The role of sediment characteristics and food regimen in a toxicity test with *Chironomus riparius*

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by

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Abstract

Toxicity tests using standardised artificial sediments are a useful tool to evaluate toxicity of pesticides to benthic organisms. Artificial sediments differ from natural sediments in physico-chemical characteristics and require feeding of test organisms, which will likely alter the exposure of sediment-associated contaminants and thus the outcome of the test. To determine the potential change in bioavailability, due to sediment physico-chemical characteristics and addition of exogenous food, between artificial and natural sediments, a microcosm study was performed using a standardised methology with the midge-larvae *Chironomus riparius*. The toxicity of the pesticide lindane was compared in artificial and two natural sediments with the same organic matter content under fed and unfed conditions. Also, feeding rates for *C. riparius* larvae were determined in two of the test sediments.

Neither sediment type (artificial vs. natural) nor the addition of food had effect on the toxicity of lindane as determined by emergence, mean development rate and survival. This shows that the *Chironomus* test is a robust test that provides a good estimate of contaminant toxicity in natural sediments, despite marked differences in sediment characteristics (e.g. N content, density and water content). However, differences in the lindane concentrations of the overlying water indicated that differences existed in the exposure concentrations of the larvae. By the end of the experiment the concentration of lindane in the overlying water was lower in the microcosms with natural sediments compared with microcosms with artificial sediment. The study also showed that the nutritional value of the artificial sediment is lower compared with the natural sediments despite equal amounts of organic matter. These differences could be due to difficulties for the larvae to explore the artificial sediment due to the realtively high density of the artificial sediment and/or the lower nutritional value of the organic matter pool.

Introduction

The use of pesticides in agriculture and other pest controlling activities (e.g. controlling of parasites and vectors of diseases) may lead to contamination of the aquatic ecosystems. The pesticides can enter streams and lakes either "accidentally" by surface run-off, groundwater leaching and precipitation while applying on land, or directly, when controlling pests in the water (e.g. tributyl-tin). Many of these substances are hydrophobic and thus strongly associates with particles which eventually reach the lake sediments by sedimentation. As a result, the sediment concentrations of pollutants can exceed by several orders of magnitude those in the water column. In the 1970s the ecological concern about contamination of the sediments resulted in development of toxicity tests using pelagic aquatic species (e.g. *Daphnia* sp.). The use of toxicity test with bentic organisms in environmental hazard assessment started some years later and has resulted in a variety of different tests and methods (Burton 1991, for review see Traunspurger and Drews 1996).

An important key to sediment toxicity assessment is bioavailability. Although sediments may contain high concentrations of toxic compounds, this condition does not necessarily lead to adverse effect on sediment-dwelling organisms living in the sediment. Bioavailability is dependent on several abiotic (e.g. physico-chemical characteristics of the sediment and the contaminant) and biotic (e.g. test organism and feeding habits) factors controlling the distribution pattern within the sediment compartments (Adams 1984, Power and Chapman 1992). Among the abiotic factors the amount of organic matter in the sediment has been widely accepted as the primary factor controlling the bioavailability of nonionic organic compounds (Di Toro et al. 1991). A higher organic matter content in the sediments increases the sorption of organic compounds to the organic particles and makes them less available. Though organic matter content has been used as a normalisation factor for the bioavailability of contaminants in sediments, other abiotic factors (e.g. organic matter composition and particle size distribution) has been shown to affect bioavailability (Suedel et al. 1993, Kukkonen and Landrum 1996). As mentioned, bioavailability is also dependent on biotic factors like feeding habits of the organism. Bentic organisms can accumulate contaminants through (i) uptake from pore water and overlying water across body walls and across respiratory surfaces, and (ii) through ingestion of contaminated sediment particles (Knesovitch et al. 1987, Power and Chapman 1992). The relative importance of these routes depends both on the partitioning of the contaminant over the different compartments in the sediment (e.g. inorganic particles, organic matter and interstitial water) and the feeding habits of the organism (e.g. food source and feeding rate) (Leppänen 1995). For example, selective feeding on sediment particles with a high concentration of contaminant increases its bioavailability, as shown by Harkey et al. (1994 b) with *Diporeia* spp..

Streloke and Köpp (1995) evaluated an international ring test (interlaboratory study) for a long-term toxicity test with the dipterian larvae *Chironomus riparius*. The purposes of the new test system were to establish a reliable and reproducible toxicity test for pesticides in sediments using a standardised artificial sediment (i.e. sediment that has been constructed in the laboratory) and microcosms. The artificial sediment consists of sand (70% by dry weight), kaolin clay (20%) and pulverised peat (10%) (OECD 1994). The major advantages of using an artificial sediment in a laboratory-based test is the reproducibility, the absence of indigenous fauna and possible contaminants (Suedel and Rodgers 1994). However, these advantages should be balanced against the "realism" of an artificial sediment. When developing the *Chironomus* toxicity test, Hamer (1995) compared the toxicity (i.e. bioavailability) of two pesticides in the artificial sediment and in a set of different natural sediments and found that the toxicity was "sufficiently similar" in all sediments despite a wide range of particle sizes and organic matter contents among the sediments. Despite these similarities of toxicity among sediment types found by Hamer (1995), the study showed large variability that could be explained by differences in physico-chemical characteristics of the sediments and thus differences in bioavailability (see above). It is therefore important to know to what extent the artificial sediment reflects natural sediments in terms of sediment physico-chemical characteristics. For example, Goedkoop and Johnson (1997) observed that the *Chironomus* larvae only explored the top 2-5 mm of the artificial sediment and attributed this to the high density of the sediment.

Peat, used in the *Chironomus* test as the organic matter constituent, is a nutrient poor substrate (high C/N-ratio) which requires food addition during the test to assure proper larval development. *C. riparius* is a sediment-dwelling midge whose larvae feed on surficial

sediment and suspended matter. Food addition in toxicity test with *Chironomus* sp. has been shown to be crucial for both natural and artificial sediments for obtaining sufficient survival of non-exposed larvae (Ankley et al. 1994, Suedel and Rodgers 1994 a and b, Naylor and Rodrigues 1995). However, addition of uncontaminated food might alter the exposure of sediment-associated contaminants to organisms ingesting sediment (Harkey et al. 1994 a). A toxicity test without or with minimum food addition may thus be a better estimate of the potential toxicity of pesticides to the organism (Burton et al. 1992). Change in larval behaviour due to the dense artificial sediment and the addition of food may result in a exposure condition far from natural.

The objectives of this study were to determine how sediment characteristics (e.g. organic matter composition and density), other than organic matter content, and food addition affected the bioavailability of pesticides in a standardised toxicity test. This was done by selecting two natural sediments with the same organic matter content as the artificial sediment but with different physico-chemical characteristics and by performing a set of standardised toxicity tests with *C. riparius* with and without food additions. The contaminant used in this study was lindane. Lindane is an organochlorine insecticide with a log K_{ow} of 3.7, which means that is has a strong sorption capacity to sediments (Saleh et al. 1982). The toxic route of lindane is through the nervous system and the symptoms after exposure are hyperactivity followed by paralysis and death. Additionally, differences in the suitability of natural and artificial sediments to serve as food was investigated by determining the feeding rate of *C. riparius*.

Materials and methods

Test organisms

Chironomus riparius larvae were cultured in a laboratory culture at room temperature with a photoperiod of 16:8 (light:dark). Larvae were fed with Tetraphyll® (TetraWerke, Germany), added to the overlying water. The container was placed in a cage, allowing adult midges to swarm and produce eggmasses.

Chemicals

^{14}C lindane (γ-hexachlorocyclo-hexane, specific activity 3.81 Mbq/mg, Internationale Isotope München) dissolved in acetone (pesticide grade) was used as contaminant. Scintillation cocktail (OptiPhase 'highsafe' 2) and internal standards (^{14}C-W standards) were obtained from Wallac OY (Finland).

Sediment preparations

Artificial sediment was prepared according to OECD-guideline 207 (OECD 1994) by mixing dry ingredients consisting of seasand (Merck, > 50 µm), kaolin clay (KEBO lab), finely pulverised peat (Original solmull®, Hasselfors Garden AB, <150 µm), and $CaCO_3$ (*p.a.*, Merck). The relative contribution of the ingredients was 68.3, 19.7, 10.3 and 1.7% of the total dry weight, respectively. The sand fraction used in this experiment was slightly coarser than outlined by the OECD guideline which requires that more than 50% of the sand fraction should be < 200 µm. Drying of the ingredients were conducted at 105˚C for 24 hours, except for peat and kaolin clay which were dried at 40˚C until constant weight was achieved. Oxygen-saturated M7-medium (a water medium prepared according to Heimbach, 1995) was added to dried ingredients and the slurry was thoroughly mixed. The sediment was kept in a refrigerator (4˚C) for 9 days to let the dry ingredients absorb the medium.

The natural sediments were collected in Lake Erken, a mesotrophic lake situated 60 km NNE of Stockholm. Surficial sediment was collected during late February at two profundal and one littoral sampling station. At 12 and 16 meters depth, the top two centimetres were collected with an Ekman sampler by removing the overlying water with a suction-device and carefully collecting the sediment with a spoon. At the littoral sampling station (5.5 m), the hole grab was collected. The sediments were sieved (0.5 mm) to remove coarse detritus and macrofauna. The sieved residue mainly consisted of *Gloeotrichia echinulata* colonies. Sieved sediments were then frozen (48 h, -20˚C) to kill ambient meio- and microfauna and later on thawed at 4˚C prior to the start of the experiment.

Two sediments with an identical organic content as the artificial sediment were prepared by different mixing procedures: (i) mixing sediment from 16 m with sediment from 5.5 m

Table 1. Physico-chemical characteristics of the three experimental sediments and the added food (Tetraphyll®) used in the study. Values are means ± standard deviations with number of replicates (n) in parenthesis.

Sediment type	Loss on ignition (% dw)	C content (%dw)	N content (%dw)	C/N	Water content (%)	Density (g/cm^3)
Artificial (A)*	11.5 ± 0.1 (n=3)	5.73 ± 0.11 (n=3)	.12 ± 0.01 (n=3)	48.6 ±0.6 (n=3)	49.6 ± 0.2 (n=3)	1.42 ± 0.01 (n=3)
Natural 1 (N1)**	10.9 ± 0.4 (n=5)	6.06 ± 0.72 (n=5)	0.84 ± 0.11 (n=5)	7.2 ± 0.1 (n=5)	76.5 ± 2.8 (n=5)	1.16 ± 0.02 (n=4)
Natural 2 (N2)***	10.0 ± 1.3 (n=4)	4.80 ± 0.58 (n=4)	0.67 ± 0.08 (n=4)	7.2 ± 0.1 (n=4)	74.1 ± 1.9 (n=4)	1.18 (n=1)
Tetraphyll®	-	44.34 ± 0.12 (n=2)	7.79 ± 0.01 (n=2)	5.7 ± 0.1 (n=2)	-	-

* Prepared according to OECD-guideline 207
** Prepared by mixing sediment from 16 meters depth with sediment from 5.5 meters depth
*** Prepared by mixing sediment from 12 meters depth with sand (> 50 µm)
dw = dry weight

at a ratio of 2.4:1, and (ii) mixing sediment from 12 m with sand (> 50 µm) at a ratio of 17.9:1. The two sediments are here referred to as the N1-sediment and N2-sediment, respectively.

Loss on ignition was determined after combustion in a Nabertherm® oven (Mod N54E) at 550˚C for 2 h (SS, 028113 1981). C and N contents were determined after combustion in an Elemental analyser (Carlo Erba Strumentazione, Mod. 1106). Density was determined with density-bowls (SS, 028113 1981). Physico-chemical characteristics of the three sediments are listed in table 1.

The two natural sediments (N1 and N2) were quite similar with respect to all physico-chemical characteristics, but differed markedly from the artificial sediment with respect to N content, water content and density. All three sediments had similar loss on ignition values and C contents but the natural sediments showed a factor of seven (N1) to six (N2) times higher N content than the artificial sediment, resulting in a higher C/N-ratio for the artificial sediment. The C/N-ratio of the natural sediments were similar to that of the added food. The water content of the artificial sediment was substantially lower than that of the N1 and N2 sediments. Consequently, the artificial sediment had a higher density than the N1 and N2 sediments.

Effects of sediment type and food addition on bioavailability

A three-way factorial design was used to study the effects of sediment type and food addition on bioavailability (i.e. toxicity) (figure 1). Three different sediments (here referred as sediment types) with the same organic matter content (10 % loss on ignition) were prepared (see above) and experiments were run with and without food additions (fed vs. unfed) at 4 concentrations of lindane. Each treatment was performed with four replicates. The experimental endpoints were emergence, mean development rate, larval survival and length of the surviving larvae after 33 days of incubation. Emergence was calculated as the sum of emerged adult midges per microcosms divided by the number of larvae introduced. Mean development time represents the mean time span between application of the test substance (day 0) and the emergence of the experimental cohort of midges. The mean development rate is the reciprocal of the development time (unit: day^{-1}) and represents the portion of larval development which takes place per day. Furthermore, survival was calculated as the sum of emerged adult midges and surviving larvae divided by the number of larvae introduced.

The contaminant concentration in the overlying water was also quantified in order to investigate if there were any differences in contaminant distribution among the sediment types and between food regimen. Determination of lindane concentrations in the overlying water were performed by scintillation counting (Beckman Ls 6000TA) after addition of 10 ml of scintillation cocktail to 1 ml of the water samples. Internal standards were used to correct for quenching. The mean background radiation (controls) was subtracted from the lindane-

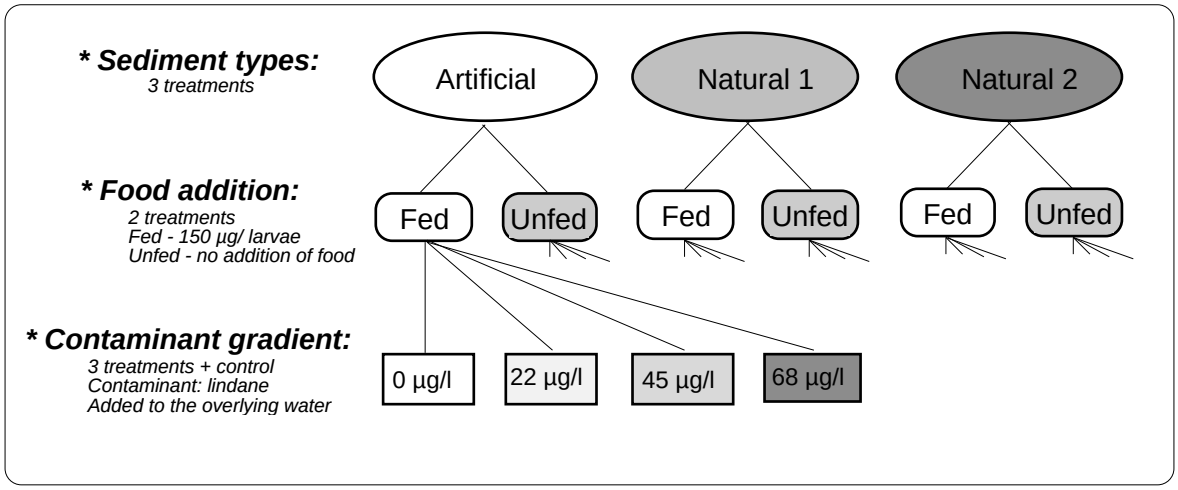

Figure 1. Experimental design.

treated microcosms. The contribution of lindane metabolites to the measured radioactivity was not quantified.

The experimental setup used was similar to that used in the International Toxicity Ring-test (Streloke and Köpp 1995) with the following three modifications: (i) glass beakers with a volume of (175 ml and a bottom area of 28 cm^2 were used instead of 2-liter vessels, (ii) aeration was more intense (approximately 3x), and (iii) the larvae were fed 150 µg Tetraphyll® individual^{-1} d^{-1} instead of 1 mg individual^{-1} and d^{-1}. Goedkoop and Johnson (1997) showed that this food regimen was sufficient for an optimal development and survival of the control larvae.

The experimental vessels were filled with an approximately 1-cm thick layer of sediment, corresponding to 30.1 ± 0.1 g (wet weight) of artificial sediment, 24.7 ± 0.4 of the N1-sediment and 25.1 ± 0.3 g of the N2-sediment. Aliquots of each sediment were randomly taken for analysis of density, loss on ignition, carbon-, nitrogen- and water content. The microcosms were then placed in a freezer (-20˚C) for 7 h. This was done to prevent resuspension and stratification of the sediment upon the addition of the M7-medium. 150 ml cold and well aerated M7-medium was added and the microcosms were placed in a climate room (19.4 (0.1˚C) with a photoperiod of 16:8 h (light:dark) and aeration was turned on. Each microcosm was aerated individually by a capillary tubing (i.d. 0.76 mm) and a syringe fixed in the centre of the microcosm lid with the end approximately 5 cm above the sediment surface (figure 2). Aquarium pumps were used for aeration 15 minutes for every half hour with a bubble frequency of approximately 6 bubbles s^{-1}. The microcosms were allowed to acclimate for 9 days.

On day -4 (i.e. four days before the addition of lindane), four newly laid eggmasses from the *C. riparius* laboratory culture were transferred to Petri dishes filled with well aerated water from the culture and allowed to hatch. On day -1, 150 µg individual^{-1} (600 µg Tetraphyll®/microcosm) of food was added to the microcosms with food addition and four randomly selected larvae were transferred to each microcosm with a micropipette. This larval density corresponded to 2000 ind. m^{-2}, in accordance with the international ring test methodology. Aeration was switched off over night (18 h) to allow larvae to settle. Larvae in treatments with food additions were fed 150 µg individual^{-1} d^{-1} three times weekly by applying 0.5 ml food suspension consisting of dried and pulverised Tetraphyll® in deionized water. Microcosms without food addition received an equal volume of deionized water. During food additions the aeration was turned on to assure appropriate mixing.

One day after the addition of larvae (day 0) lindane was added to the overlying water of the microcosms. 30 µl of lindane stock solutions were added to obtain final concentrations of 22, 45 and 68 µg/l lindane as initial concentrations in the overlying water. 30 µl of each lindane solution were pipetted directly into scintillation vials for determination of the lindane addition by liquid scintillation counting. The controls (no lindane additions) received 30 µl of acetone (vehicle). 1 ml of the overlying water of each microcosm was transferred to scintillation vials at 0.2 (5 h), 3.2 (77 h), 9, 20 and 30 days after lindane application for determination of the lindane concentration in the overlying water.

Aeration was checked and temperature

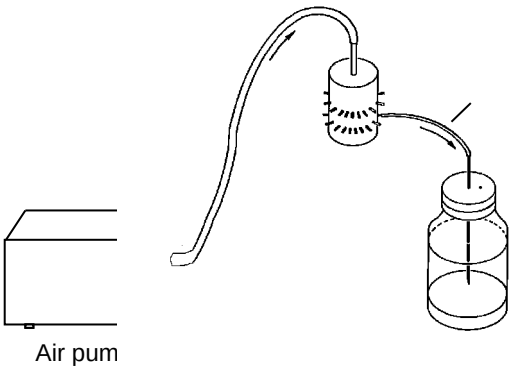

Figure 2. Experimental set-up.

was scored on a daily basis during the whole experiment. Oxygen concentration and pH of the overlying water were measured on day 11 and day 25 in the controls (dissolved oxygen meter, YSI model 51B and MeterLab PHM210 were used for the two measurements, respectively). Starting on day 10, daily checks on *Chironomus* emergence were made. Adult midges and their exuviae were removed and sex was scored. At the termination of the experiment (day 33), the aeration was switched off and sediments were examined for surviving larvae by sieving (0.5 mm meshsize). Surviving larvae were scored and preserved in 70 % ethanol for later measurements of body length. Body length measurements (from the labrum to end of the feet of the last segment) were carried out using a binocular microscope with a precision of 0.25 mm.

Chironomus feeding activity

Larval feeding activity in artificial sediment and natural sediment (N1, see above) was compared by determining the gut passage time of *C. riparius* larvae of the last larval development stage (4th-instar). This was done by marking these sediments with fluorescent particles according to Johnson et al. (1989). Johnson et al. (1989) determined the gut passage time for *Chironomus plumosus* larvae in a natural sediment to be 1.5 hours (at 19(C). Pilot studies had indicated a somewhat longer gut passage time for *C. riparius*. Each treatment was done with 7 replicates (1 larva per replicate) and the experiment was conducted in light.

Glass scintillation vials (20 cm^3, inner diameter of 2.5 cm) were filled with 5 g (wet weight) of well-aerated sediment and frozen (4 h) to prevent resuspension of sediment upon medium addition (see above). 12 ml of cold (4˚C) oxygen-saturated M7-medium was added to each vial and the microcosms were placed in a climate room (19˚C). Aeration (as above) was switched on and all microcosms were allowed to acclimate for three days. One larvae from a life-stage synchronised culture (20˚C) was added to each microcosm 26 h before the start of the experiment to allow the larvae to acclimate (e.g. burrow and construct a tube). Additional sediment from both sediments was marked with fluorescent particles (0,1 % dry weight/wet weight, 2-7 µm, sp gr 1.4; Radiant Color, Richmond, California). At the start of the experiment 0.5 g of marked sediment was added to the overlying water of the microcosms, forming a surficial sediment layer. The N1 sediment was added with a 5 ml pipette and the more dense artificial sediment was added with modified 10 ml syringes which were pre-filled with approximately 0.3 ml of the sediment to facilitate the addition.

The larvae were sacrificed after 20, 40, 100, 140, 180, and 220 min., respectively. The overlying water was removed with a pipette and approximately 5 ml of CO_2-saturated water was added to anaesthetise the larvae and prevent sediment regurgitation (Johnson et al. 1989). Microcosms were then preserved by adding 5 ml of formalin (4%). *Chironomus* alimentary tracts enclosed in visceral tissue were removed under a microscope and fixed in Euparal® on microscope slides. Larvae with empty guts and larvae not containing any marked sediment were excluded from the analysis. These individuals were likely to be pre-pupae that had stopped feeding prior to emergence. The length of the gut containing marked sediment and the total gut length were measured with an epifluorescence microscope (100x magnification) equipped with a measuring eyepiece.

Statistical analyses

Emergence data were arcsin-transformed prior to statistical analysis. Data of the lindane concentration in the overlying water were log-transformed prior to statistical analysis. Data were analysed using ANOVA with (α set at 0.05) and Scheffé's post hoc tests for pairwise comparisons. Treatments with and without food additions were analysed separately using two-way ANOVA for the effects of sediment type (A, N1 and N2) on experimental endpoints. Two-way ANOVA were also used to analyse effects of food additions within each sediment treatment. Differences in lindane concentrations of the overlying water were

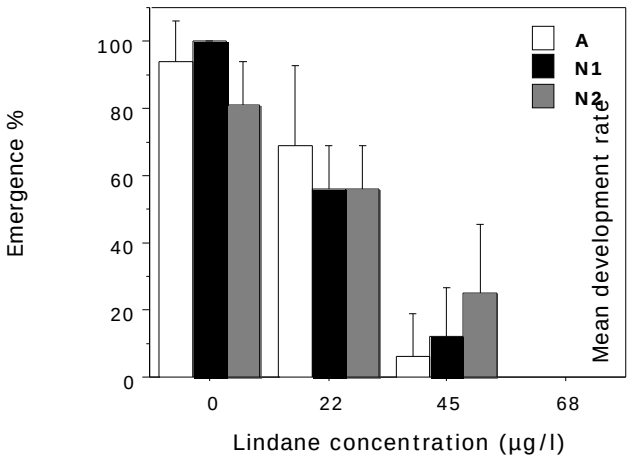

Figure 3. Chironomus emergence as percent of total number of introduced larvae (mean + S.D., n=4) in microcosms with food addition at different concentrations of lindane. Microcosms contained artificial (A) or natural (N1 and N2) sediment.

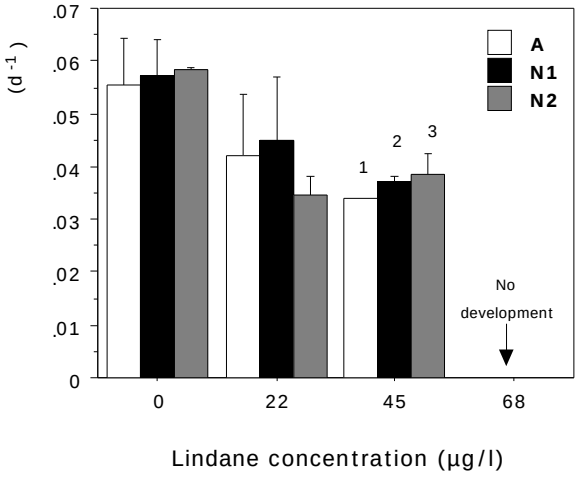

Figure 4. Mean development rate per day (mean + S.D.) of emerged midges in microcosms with food addition at different concentrations of lindane. Microcosms contained artificial (A) or natural (N1 and N2) sediment. Numbers over bars indicate the number of replicates. If no number n=4.

analysed in the 22 µg/l lindane treatments by two-way ANOVA.

Differences in larval length between treatments were analysed using Mann-Whitney *U*-test by comparing the length of surviving larvae in the treatments with 0 and 22 µg/l lindane without food addition. These treatments had low emergence and high survival and thus a sufficient amount of surviving larvae for statistical analysis by Mann-Whitney *U*-test.

Results

Effects of sediment type and food addition on bioavailability

Neither emergence nor mean development rate were affected by sediment type in fed treatments (Two-way ANOVA, $P > 0.05$ for both) and no significant interaction was found between sediment type and lindane treatment ($P > 0.05$ for both) (figures 3 and 4). As expected, emergence and mean development rate successively decreased with increasing lindane concentrations (Two way ANOVA, $P < 0.0001$ for both). No emergence occurred in the unfed treatments, whereas emergence in the controls with food additions consistently was 100% in N1, 81.3 ± 12.5% in N2 and 93.4 ± 12.5% in the artificial sediment. No emergence occurred in the treatments with the highest lindane concentration (68 µg/l). The mean development rate in the controls was similar for all three sediments, ranging 0.055 - 0.058 d^{-1}.

Chironomus emergence was significantly affected by addition of food in all sediments (Two-way ANOVA, $P < 0.0001$ in all sediments). Food addition also showed a significant interaction with lindane concentration ($P < 0.0001$).

Survival was not affected by sediment type (Two-way ANOVA, $P > 0.05$ for both fed and unfed treatments) and no significant interaction was found between sediment type and lindane concentration. As expected, lindane concentration significantly affected survival in both unfed and fed treatments (Two-way ANOVA, $P<0.0001$) and survival successively decreased with increasing lindane concentration (figure 5). Survival in the controls ranged from 100 to 50% and survival in the 68 µg/l lindane treatment ranged from 50% to 0%, all sediments and both fed and unfed treatments included.

Food addition had a significant effect on survival only in the artificial sediment (Two-way ANOVA, $P = 0.0482$) (figure 5). Contrast test revealed significantly higher survival in the fed treatments of the artificial sediment than in unfed treatments (Scheffé's, $P = 0.0482$). Mean survival in the control containing artificial sediment was 93.8 ± 12.5% in fed treatments and 68.8 ± 12.5% in unfed treatments.

Sediment type significantly affected the body length of surviving larvae in the controls and the 22 µg/l lindane treatments without food additions (figure 6). Larvae in the artificial sediment were smaller than larvae in the N1 sediment (Mann-Whitney *U*-test, $P = 0.04$ and

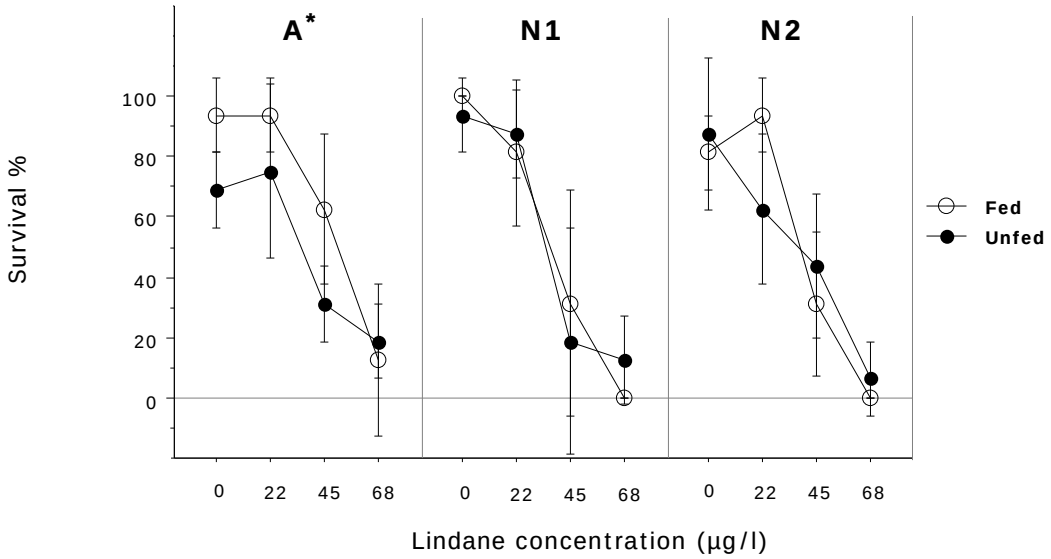

*Figure 5. Effect of food additions on Chironomus survival (mean ± S.D.) in artificial sediment (A) and in two natural sediments (N1, N2) at different concentrations of lindane. * indicates significant effect of feeding (two-way ANOVA, P < 0.05).*

P = 0.02 for the control and 22 µg/l treatment, respectively). Mean larval length per replicate in controls ranged from 11.0 to 9.7 mm in the artificial sediment and from 13.1 to 10.1 mm and from 12.6 to 9.8 mm in the N1 and N2 sediments, respectively. No difference in body length was found between larvae from the artificial sediment and the N2 sediment (P > 0.05). Larvae from the N2 sediment were shorter than larvae from the N1 sediment in the 22 µg/l lindane treatment (P = 0.02).

Control microcosms with food additions of all sediments fulfilled the test criteria according to guideline of the International toxicity ring test (Streloke and Köpp 1995) with an emergence greater or equal to 70 % and a mean development rate greater than 0.05. Oxygen concentrations in overlying water varied between 7.9 to 8.3 mg/L and the pH ranged between 7.3 to 8.0 during the experiment. Experimental temperature was 19.4 ± 0.1°C. Visual observations of the sediments in the microcosms revealed that the larvae burrowed deeper in the natural sediments than in the artificial sediment. The surface of the natural sediments appeared homogenous, while the surface of the artificial sediment was stratified with a fluffy layer on the top, most likely consisting of organic matter used by the larvae for tube construction.

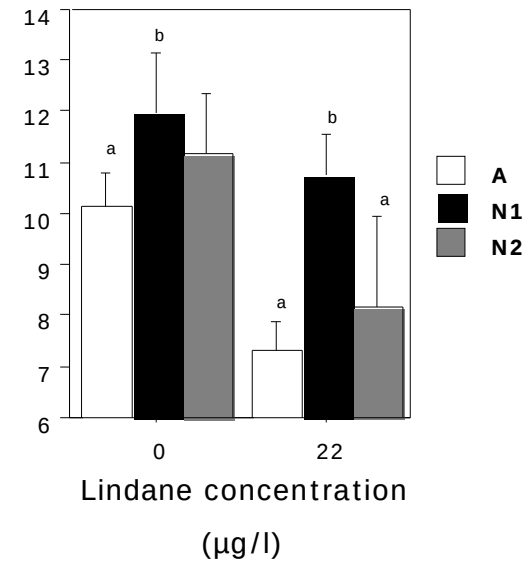

Figure 6. Length (mean + S.D.) of surviving larvae in microcosms with artificial (A) or natural (N1, N2) sediments and without food additions. Letters over bars indicate significant differences in larvae length between microcosms with different sediments within each lindane concentration (Mann-Whitney U-test, P < 0.05).

Lindane concentration in overlying water

Lindane concentration in the overlying water was significantly affected by sediment type in both fed and unfed treatments (two-way repeated measures ANOVA, P < 0.0001 for fed and P < 0.0069 for unfed treatments) and, as expected, also by time (P < 0.0001 for both fed and unfed treatments). After t = 0.2 days, the lindane concentrations were 17.1 ± 0.3 µg/l (corresponding to 78% of the added amount), 17.8 ± 0.5 (81%) and 17.7 ± 0.1 µg/l (80%) for the fed treatments of the artficial, N1 and N2

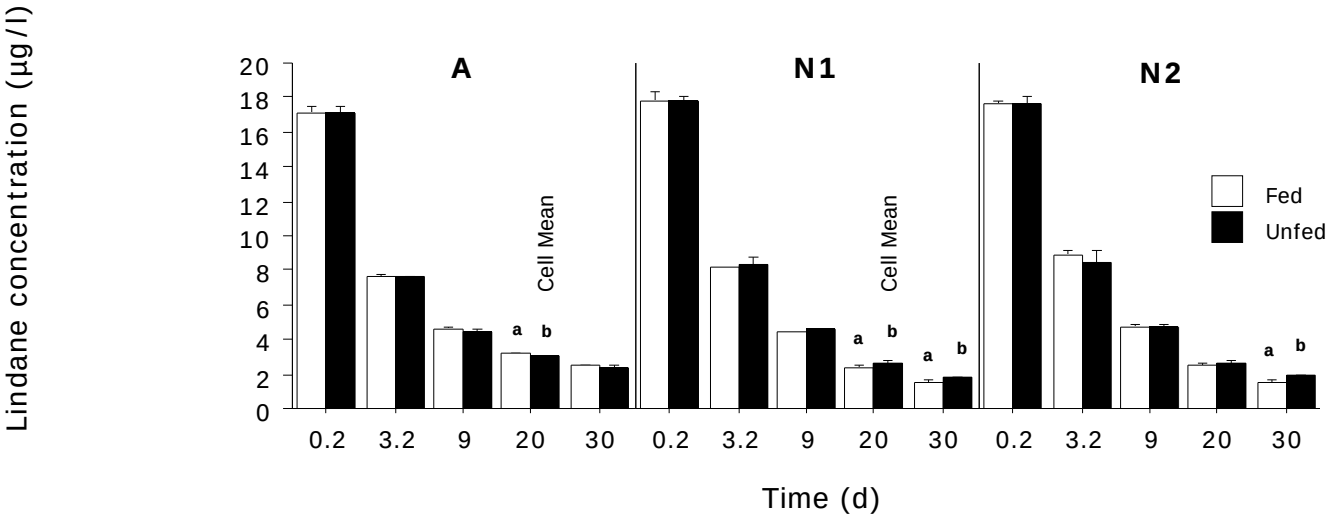

Figure 7. Concentration of lindane in overlying water over time (mean + S.D.) in fed and unfed treatments with an initial lindane concentration of 22 µg/l. Letter codes indicate significant differences between artificial (A) and two natural (N1 and N2) sediments within each time interval (Scheffé's, P < 0.05).

sediments, respectively. Lindane concentrations decreased exponentially over time and at the end of the experiment (t = 30 d) the lindane concentrations were 2.4 ± 0.1 (11%, artificial sediment), 1.6 ± 0.1 (7%, N1 sediment) and 1.6 ± 0.1 µg/l (7%, N2 sediment). A similar decrease was found in the unfed treatments. Effect of sediment type showed a significant interaction with time in both fed and unfed treatments (P < 0.0001 for both). ANOVA analysis (one-way) within each time interval and subsequent pairwise comparison between sediment types showed significantly higher lindane concentrations in the overlying water of the artificial sediment at t = 20 and 30 days compared with the two natural sediments (figure 7). At t = 3.2 days, lindane concentrations in the overlying water of the artificial sediment in treatments with food addition were lower than that of the natural sediments. The N2 sediment had higher lindane concentrations in the overlying water after 3.2 and 9 days in the fed treatments, and on day 30 in the unfed treatments, compared with the N1 sediment.

Food additions had an overall significant effect on the lindane concentration in the overlying water of the N1 sediment (two-way repeated measures ANOVA, P = 0.0020). No overall effect of food addition was found in the artificial sediment and the N2 sediment (P = 0.0977 and P = 0.0790). The overall effect of food additions showed a significant interaction with time for both natural sediments (P = 0.0002 and P = 0.0023 for N1, and N2, respectively). One-way ANOVAs within each time interval and subsequent pairwise comparison showed significantly higher lindane concentrations in overlying water of the unfed treatments, on day 20 and 30 for N1 and on day 30 for N2, compared with fed treatments (figure 8). On day 20, lindane concentration in the overlying water of the artificial sediment was higher in the fed treatments compared with the unfed. At t = 30 days overlying water of the fed treatments of the N1 and N2 sediments contained 1.6 ± 0.1 and 1.6 ± 0.1 µg/l lindane, respectively, whereas unfed treatments contained 1.8 ± 0.1 and 1.9 ± 0.1 µg/l, respectively.

Chironomus ***feeding activity***

The first occurrence of a full gut (i.e. 100% of the gut-length occupied by fluorescently marked sediment) was found after 140 minutes in the natural sediment, and 7 out of 13 samples had full guts within the time interval 140-220 minutes (figure 9). In the artificial sediment, the larvae never showed any full guts and the two maximum values of fluorescently marked sediment in the gut of the larvae occurred after 100 minutes (81 and 70 %). At the end of the experimental period (t = 220 minutes) the maximum values were only 51 % for the larvae in the artificial sediment. No comparison of the gut passage times of *Chironomus* larvae in the two sediments was made due to the large variation between replicates. The coefficient of variation for these measurements ranged between 36 and 113 %.

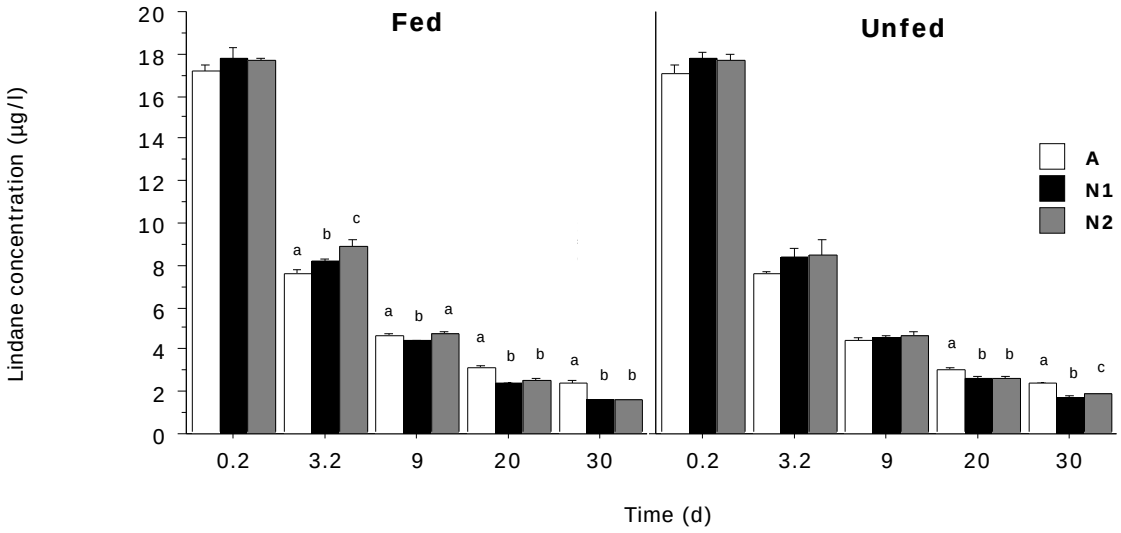

Figure 8. Concentration of lindane in overlying water over time (mean + S.D.) in microcosms with artificial (A) and two natural sediments (N1 and N2) with an initial lindane concentration of 22 µg/l. Letters over bars indicate significant differences between fed and unfed treatments within each time interval (Scheffé's, P < 0.05). For sediment characteristics see table 1.

A total of 32 samples of 84 were excluded from the data set. 13 samples were excluded due to pupated larvae or empty guts and 19 samples did not contain any marked sediment (pre-pupae).

Discussion

The present study showed that neither sediment type nor the addition of food had effect on the toxicity of lindane as determined by emergence, mean development rate and survival. This shows that the *Chironomus* test is a robust test that provides a good estimate of contaminant toxicity in natural sediments, despite marked differences in sediment characteristics (e.g. N content, density and water content). However, differences in the lindane concentrations of the overlying water indicated that differences existed in the exposure concentrations of the larvae. The study also showed that the growth of the larvae in the natural sediments was greater than that in the artificial sediment, despite equal amounts of organic matter. This difference could be due to difficulties for the larvae to explore the artificial sediment and/or a lower nutritional value of the organic matter pool (see below).

The three experimental sediments showed similar results for all toxicity endpoints except for survival. The reduced survival in the controls without food addition of the larvae in artificial sediment compared with larvae in the fed controls was contributed to a low nutritional value (i.e. no reduction in survival due to lindane exposure) (figure 5). The differences in N-content, density and water content of the three sediments did not affect the bioavailability of lindane to the larvae. All sediments had equal amounts of organic matter and the bioavailability of lindane was the same which indicates that the results of the toxicity tests in this study are consistent with that bioavailability is, if not solely, largely dependent on the organic matter content of the sediments as stated by Di Toro et al. (1991). However, the present study only involves one contaminant and the results may only be extrapolated to neutral organic contaminants with similar chemical properties as lindane.

Although the sediment type had no effects on toxicity there were differences in lindane concentration of the overlying water and thus the exposure of lindane to the larvae. The concentration of lindane in overlying water is a indirect measure of the concentration in the sediment. Karlsson (1998) showed that the main process affecting the concentration of lindane in the overlying water of a artificial sediment (10% organic matter) was adsorption to the sediment. After 30 days of incubation, Karlsson (1998) found 62 % of the added label associated with the sediment, 11 % in the overlying water and a total loss of lindane of 27%. Karlsson (1998) also found that the major route of loss of lindane from a microcosm was volatilisation. Other processes, like microbial degradation, did not contribute to any greater extent to the loss of lindane (Karlsson 1998).

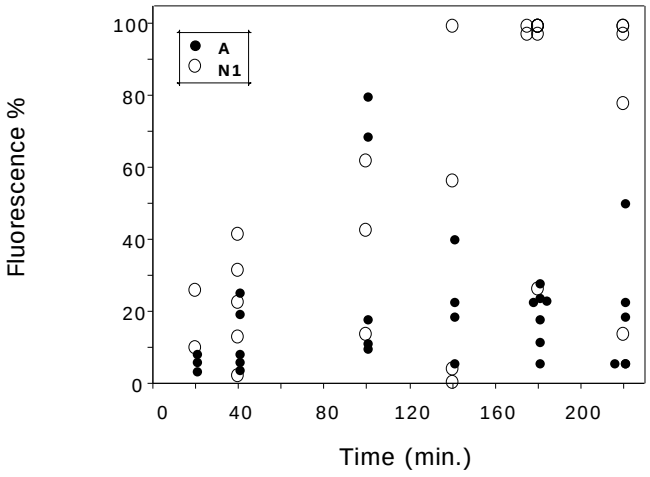

Figure 9. Ingestion of fluorescently marked sediment over time by Chironomus larvae in artificial (A) and natural sediment (N1), expressed as percentage of gut length occupied by marked sediment.

Assuming that volatilisation of lindane was the same in microcosms with the three different sediments in the present study, comparisons of the concentrations of lindane in the sediments can be made from the concentrations of lindane in the overlying water.

The distribution of lindane from the overlying water to the sediments over time were similar in the two natural sediments and differed from that of the artificial sediment (figure 7). In treatments with food addition, the lindane concentration in the overlying water of the artificial sediment was initially (t = 3.2 days) lower than that of the natural sediments. The unfed treatments followed the same trend. In the later part of the experiment (t = 20 and 30 days) the concentrations of lindane in the overlying water was consistently higher in the artificial sediment compared with the natural sediments. The lower initial concentrations of the artificial sediment compared with the natural sediments can be interpreted as faster sorption to the sediment and the observed differences in lindane distribution may be an effect of differences in sediment physico-chemical characteristics, for example particle size distribution and/or differences in the qualitative composition of the organic carbon pools (Meyer et al. 1993, Kukkonen and Landrum 1996). The two natural sediments had similar physico-chemical characteristics and, consequently, a similar distribution pattern of lindane over time.

The concentration of lindane in overlying water was also affected by the addition of food. (figure 8). The N1 sediment had lower concentrations in the overlying water of the unfed treatments on day 20 and 30, the N2 sediment on day 30 and the artificial sediment had higher concentration on day 20, compared with the fed treatments, respectively. A possible explanation could be that the added food decreased the lindane in the overlying water by absorbing lindane while passing through the water column, but then the effect should be the same in all sediments, which was not the case. The only sediment that caused an overall reduction of lindane in the overlying water by food additions over the whole time interval was the N1 sediment.

However, the differences in exposure concentrations between sediments described above were not large and thus no difference in toxicity was seen in the toxicity tests. For example, the final concentrations in sediments with an initial lindane concentration of 22 µg/l were 0.068 µg/g sediment in the artificial sediment and 0.087 µg/g sediment in the two natural sediments, when accounting for the 27% loss of lindane (Karlsson 1998). This indicates that by the end of the experiment, exposure concentration in the artificial sediment was 80% of that in the natural sediments.

Food addition in toxicity test with *Chironomus* has been shown to be crucial for both natural and artificial sediments for obtaining sufficient survival in control sediments (Ankley et al. 1994, Suedel and Rodgers 1994 a and b, Naylor and Rodrigues 1995). Ankley et al. (1994) tested *Chironomus* survival (10 d) in 50 uncontaminated natural sediments under fed and unfed conditions and found that survival in 75% of the unfed sediments were not acceptable, whereas in fed sediments acceptable survival (70%) were reached in 95% of the sediments. However, the dependence of food addition is dependent on the endpoints measured. For example, sub-lethal endpoints like emergence and mean development rate are more dependent on nutrition than survival, as shown in this study where no emergence occurred in unfed treatments whereas survival ranged 100–50%. Addition of food was necessary for emergence and positively affected mean development rate in both artificial and natural sediments. Without exogenous food addition, none of the sediments supplied enough food for a proper larval development.

Difference in nutritional values between the artificial and the natural sediments was indicated by the observed lower survival in the artificial sediment than the natural sediments. Additionally, the larvae in the N1 sediments showed a faster growth compared with the larvae in the artificial sediment (figure 6). Surprisingly, the growth of the larvae in the N2

sediment did not differ from the growth of the larvae in the artificial sediment. The N1 and N2 sediments were collected at different sampling sites but within the same lake and the main constituent of the two sediments in the preparation was profundal sediment (sediment from 12 and 16 m depth). This would have given the two sediments similar organic matter pools and thus similar nutritional values. However, the N1 sediment contained sediment from a littoral sampling site which could have contained organic material with better food quality e.g. richer in essential fatty acids. These differences in survival and growth described above suggests differences in nutritional values between the artificial sediment and the natural sediments. The C/N ratio of the artificial sediment was about 6 times higher than that of the natural sediments, thus indicating a more nutrient rich substrate in the natural sediments. Another factor that could have contributed to the observed differences in survival and larvae length is the density of the sediments. The lower density of the natural sediments makes burrowing and food searching easier and the total volume of explored sediment larger which was confirmed by visual observations of the larvae in the sediments and the feeding rate experiment. The result of the feeding rate experiment was hard to interpret due to the large variation, but the test indicated that the larvae preferred the natural sediment (N1). The large variability of the results may be due to uneven distribution of the added fluorescently marked particles in the sediment or feeding on deeper unmarked sediment.

The use of other organic matter sources than peat could lower the needs for addition of exogenous food during toxicity tests. For example, Suedel and Rodgers (1994 a) used humus consisting of decaying plant material and manure, which are more representative of naturally occurring organic matter than peat. An important issue of the choice of organic matter component in standardised toxicity tests is the variation of the composition between different sources. For example, different sources of peat could vary with respect to important characteristics (e.g. nutritional values) and thus make standardisation of feeding regimes difficult. A well-defined formulated sediment which could be used internationally requires an organic component that is independent of spatial and temporal variations and have high nutritional values for the test organism. This would reduce the needs of adding exogenous food and facilitate standardisation of the feeding regimes.

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