binding to organic sediment.

Fenoxycarb had no influence on the water chemistry, directly or indirectly, through effects on the periphyton or microbial community. The data for water chemistry were comparable to values reported from earlier experiments in artificial indoor streams (Jungmann et al., 2001; Brust et al., 2001).

No effects of fenoxycarb on the aufwuchs could be detected (maximum biomass and AUC showed no concentration–effect relationship). Only in the highest treatment (50 $\mu\text{g/L}$) at one sampling date was the biomass of aufwuchs higher. The E_bC₅₀ (96 h) for the green algae *Scenedesmus subspicatus* (Chlorophyta: Chlorophyceae) was 1100 $\mu\text{g/L}$ (Novartis, pers. comm.), thus direct effects on green algae can be excluded.

Fenoxycarb as a juvenile hormone analog interrupts the molting of insects, thus mayflies are potentially affected as “non target organisms”. Effects of fenoxycarb on emergence and survival were found at treatments of 5 $\mu\text{g/L}$ and higher. In the stream the LC₅₀ (based on the number of dead larvae) was 3.3 and 2.5 $\mu\text{g/L}$ in the enclosures. No acute or chronic effect data from the laboratory are available for mayflies. Tada and Hatakeyama (2000) investigated the effect of fenobucarb, a carbamate insecticide without juvenile

hormone activity used in rice fields, on the growth and emergence of two mayfly species in model streams. In their simple system (caged mayflies only with one tile with periphyton as the food source) and semistatic exposure they found a nearly total inhibition of emergence at 8 and 16 µg/L. Miura and Takahashi (1987) described effects of fenoxycarb on late larval stages (10th and 11th) of the familiar bluet damselfly *Enallagma civile* (Odonata: Coenagrionidae) over a period of 40 days. Even in the lowest tested concentration of 0.1 µg/L more than 50% of the larvae died (control mortality not mentioned, 94.5% mortality at 10 µg/L). Because of differences in the species used and the exposure regime (continuous exposure versus decreasing concentration in our experiment) these findings are difficult to compare. The developmental stage of the animals used in the tests plays an important role in the effectiveness of substances with this specific mode of action. This is shown by some authors for the effectiveness of fenoxycarb against mosquitoes (Mulla et al., 1985; Schaefer et al., 1987) and also for the green lacewing *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), an aphid predator used in integrated pest management (Liu and Chen, 2001). In a 21-day reproduction test with *Daphnia magna* (Crustacea: Branchiopoda) a NOEC of 0.0016 µg/L was determined with a continuous exposure regime. In another test with a decreasing concentration (half-life of 10 h) a NOEC of 13 µg/L was determined (Hosmer et al., 1998).

Table 5 gives an overview of the ecotoxicological effects of fenoxycarb. The acute toxicity for algae, *Daphnia*, and fish is about 400–1100 µg/L. The long-term toxicity for *Oncorhynchus mykiss* (Salmoniformes:

Salmonidae) (early life stage, 60 days post hatch) and *Daphnia magna* (21-day reproduction) was 48 and 13 µg/L, respectively. But in a *Daphnia* 21-day-reproduction test the concentration decreases, with a half-life of 10 h. Under continuous exposure the NOEC decreases to 0.0016 µg/L. For the Southern house mosquito *Culex quinquefasciatus* (Diptera: Culicidae) the LC₅₀ is 1.6 µg/L and the EC₅₀ (inhibition of emergence) is 0.073 µg/L. In a pond microcosm study the NOEL was 1.1 µg/L (Hosmer et al., 1998).

The results *E. ignita* indicate that, even though no fenoxycarb could be found after 19 days in the water of the highest treatment (LOD: 0.5 ng/L), the substance or its metabolites must have been present in the sediment of the stream and caused effects during metamorphosis (incomplete emergence and morphological abnormalities). Morphological abnormalities induced by fenoxycarb have been described by some authors for terrestrial insects (Moreno et al., 1993a, b). Miura and Takahashi (1987) described fenoxycarb-induced abnormalities for several water organisms: *Notonecta unifasciata* (Hemiptera: Notonectidae), *E. civile* (Odonata: Coenagrionidae), and *Pantala hymenaea* (Odonata: Libellulidae). However, for the mayfly such effects have not been described previously.

Acknowledgments

The authors thank R. Hartmann and T. Brethfeld for excellent technical assistance. Special thanks are extended to Novartis for providing the insecticide fenoxycarb and to M. Urban (Syngenta Crop Protection AG)

Table 5
Overview of the effects of fenoxycarb on different organisms

Species	Parameter (test duration, endpoint)	Concentration (µg/L)	Reference
Algae			
<i>S. subspicatus</i>	E _b C ₅₀ (96 h, static, biomass)	1100	Novartis, pers. com.
Invertebrates			
<i>D. magna</i>	EC ₅₀ (48 h, static, immobilization)	400	Hosmer et al. (1998)
	NOEC (21 days, flowthrough, pulse-dosed, reproduction)	13	Hosmer et al. (1998)
	NOEC (21 days, flowthrough, continuous, reproduction)	0.0016	Hosmer et al. (1998)
<i>C. quinquefasciatus</i>	LC ₅₀ (time until emergence or death, 4th instar larvae)	1.6	Schaefer et al. (1987)
	EC ₅₀ (time until emergence or death, early 4th instar larvae, inhibition of emergence)	0.073	Mohsen and Al-Chalabi (1989)
Vertebrates			
<i>O. mykiss</i>	LC ₅₀ (96 h, flowthrough)	660	Syngenta (2001)
	NOEC (ELS, 60 days post hatch, flowthrough, fry length and weight)	48	Novartis, pers. com.
Microcosm studies			
Microcosm	NOEL (112 days, pond)	1.1	Hosmer et al. (1998)
Artificial indoor streams	NOEC (98 days, mayfly, abnormalities at abdomen, 72 days after application, DT ₅₀ < 5 days)	5 (nominal)	This paper

and D. Schudoma (Umweltbundesamt) for valuable discussions of the results. Arnd Weyers and two anonymous reviewers gave helpful comments on the preparation of the manuscript. The project (UFO-PLAN, Ref. No. 295 63 075) was funded by the Federal Environmental Agency (Umweltbundesamt, Berlin, Germany).

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