Bull. Environ. Contam. Toxicol. (2004) 72:358–364

DOI: 10.1007/s00128-003-9042-z

Environmental Contamination and Toxicology

Effects of Methiocarb on *Chironomus riparius* Survival and Growth With and Without Tube-Building

A. R. R. Péry, R. Mons, V. Ducrot, J. Garric

Laboratoire d'Écotoxicologie, Cemagref, 3b quai Chauveau, 69009 Lyon, France

Received: 28 April 2003/Accepted: 15 October 2003

Chironomids are non-biting midges which constitute the most widely distributed insects in freshwater ecosystems. They represent a prominent part of benthic communities in virtually all freshwater habitats. Most of the life cycle of *Chironomus riparius* is sedimentary, which explains why it is commonly used to assess the toxicity of field or spiked sediments. This does not mean that it is not sensitive to chemicals present in the overlying water, as the first instar of the larval stage and the pupal stage are aquatic. Also, even when they are in the sediment, the chironomids can be affected by chemicals in the water, most probably through the pore water of the sediments (Hoke et al. 1993).

Chironomids possess protective responses against water pollution. First, tube building can protect them from predation (Macchiusi and Baker 1992), but also from chemicals. Halpern et al. (2002) showed that the tube of the midge *Chironomus luridus* substantially decreased the effects of copper and chloramine on survival. Second, chironomids are particularly resistant when exposed to metals, probably through the production of metallothionein (Gillis et al. 2002), and to some organic compounds, like 2,4,5-trichlorophenol for which they show no sublethal effects (Ristola et al. 1999).

Assessing the effects of pesticides on chironomids is a crucial issue, both in terms of protecting ecosystems and population control. Indeed, recently, chironomids occurred as pests in Israel (Halpern et al. 2002). In the present study, we tested the effects of methiocarb on survival and growth of *Chironomus riparius* in the overlying water. We also studied the protective effects of tube building relative to growth and survival. This study followed a previous one (Péry et al. 2003a), in which we showed that effects of methiocarb on survival were related to its concentration in the overlying water, with an estimated no effect concentration between 200 and 262 μg/L for third and fourth instars larvae and an estimated no effect concentration of about 6 μg/L for second instar larvae.

MATERIALS AND METHODS

Chironomus riparius organisms came from our laboratory culture. The test beakers were filled with 0.1 L artificial sediment (silicate) and 0.3 L water (taken

Correspondence to: A. R. R. Péry

from an uncontaminated spring near our laboratory with pH 7.7 specific conductivity 400 µS/cm, only traces of chemicals except for aluminium, with a concentration of 21 µg/L and atrazine, with a concentration of 0.03 µg/L), three days before the beginning of the test. During the test, the beakers were set in a water bath at 21°C with a 16:8h light:dark photoperiod. Test water was gently aerated. Specific conductivity, temperature, pH, dissolved oxygen, nitrates and ammonium concentrations were measured daily. Midges were fed each day with Tetramin® fish food (Tetrawerke, Melle, Germany). Tests organisms received 0.6 mg/larva/day, which corresponds to *ad libitum* conditions (Péry et al. 2002). Methiocarb (from Cil Cluzeau, Saint Foy La Grande, France, with purity 98.5 %) was introduced in the beakers by adding 0.1 L water at day 0 of the tests with pesticide concentrations corresponding to the nominal exposure concentrations selected. This additional amount of water was introduced very slowly to avoid perturbation of the sediment.

Tests were performed to assess growth effects and protection effects of the tube on adverse survival and growth effects.

Growth effects were assessed by measuring length daily. At day 0 of the test, 10 organisms were introduced randomly into each glass-beaker. Twenty organisms were also taken randomly and measured just before the beginning of the test. During the test, three beakers per condition were randomly chosen each day for measurements, which corresponded to about 30 length measurements per data point. Organisms were killed using a solution of 20 % formaldehyde and 80% water. They were kept in this solution less than 10 seconds to avoid distortion of the shape. Then length was measured using a binocular microscope fitted with a calibrated eye-piece micrometer. We performed growth tests with second, third and fourth instar larvae to assess any significant difference between instars. They were respectively two, four and six days old (since hatching) at day 0. Instar stage was checked with head capsule width measurements. The tests lasted three days each. Methiocarb nominal exposure concentrations were control, 10, 20, 30, 60, 80 and 100 µg/L.

Protection against adverse survival effects was assessed by counting organisms after two days of exposure. The chironomids were separated in two groups. For the first group, 10 fourth instar larvae (six days since hatching) were put in each beaker one day before the beginning of the test to let them build tubes under clean water conditions. For the second group, 10 fourth instar larvae (seven days since hatching) were introduced into triplicate beakers after introduction of the pesticide. Nominal methiocarb exposure concentrations were control, 67.5, 125, 250, 500 and 1000 µg/L.

Protection against adverse growth effects was assessed by measuring organisms as presented previously after four days of exposure. As previously, the chironomids were separated into two groups. For the first group, 10 third instar larvae (four days since hatching) were put in each beaker one day before the beginning of the test to let them build their tubes. For the second group, 10 third instar larvae (five

days since hatching) were introduced into triplicate beakers after introduction of the pesticide. Nominal methiocarb exposure concentrations were control, 10, 20, 30, 60, 80 and 100 µg/L.

Mean length and survival values obtained during the growth tests were compared using Student-t-tests with the mean values of each replicate. As for the experiment to compare survival with and without tube-building, we compared both treatments using a Chi-square test with the total number of survivors for each concentration.

RESULTS AND DISCUSSION

During all the experiments, temperature was constant (21+/-0.5°C) as was pH (between 8.1 and 8.4). Specific conductivity was between 300 and 400 µS/cm, and the percentage of dissolved oxygen was always above 80 %. Nitrate and ammonia level were always below 2 mg/L.

Table 1 presents the survival NOAEC (No Observed Adverse Effect Concentration) and LOAEC (Lowest Observed Adverse Effect Concentration) obtained during the growth tests together with no effect concentration estimations from previous survival tests performed with *Chironomus riparius* (Péry et al. 2003a). For third and fourth instars, survival effects appear for a lower concentration (80 µg/L) than the no effect concentration we had estimated (250 µg/L). In contrast, for second instar, survival effects appear above a higher concentration (30 µg/L) than the no effect concentration we had estimated (6 µg/L). We do not have any clear explanation to account for the difference between the present and the former survival results. One reason could be the inappropriate range of tested concentrations in our previous paper, no exposure concentrations between 60 and 280 µg/L having been tested.

The results of the growth tests for second, third and fourth instar larvae are presented in figures 1, 2 and 3 respectively. For second instar larvae, data from the control and 10 µg/L treatments were combined, because their mean values did not differ by more than 0.1 mm; this difference being not significant ($p>0.5$). Also, control, 10 µg/L and 20 µg/L treatments for third and fourth instars were combined. Growth was inhibited during the whole test or only for a short period at the beginning of the test, with growth thereafter being similar to the one for the control (growth curves are parallel). Each time growth inhibition during the whole test was observed, there was also mortality for the corresponding concentration. As for the observed delay, we tested, in a previous experiment (Péry et al. 2003b), the influence of natural sediment characteristics on the growth patterns, and we also observed a delay at the beginning of the test for certain sediments. We proposed an explanation based on the works by Naylor and Rodrigues (1995), who observed that larvae could feed only once they had constructed a tube. For different natural sediments, sediment particle type could influence the ease to tube-building and consequently the time necessary for this activity. The difference in times to construct tubes and to begin feeding could thus explain the delay observed since the beginning of the experiment. Similarly, we believe that here,

methiocarb induced tube-building difficulties. After tube building, methiocarb has no more effect on growth.

The results of the survival tests performed to assess the protective effects of tube building are presented in Table 2. There is no significant difference ($p<0.05$) between the two groups. Moreover, all the dead organisms were found at the surface of the sediment. In the group of organisms introduced in the beakers after introduction of the pesticide, no tube was found. On the contrary, we found tubes for the other group, which means that organisms had left their tubes to die. These results suggest the poor protection of the tube against effects of methiocarb on survival. This could be dependent on the chemicals tested. Indeed, Halpern et al. (2002) showed that tubes are able to protect *Chironomus luridus* against copper and monochloramine.

The hypothesis we formulated about effects on growth (increase of the time necessary for tube-building and growth inhibition when exposure concentration reaches lethal level) is consistent with the results of the growth tests we performed to assess the protective effects of tube building. Results are presented in Figure 4. There is no growth effect for the organisms which were already in tubes when pesticide was introduced except for the concentrations associated with mortality. This explained the slight differences between the two groups of organisms.

Table 1. Survival NOAEC and LOAEC values obtained here during growth tests together with the no effect concentration (NEC) estimates obtained in previous survival experiments (Péry et al. 2003a)

Instar	NOAEC (µg/L)	LOAEC (µg/L)	Estimated NEC (µg/L)
Second	30	60	6
Third	60	80	250
Fourth	60	80	250

Table 2. Results of the survival tests as a function of concentration and group (pesticide introduced first or organisms introduced first).

Concentration (µg/L)	Organisms before pesticide	Pesticide before organisms
0	30/30	30/30
67.5	19/30	18/30
125	5/30	10/30
250	0/30	0/30
500	0/30	0/30
1000	0/30	0/30

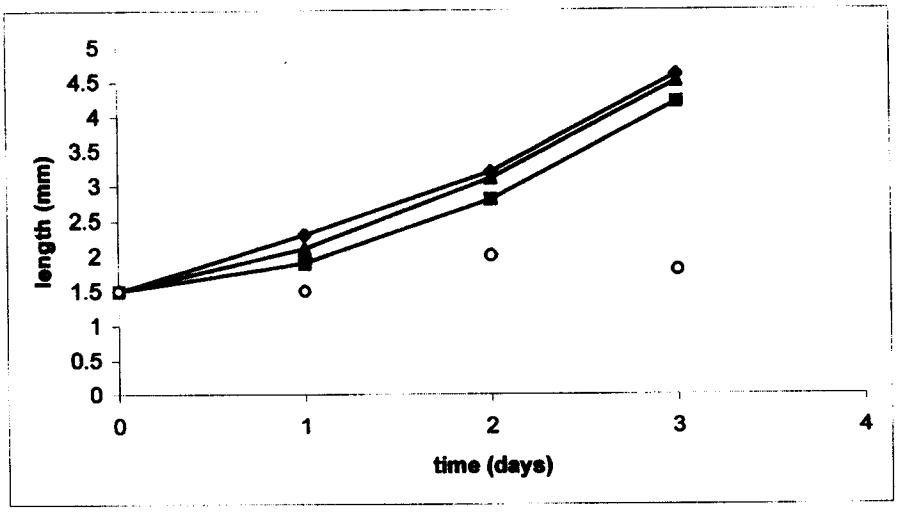

Figure 1. Growth pattern for second instar larvae exposed to methiocarb (diamonds: control and 10 µg/L ; triangles: 20 µg/L ; squares: 30µg/L ; white circles: 60 µg/L).

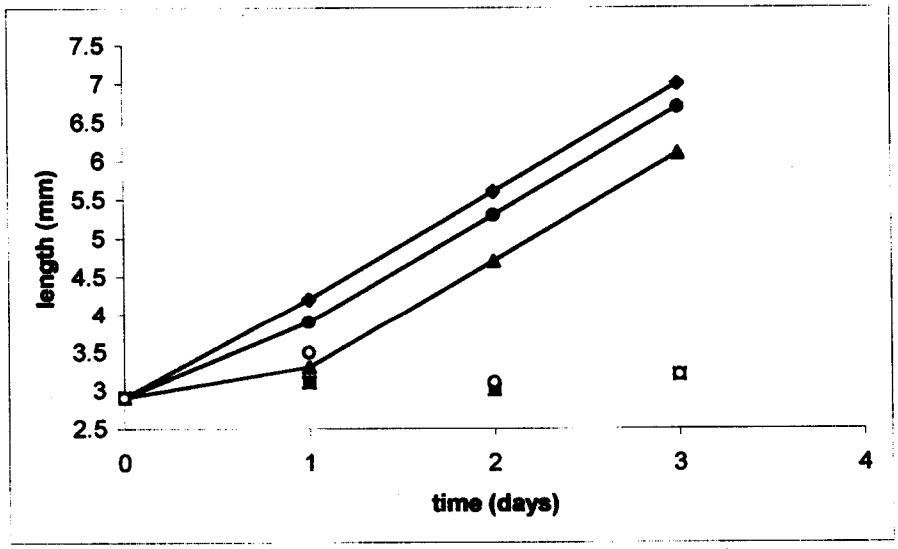

Figure 2. Growth pattern for third instar larvae exposed to methiocarb (diamonds: control, 10 and 20 µg/L ; black circles: 30 µg/L ; triangles: 60 µg/L ; squares: 80µg/L ; white circles: 100 µg/L).

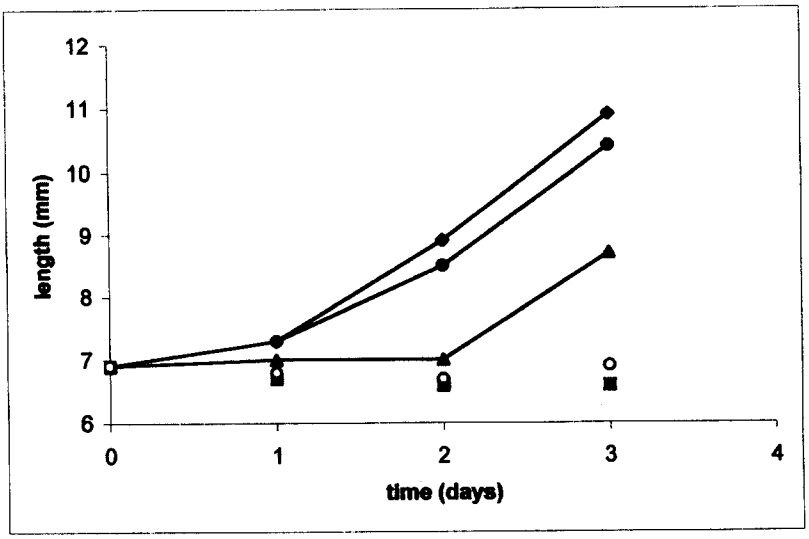

Figure 3. Growth pattern for fourth instar larvae exposed to methiocarb (diamonds: control, 10 and 20 µg/L ; black circles: 30 µg/L ; triangles: 60 µg/L ; squares: 80µg/L ; white circles: 100 µg/L).

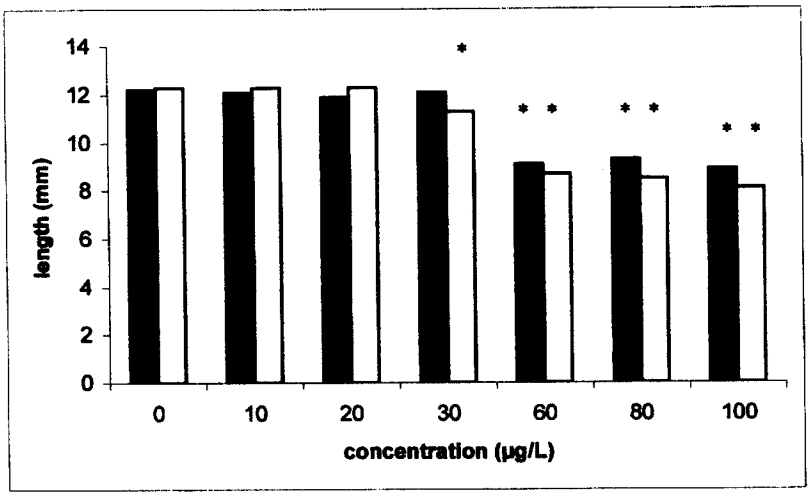

Figure 4. Mean end-of-the-test length for the test of the protective effects of tube-building (black: pesticide introduced one day after the organisms, white: organisms introduced after the pesticide). The symbol * accounts for significant differences ($p<0.05$) compared to the control.

REFERENCES

Gillis PL, Diener LC, Reynoldson TB, Dixon DG (2002) Cadmium-induced production of a metallothioneinlike protein in *Tubifex tubifex* (Oligochatea) and *Chironomus riparius* (Diptera): correlation with reproduction and growth. Environ Toxicol Chem 21: 1836-1844

Halpern M, Gasith A, Broza M (2002) Does the tube of a benthic chironomid larva play a role in protecting its dweller against chemical toxicants. Hydrobiologia 470:49-55

Hoke RA, Giesy JP, Zabik M, Unger M (1993) Toxicity of sediments and sediment pore waters from the Grand Calumet River-Indiana Harbor, Indiana area of concern. Ecotox Environ Saf 26:86-112

Macchiusi F, Baker RL (1992) Effects of predators and food availability on activity and growth of *Chironomus tentans* (Chironomidae, Diptera). Freshwater Biol 28:207-216

Naylor C, Rodrigues C (1995) Development of a test method for *Chironomus riparius* using a formulated sediment. Chemosphere 31:3291-3303

Péry ARR, Ducrot D, Mons R, Miège C, Gahou J, Gorini D, Garric J (2003a) Survival tests with *Chironomus riparius* exposed to spiked sediments can profit from DEBtox model. Wat Res 37:2691-2699

Péry ARR, Mons R, Flammarion P, Lagadic L, Garric J (2002) A modelling approach to link food availability, growth, emergence, and reproduction for the midge *Chironomus riparius*. Environ Toxicol Chem 21:2507-2513

Péry ARR, Sulmon V, Mons R, Flammarion P, Lagadic L, Garric J (2003b) A model to understand the confounding effects of natural sediments in toxicity tests with *Chironomus riparius*. Environ Toxicol Chem 22(10): in press

Ristola T, Kukkonen JVK, Pellinen J (1999) Body residues and responses of the midge *Chironomus riparius* to sediment-associated 2,4,5-trichlorophenol in subchronic and chronic exposures. Arch Environ Contam Toxicol 37:42-49