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Sensitivity of characteristic riverine insects, the caddisfly *Cyrnus trimaculatus* and the mayfly *Ephoron virgo*, to copper and diazinon

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"Capsule": *This study reports effects of two model toxicants on indigenous insect species from large European rivers.*

Abstract

This study reports the effects of two model toxicants, copper and diazinon, on two characteristic riverine insect species, the caddisfly *Cyrnus trimaculatus* and the mayfly *Ephoron virgo*. It was demonstrated that these species are very sensitive to both compounds in comparison with aquatic insects traditionally used in ecotoxicity tests. For diazinon, the 96-h LC_{50} value of *Cyrnus trimaculatus* (1.1 μg/l) is lower than for any other insect species known from the literature and for copper it was demonstrated that *Ephoron virgo* is among the most sensitive aquatic insect species. The observed low LC_{50} values stress the importance of using these indigenous species in assessing the risk of environmental contaminants in large European rivers and in defining conditions for ecological recovery. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Ephoron virgo; *Cyrnus trimaculatus*; Diazinon; Copper; Sensitivity ranking

1. Introduction

During the last century, biodiversity of large river ecosystems in Western Europe has declined strongly as a result of both habitat loss and water quality deterioration. Rehabilitation of these rivers is nowadays a major concern of environmental management and to this purpose several action programmes have been started (like the 'Rhine Action Programme', which was set up in 1987) or are being initiated. To support such programmes, test organisms are needed that indicate the progress of recovery. The selection of these test organisms should be based on both the sensitivity to stressors and the representativity for the ecosystem of concern. For large rivers, in particular, the number of typical riverine insect species, such as mayflies, stoneflies and caddisflies, has been greatly reduced (Bij de Vaate, 1995; Ketelaars and Frantzen, 1995; Nijboer and Verdonschot, 1997) and, therefore, such species play a key role in assessing the ecological status of river communities. However, the use of such indigenous insect species in standardised monitoring programmes is still hampered by a lack of ecotoxicological knowledge, limiting the possibility of explaining why they are present or absent. The present project aims, therefore, to determine the sensitivity of three riverine insect species (the caddisflies *Hydropsyche angustipennis* and *Cyrnus trimaculatus* and the mayfly *Ephoron virgo*) to various environmental stressors. Within this framework, the present study describes the short-term effects of two model toxicants, copper and diazinon, on the caddisfly *Cyrnus trimaculatus* and the mayfly *Ephoron virgo*, following a previous study on *Hydropsyche angustipennis* (Van de Geest et al., 1999).

Ephoron virgo is a mayfly species typical for large rivers (Kureck, 1996). *Cyrnus trimaculatus* usually appears in the lower reaches of large rivers, but also occurs in ponds and lakes (Edington and Hildrew, 1981). In undisturbed river systems, both species play an important ecological role, e.g. in decomposing organic material or as predator and as a food source for fish and birds. In polluted rivers, like the River Meuse at the Dutch–Belgian border, however, these species have disappeared during the last century and have not yet returned (Bij de Vaate, 1995). Based on their past and

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present distributions, these insects are likely to be useful test species for indicating ecological recovery.

In order to gain insight into the sensitivity of these species to environmental contaminants, the effects of copper and diazinon were determined in newly developed standardised short-term ecotoxicity tests (Greve et al., 1998, 1999). These compounds were selected as model toxicants based on their occurrence in large European rivers (RIWA, 1993–1997) and differences in mode of toxicity. By testing these different model toxicants it is expected that basic ecotoxicological information for *Ephoron virgo* and *Cyrnus trimaculatus* will become available. This information will be compared with ecotoxicological data from the literature, in order to determine the sensitivity of both species relative to other organisms. In this way, the potential role of the newly developed ecotoxicity tests in environmental risk assessments will be evaluated.

2. Materials and methods

The effects of copper and diazinon on larvae of the caddisfly *Cyrnus trimaculatus* and the mayfly *Ephoron virgo* were estimated by determining the survival after 96-h exposure to different concentrations of both compounds in standardised laboratory tests (Greve et al., 1998, 1999). Each experimental treatment consisted of a glass vessel (180-ml) filled with 100 ml Dutch Standard Water (DSW; NEN, 1980), a standardised synthetic analogue of common Dutch surface waters, containing 200 mg $CaCl_2.2H_2O$, 180 mg $MgSO_4.7H_2O$, 100 mg $NaHCO_3$ and 20 mg $KHCO_3$ per litre demineralised water (pH 8.2, hardness 210 mg/l $CaCO_3$, alkalinity ca. 1.2 meq/l). For *Cyrnus trimaculatus*, ten 20–25-day-old second instar larvae, originating from a laboratory culture (Greve et al., 1998), were placed in each vessel and fed with 1 ml of a suspension of 150 mg dried and ground *Urtica*, 75 mg Trouvit, 40 mg Tetraphyll and 2.5 ml fresh algae (*Scenedesmus* sp.) in 25 ml DSW to avoid mortality by starvation. During the experiments with *Cyrnus trimaculatus*, all vessels were continuously but gently aerated. For *Ephoron virgo*, twenty 2-day-old larvae, originating from field-collected egg masses (Greve et al., 1999), were placed in each vessel and fed with 0.1 ml of a suspension of 750 mg dried and ground *Urtica* in 25 ml DSW. In these tests, no aeration was supplied. In all experiments, vessels were closed with perforated plastic foil in order to avoid evaporation, a 16:8 h light:dark regime was provided and the temperature was maintained at 20°C.

Toxicants were added at the start of the experiments. Copper was added as $CuCl_2$ (Titrisol, Merck) and diazinon (derived from Luxan Inc., 99.7% purity) was added using a generator column-derived stock solution (Bleeker et al., 1998). For both compounds, a series of concentrations (including controls) was tested at least in duplicate. Water samples (3×1 ml in copper experiments; 1×10 ml in diazinon experiments) were taken after 1 h and at the end of each experiment in order to determine actual toxicant concentrations in the water. Depending on the copper concentration in the water, samples were analysed by air–acetylene flame or graphite furnace atomic absorption spectrometry (Perkin-Elmer 1100B equipped with an impact bead and Perkin-Elmer 5100PC/HGA600/AS60 equipped with Zeeman background correction, respectively) after acidification with 20 µl 65% nitric acid (Merck P.A.). Since no filtration was applied, actual concentrations reflect total copper concentrations in the water. Quality control of copper analysis was carried out by analysing blanks and reference material.

Before water samples were analysed for diazinon, 50 µl of a solution of 1.14 mg chlorpyrifos/l hexane was added as an internal standard to each 10-ml water sample and extraction was performed with 3×1 ml hexane (Rathburn dest.). The concentration of diazinon was quantified by gas chromatography (GC) using a calibration series of diazinon in hexane. Analysis of samples from the *Ephoron virgo* experiments was conducted on a GC–mass spectrometer (MS) according to Van der Geest et al. (1999). For the analysis of samples from the *Cyrnus trimaculatus* experiments, this method was replaced by GC–NPD measurements which are equally effective but less expensive and time consuming. Samples were measured using a Carlo Erba GC 8000 series Gas Chromatograph equiped with a Carlo Erba NPD-80-FL NP-detector and a J&W Scientific (DB-1701, 30 m long, 0.25-µm film thickness and 0.25-mm inner diameter) column. From the actual toxicant concentrations in the water at the start and the end of each survival test, average exposure concentrations were calculated assuming an exponential decrease of the toxicant concentration over time. Survival was expressed as percentage of the corresponding controls and plotted against the actual toxicant concentration in the water. From the obtained dose–response plots, LC_{10} and LC_{50} values and their corresponding 95% confidence limits were calculated by a non-linear curve-fitting procedure with the computer program Kaleidagraph (Synergy Software) using the logistic response model after (Haanstra et al., 1985):

$$Y = c/(1 + e^{b^*(X-a)}) \quad (1)$$

in which Y = survival (%), c = survival in control (set to 100%), a = log LC_{50} (µg/l), b = slope and X = log concentration (µg/l).

3. Results and discussion

The mayfly *Ephoron virgo* and the caddisfly *Cyrnus trimaculatus* appeared to be suitable organisms for short-term toxicity testing of individual toxicants. Control survival was within acceptable limits (average after 96 h, 98 and 78%, respectively) and clear dose–response relationships were observed for survival after 96 h exposure to both diazinon (Fig. 1) and copper (Fig. 2). From these results, LC_{10} and LC_{50} values and the corresponding 95% confidence limits based on measured toxicant concentrations were calculated (Table 1). The 96-h diazinon LC_{50} value for *Cyrnus trimaculatus* is ca. 10 times lower than for *Ephoron virgo*, but for copper the reverse was found and *Ephoron virgo* appeared to be ca. 10 times more sensitive than *Cyrnus trimaculatus*. Such species- and toxicant-specific variation in sensitivity is, however, even described for taxonomically closely related species. Pantani et al. (1997) assessed the acute toxicity of 26 chemicals towards two amphipod species, *Gammarus italicus* and *Echinogammarus tibaldii*. It was demonstrated that for 15 compounds *E. tibaldii* was the most sensitive species and that for eight compounds *G. italicus* was the most sensitive species. This variation demonstrates that a ranking of sensitive and tolerant species is highly toxicant dependent and emphasises the need of incorporating more than one model toxicant when the sensitivity of species is compared. Therefore, for both copper and diazinon a comparison with effect concentrations from the literature is made to gain insight in the sensitivity of both species relative to other organisms. Fig. 3 shows the 96 h diazinon LC_{50} values for all freshwater macroinvertebrate and fish taxa listed in the US EPA aquatic toxicity database AQUIRE (AQUA/INFO, 1997). Large differences in sensitivity of different species to diazinon are apparent: the 96-h LC_{50} of diazinon for the most tolerant species listed, the fish *Carassius carassius*, and for the most sensitive species, the amphipod *Gammarus fasciatus* are, respectively, 12,267 and 0.2 µg/l (Bathe et al., 1975; Johnson and Finley, 1980). In general, insects and crustaceans are the most sensitive taxa and fish, molluscs and oligochetes are the least sensitive taxa to this insecticide. This ranking of sensitivities based on laboratory-derived short-term LC_{50} values is generally confirmed by mesocosm studies in which the effects of organophosphate insecticides on aquatic communities have been studied: in experimental ditches treated with diazinon (Arthur et al., 1983) and chlorpyrifos (Van den Brink et al., 1996) it was demonstrated that indeed arthropods suffer most from the presence of organophosphates. The higher sensitivity of arthropods to diazinon is as expected since the individual species of this group are related to the target organisms of this insecticide. However, large differences exist also between taxonomically closely related species e.g.: *Gammarus fasciatus* is almost 1000 times more sensitive to diazinon than *Gammarus lacustris* (Sanders, 1969; Johnson and Finley, 1980). Within the aquatic insects, a difference of two orders of magnitude in LC_{50} values is noted between the most sensitive and

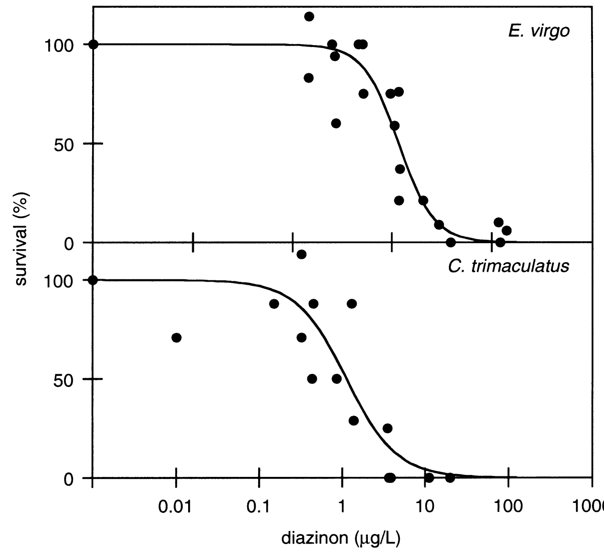

Fig. 1. Survival of 20–25-day-old *Cyrnus trimaculatus* and 1–2-day-old *Ephoron virgo* larvae after 96 h exposure to different concentrations of diazinon, plotted as percentages of the corresponding controls against measured diazinon concentrations (in µg/l). The lines represent curve-fits after Haanstra et al. (1985).

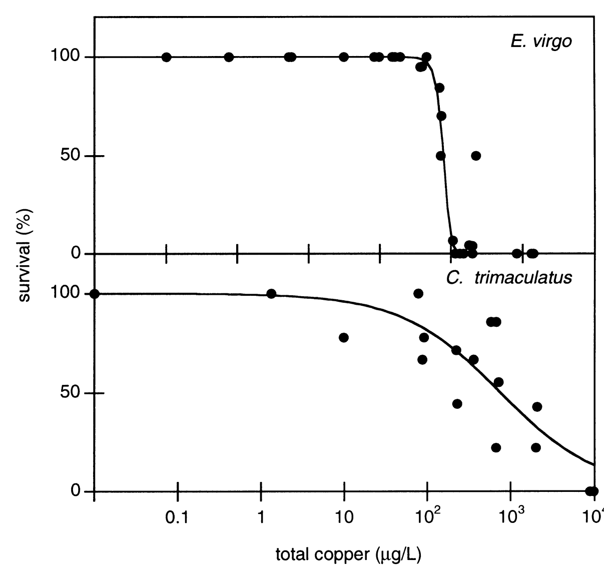

Fig. 2. Survival of 20–25-day-old *Cyrnus trimaculatus* and 1–2-day-old *Ephoron virgo* larvae after 96 h exposure to different concentrations of copper, plotted as percentages of the corresponding controls against measured total copper concentrations (in µg/l). The lines represent curve-fits after Haanstra et al. (1985).

the most tolerant species. The three insect species selected in the present project, the mayfly *Ephoron virgo*, the caddisfly *Cyrnus trimaculatus* (this study) and the caddisfly *Hydropsyche angustipennis* (Van der Geest et al., 1999), indicated in Fig. 3 by arrows, are among the most sensitive species to diazinon. The 96-h LC_{50} for *Cyrnus trimaculatus* is even lower than for any other insect species known from the literature.

Also for copper, a comparison with 96-h LC_{50} values from the literature for other aquatic taxa is made

Table 1
Ninety-six hour LC_{10} and LC_{50} values for diazinon and copper[a]

		96-h effect concentrations (μg/l)	
		Cyrnus trimaculatus	*Ephoron virgo*
Diazinon	LC_{10}	0.2 (0.1–0.6)	5.3 (3.0–9.0)
	LC_{50}	1.1 (0.7–1.7)	11.8 (9.9–14.1)
Copper	LC_{10}	38 (6–225)	61 (49–77)
	LC_{50}	759 (402–1433)	77 (71–84)

[a] Confidence limits (95%) are given in parentheses.

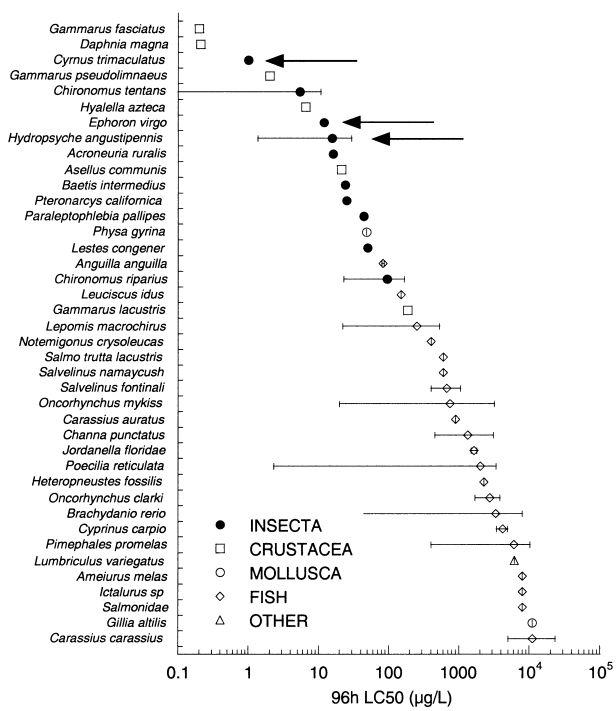

Fig. 3. Acute toxicity of diazinon (96-h LC_{50} values in μg/l) to several aquatic taxa. Data from AQUA/INFO (1997). If more than one LC_{50} value was listed in AQUA/INFO for a specific species, the average value is presented and error bars indicate the lowest and the highest reported value. The arrows indicate test organisms used in this project.

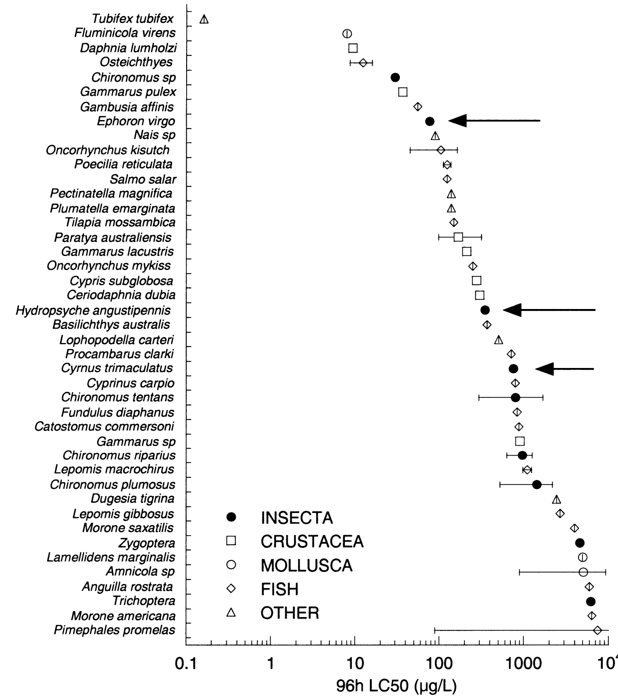

Fig. 4. Acute toxicity of copper (96-h LC_{50} values in μg/l) to several aquatic organisms. Data from AQUA/INFO (1997). If more than one LC_{50} value was listed in AQUA/INFO for a specific species, the average value is presented and error bars indicate the lowest and the highest reported value. The arrows indicate test organisms used in this project.

(Fig. 4) and again a wide variety of effect concentrations, ranging from 0.16 to 13,560 μg/l for, respectively, the oligochete *Tubifex tubifex* and the fish *Pimephales promelas*, is noted (Brungs et al., 1976; Das et al., 1993). In contrast to diazinon, however, no general classification of sensitive and tolerant taxa can be made: in all taxa, both sensitive and tolerant representatives are found. The three species selected in this project (indicated again by arrows in Fig. 4) are among the most sensitive insects, and in the middle range of all copper toxicity data.

Although comparative toxicity data as presented above provide important information on variation in responses of aquatic organisms, one must keep in mind that differences in sensitivity between species can be strongly influenced by several (a)biotic factors and that such a ranking is, therefore, to some extent speculative. One major factor influencing toxicity, for example, is the size or age of the test organism (Hutchinson et al., 1998; Legierse, 1998). For diazinon, it is demonstrated that differences between first and last instars of one species are often even bigger than differences between species (Stuijfzand, 1999). However, other factors like the exposure regime (Kallander et al., 1997), water characteristics (Bailey et al., 1997) and (bio)transformation of the parent compound (Keizer et al., 1993) can also strongly influence the response of individual species to diazinon. For copper, physicochemical and environmental water characteristics like pH (Meador, 1991), hardness (Winner and Gauss, 1986) and presence of organic matter (Meador, 1991; Pantani et al., 1995; Stuifzand, 1999) can possibly have an even stronger impact on toxicity. These modifying factors not only complicate the ranking of sensitive and tolerant species (groups) as presented in Figs. 3 and 4, but also limit the applicability of using standard test organisms as substitutes for sensitive indigenous species in, for example, bioassays. Since these responses are species specific, standard (substitute) test species may react in another way to natural environmental characteristics than indigenous species. It is, therefore, argued to use species that are representative for the ecosystem of concern and that risk assessments should be based on the sensitivity of these species.

The insect species selected in the present project were among the first that disappeared from contaminated rivers, like the River Meuse, and among the last to return after recovery takes place. Although only two model toxicants were tested, indications were found that indeed water quality hampers the distribution of these species: in the River Meuse, a more or less continuing load of low copper (average of 5 μg/l, with incidental peaks of 60 μg/l) has been noted over the past 5 years (RIWA, 1993–97), thereby exceeding the Dutch environmental standard of 3.9 μg/l (Ministry of Transport, Public Works and Water Management, 1997). The highest peak concentrations are within the effect concentration range of both species (LC_{10} of 38 and 61 μg/l copper for *Cyrnus trimaculatus* and *Ephoron virgo*, respectively). For diazinon, incidental peak concentrations (ca. 1 μg/l) in the River Meuse also exceed the Dutch environmental standard (0.04 μg/l; Ministry of Transport, Public Works and Water Management, 1997) and reach levels at which acute lethal effects on first instar *Cyrnus trimaculatus* larvae may occur (96-h LC_{10} 0.2 μg/l). In more than 15% of all measurements in the River Meuse pesticides are present (RIZA, 1996) and since peak concentrations of diazinon usually occur in combination with other organophosphates (RIWA, 1993–97; RIZA, 1996) an even higher impact may be expected than that based on diazinon toxicity alone.

The low short-term LC_{50} values of the three species selected in this study illustrate the sensitivity of these species to environmental contaminants and are in agreement with their field-observed distribution pattern. This stresses the importance of using these species in assessing the risk of environmental contaminants in large rivers.

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