Ecological quality assessment of Dutch surface waters using a new bioassay with the cladoceran *Chydorus sphaericus*

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Routine chemical monitoring gives insight in the presence of contaminants in surface waters, but not in their joint ecological effects. Therefore ecological water quality is assessed with bioassays. Recently, a new bioassay using the chydorid *Chydorus sphaericus* has been developed. Working with smaller volumes, materials and being less time consuming than the traditional *Daphnia magna* test regarding the culture and experimental design, the 'Chydotox-test' shows a comparable sensitivity. The new Chydotox-test is a promising alternative for the existing *Daphnia* sp. acute immobilisation test (OECD 1984).

Keywords: ecological water quality, bioassay, *Chydorus sphaericus*, *Daphnia magna*, toxicity

Routine chemical monitoring gives insight in the presence of contaminants in surface waters. Yet, for many compounds no reliable analytical method is available and many known compounds are not measured. The biggest limitation of chemical monitoring however is the lack of insight in the bioavailability of the present toxicants and the joint effects of mixtures of (un)known compounds on biota (Hendriks *et al.* 1994). Therefore, bioassays are deployed as a complementary tool, giving insight in biological effects, but not in their causes.

When bioassays are incorporated in ecological quality assessments, generally a battery of tests is deployed, because of species and compound specific sensitivities. The battery of short-term bioassays used by the RIVM (in cooperation with RIZA) consists of an algal photosynthetic-efficiency test (PAM-test) a bacterial test (Microtox), and three zooplankton tests, using the daphnid *Daphnia magna* (Daphnia IQ), the rotifer *Brachionus calyciflorus* (Rotox) and the crustacean *Thamnocephalus platyurus* (Thamnotox). Though the Daphnia IQ test is sensitive, it involves the use of chemicals for fluorescence measurements and the

use of relatively large test volumes due to the size of the animal. Moreover *D. magna* is not a common representative species for Dutch surface water. Therefore an additional surface water bioassay was developed and applied using the benthic Cladoceran *Chydorus sphaericus*, a useful and sensitive test species (Koivisto *et al.* 1992, Dekker *et al.* 2002, Bossuyt & Janssen 2005, Dekker *et al.* 2006).

Chydorus sphaericus is one of the most common cladocerans in The Netherlands (Fig. 1). It is a water column inhabiting and sediment dwelling species occurring in a variety of habitats (Duigan & Kovach 1991, Fryer 1995, Van de Bund & Spaas 1996), feeding mainly on detritus. The abundance of chydorids in littoral regions of freshwater lakes makes them an important component of the aquatic ecosystem (Williams 1982). They hold a key position in the food web by converting organic material into their own body mass that becomes in turn available for predators such as juvenile fish. Being cladocerans, chydorids have the same advantages as daphnids for use in experiments, such as ease of handling and parthenogenetic reproduction. Therefore *C. sphaericus* was considered a suitable species for standardized laboratory toxicity testing and recently a sediment toxicity test using this species has been developed and applied (Dekker *et al.* 2006). Departing from this sediment test, the aim of the present study was to develop and apply a surface-water toxicity test using *C. sphaericus*. One of the main objectives was to reduce the test volume to 250 µl, which would be an advantage when testing concentrates, a common practice in assessing ecological water quality using the aforementioned test battery (Struijs & de Zwart 2003). The *C. sphaericus* laboratory culture was further optimized and the developed test was validated by determining the EC_{50} for the OECD reference compound potassium dichromate ($K_2Cr_2O_7$) used in standard *D. magna* tests. This allowed us to compare the sensitivity of *C. sphaericus* with that of *Daphnia magna*, as previously performed for copper, cadmium and ammonia (Dekker *et al.* 2006).

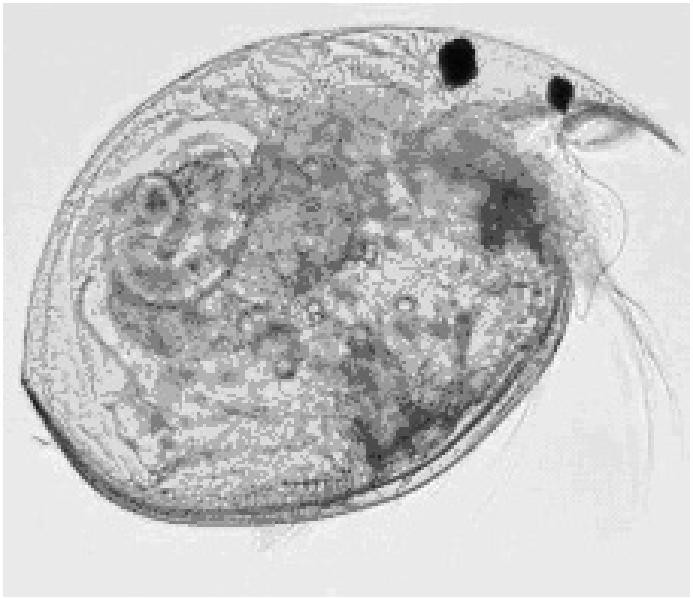

Figure 1. The benthic Cladoceran *Chydorus sphaericus*.

Finally, the bioassay was put into practice by testing series of surface water samples and it was attempted to relate the observed effects to measured toxicant concentrations.

MATERIALS AND METHODS

Chydorus sphaericus laboratory culture

The clone of *C. sphaericus* used in this study originated from one gravid female retrieved from a culture from the University of Amsterdam which was originally collected in the summer of 1998 in the Drontermeer, a eutrophic, sandy lake in The Netherlands. The animals were kept in polyethylene plastic containers filled with 100 ml of Dutch Standard Water (DSW) and about 1 g of combusted (3 h at 550°C) quartz sand (grain size 100-400 mm). Twice a week, the animals were fed 2 ml of a food suspension consisting of dried, ground nettle powder (*Urtica dioica*) from BV De Tuinen, Beverwijk, and the living diatom *Nitzschia perminuta*. This suspension was made by adding 1 g of nettle powder to 80 mL of DSW and 20 mL of concentrated *N. perminuta* culture originating from a batch culture. *N. perminuta* was batch-cultured in modified WC medium (Van der Grinten *et al.* 2005), where 6 ml of a stock solution (14.21 g/l Na_2 $SiO_3.9H_2O$) was additionally added to 3 L of WC medium. The *N. perminuta* culture was maintained at a temperature of 15°C, lightintensity of ±35 µmol/m/s and a light:dark regime of 16:8 h.

Every week, around 70% of the *C. sphaericus* culture medium was renewed, by decanting most of the medium from the container. Along with the medium, some of the animals in the culture were also removed. This partial removal prevented crowding which assured that the females continued to reproduce parthenogenetically and did not form ephippia. Every month each container was replaced by a new one that was inoculated by decanting part of the contents of an older container into it. The temperature in the culture room was maintained at 20°C and a light:dark regime of 16:8 h was applied.

Chydotox-test

The 48-h acute *C. sphaericus* test developed in this study was based on the *Daphnia* sp. acute immobilisation test (OECD 1984) and adjusted to small test volumes and to the life-history characteristics of *C. sphaericus*.

One day before the start of the experiment, adult females containing parthenogenetic eggs were collected from the culture by a mesh filter with a diameter of 250 µm and transferred into glass jars containing 200 ml of DSW medium. The jars were placed overnight in a climate room under the same culture conditions (see above). The next day, newborn neonates (<24 h) were collected by a mesh filter with a diameter of 250 µm and used for the experiments. The tests were carried out in small 2 mL HPLC vials to which 250 µl of test medium and five neonates were added. Potassium dichromate (CAS: 7778-50-9)

was chosen as model toxicant because it is recommended as a reference compound by the OECD for the *Daphnia* sp. acute immobilization test (OECD 1984) and consequently, a large set of toxicity data is available for this compound. Exposure consisted of nine different nominal concentrations in triplicate: control, 0.032, 0.1, 0.32, 0.56, 1, 1.8, 3.2 and 10.0 mg/L. Next, five <24 h neonates were added to a single droplet of DSW with a diameter of approximately 5 mm and subsequently transferred to the 250 µl test medium in the vials with a pipette. A pilot experiment with 45 vials showed that the neonates containing droplet increased the 250 µl test solution by 5.8% (SD = 1.6) and therefore slightly decreased the nominal exposure concentrations. The vials were covered with a lid to prevent evaporation and incubated for 48 h under the same conditions as the culture (see above). After 48 h the vials were placed under a reverse dissecting microscope and immobilization was determined by activation of the animals by slightly shaking the vial and monitoring them for 30 sec. Immobilization was plotted against the nominal total potassium dichromate concentration in the water. From this dose-response relationship, the EC_{50} value and its corresponding 95% confidence limits were calculated using the log-logistic curve fitting procedure of Haanstra *et al.* (1985).

Surface water tests

In 2005 routine chemical and biological monitoring was performed by the RIZA at different locations in the river Meuse. At each location 334 physical and chemical parameters (*e.g.* nutrients, metals, pesticides, PAC's and PCB's) were measured. In addition, the effect parameter acetylcholinesterase-inhibition was determined by measuring enzymatic responses of combinations of coumpounds (*e.g.* carbamates and organofosfates) with specific mechanisms of action. Water samples from the location 'Eijsden' were used to investigate the applicability of the Chydotox-test for water quality assessment. Since toxicants in Dutch surface waters rarely reach lethal concentrations, a concentration technique (Collombon 1997) for the water samples was adopted by the RIVM. Following this procedure, water samples were concentrated a thousand-fold by extracting all (organic) micro-pollutants with XAD-resins and subsequently resolving the pollutants in a smaller water volume. This XAD-concentrate can be diluted afterwards to desired test concentrations. Due to the concentration technique, confounding abiotic environmental factors such as ammonium, pH, humic acids and salinity are also circumvented.

RESULTS

Figure 2 presents the 48 h dose-response relationship for *C. sphaericus* exposed to potassium dichromate. No mortality occurred in the controls and a clear dose-response relationship was obtained, resulting in an EC_{50} value (R^2 = 0.88) of 780 µg/l (95% CI: 580-980).

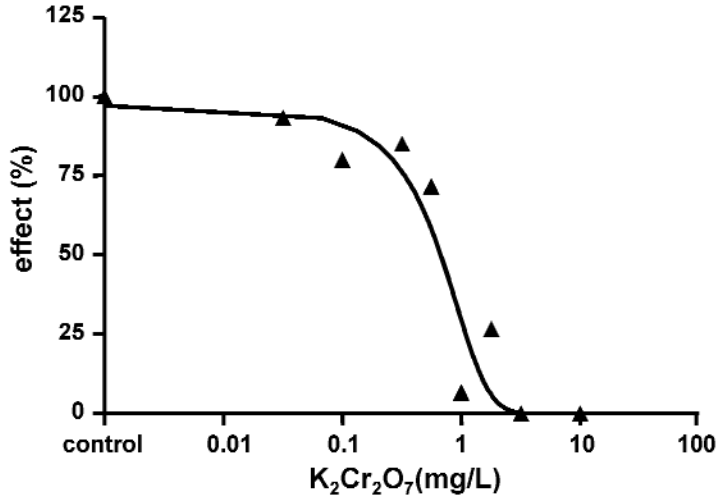

Figure 2. 48 h dose-response relationship for *Chydorus sphaericus* exposed to potassium dichromate, using immobilisation as the effect parameter.

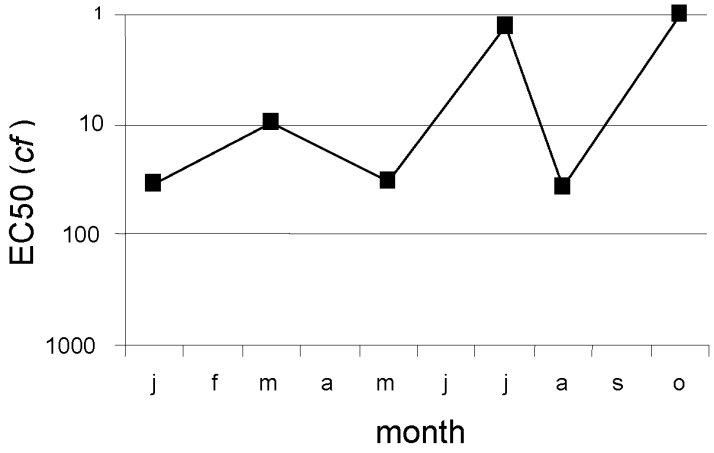

Figure 3. Time series of the toxicity of water samples from the river Meuse at Eijsden in 2005. Toxicity is expressed as the concentration factor (*cf*) of the surface water at which 50% of the exposed animals show an effect: *cf* = 1000 is concentrated a thousand-fold. The lower the *cf* the more toxic the sample.

The toxicity of the concentrated water samples from the Meuse measured with the Chydotox-tests is shown in Figure 3. Toxicity is expressed as the concentration factor (*cf*) of the surface water at which 50% of the exposed animals show an effect. Thus the lower the *cf* needed to obtain 50% effect, the higher the toxicity of the samples. Acute effects were demonstrated in the water samples from the location Eijsden in the *cf* range between 1 and 10. In july and october EC_{50} values even reached the *cf* value of 1.

Analytical-chemical measurements performed by RIZA showed that at location Eijsden the Maximum Permissible Concentrations (MPC) threshold levels for acetylcholinesterase-inhibition were exceeded in March, July and October. Plotting these data together with the results of the Chydotox-test (Fig. 4) clearly shows that when concentrations of acetylcholinesterase-inhibiters are high, toxicity to the chydorids was also high.

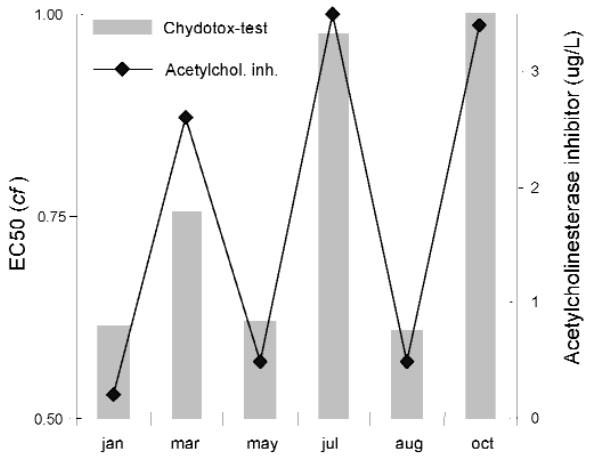

Figure 4. Results of the Chydotox-test and acetylcholinesterase-inhibition at location Eijsden in the river Meuse in 2005.

DISCUSSION

This study reports on the development and application of a newly developed acute toxicity test for surface water using the benthic cladoceran *C. sphaericus*, as an alternative for the standard *Daphnia* sp. acute immobilization test. A *C. sphaericus* laboratory culture was started and its performance under control conditions was optimized. The test was firstly validated by determining the dose-response relationship for potassium dichromate, showing a 48 h EC_{50} value for *C. sphaericus* (780 µg/l) within the range of toxicity data found in literature for *Daphnia magna* (average: 400 µg/l; range: 20-2700 µg/l; *n* = 38 (http://www.epa.gov)). It is concluded that for this OECD recommended reference compound *C. sphaericus* and *D. magna* are equally sensitive.

Using the Chydotox-test we monitored the toxicity of surface waters over time (Fig. 3). This was only possible due to the extremely small test volumes used by the Chydotox-test (250 µl), as the concentration technique results in very small sample volumes. Standard *Daphnia* tests require much larger volumes hampering the assessment of toxicity of such concentrated samples. Figure 4 also demonstrated that, despite the markedly improved water quality of Dutch surface waters in the past decade, fully diluted concentrates from the river Meuse may occasionally still be acutely toxic to macroinvertebrates.

Routine chemical monitoring gives insight in the presence of some contaminants in surface waters, but not in the joint ecotoxicological effects of all contaminants present in the water. The benefit of using bioassays for testing of surface waters is that the biological effects of (un)known (mixtures of) compounds are assessed. However, only 11% of the toxicity of XAD-concentrates from field samples could be attributed to the measured compounds, for which EC_{50} values

were obtained from databases (Hendriks *et al.* 1994). This lack of causality is expected to be caused by identified compounds for which no effect concentrations are available and from compounds that could not be identified (Lahr *et al.* 2003). In the present study, however, toxicity to *C. sphaericus* could be partly attributed to acetylcholinesterase-inhibition, assuming all cladocerans like *D. magna* show acetylcholinesterase-inhibition (Guilhermino *et al.* 2000). The specific compounds still need to be identified. Based on the results of the present study it is concluded that the newly developed Chydotox-test is a promising alternative for the existing *Daphnia* sp. acute immobilisation test (OECD 1984).

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