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Long-term Effects of 1,2,4-Trichlorobenzene on Freshwater Plankton in an Outdoor-Model-Ecosystem

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1,2,4-Trichlorobenzene (TCB) is an important industrial chemical with an estimated production in the United States of $12.8 \cdot 10^3$ and in the European Community $16 \cdot 10^3$ t $\cdot a^{-1}$ (Umweltbundesamt 1982). The product is mainly used as a solvent or an intermediate in chemical processes. TCB has a low water solubility (19 - 30 mg $\cdot 1^{-1}$), no hydrolysis (pH 3 - 9, 25°C) and a relative high volatility from aqueous phase (50 % in 6.5 hrs at 20°C) (Bundesministerium des Innern 1981). TCB residues have been detected in surface waters of the Rhine river (conc. range 60 - 115 ng $\cdot 1^{-1}$; van der Veen 1976) and in the river-bank. Laboratory toxicity tests (base-set) indicate a LC_{50} of 6 mg $\cdot 1^{-1}$ to zebrafish (Brachydanio rerio) and 109 mg $\cdot 1^{-1}$ to bluegills (Lepomis macrochirus) (Buccafosco et al. 1981). Immobilization (EC_{50}) of daphnids varied from 1.2 to 21 mg $\cdot 1^{-1}$ (Umweltbundesamt 1981, Bringmann and Kühn 1982, Calamari et al. 1983). Algae growth- and photosynthesis-inhibition tests (96 and 3 hrs EC_{50}) showed 1.4 and 3.9 mg $\cdot 1^{-1}$ TCB as effective for Selenastrum capricornutum (Calamari et al. 1983).

The present experiment had three objectives: to evaluate the effects of TCB to a natural freshwater plankton community in comparison to controls (influence upon the physico-chemical conditions, primary and secondary toxic effects upon diversity and abundance of planktonic organisms in a natural pond), to check the transferability of laboratory toxicity data to the complex outdoor system, and to relate the results of this study to previous investigations conducted with other micropollutants using comparable methodology (Schauerte et al. 1982a; Lay et al. 1984a, b).

MATERIALS AND METHODS

The experiment was performed during a five weeks period from Sept. - Oct. 1981 in a water meadow in Southern Germany. To create the ecosystem, a natural pond (3 m x 3 m x 1 m depth), very rich in Daphnia and phytoplankton species, was divided into discrete compartments

by the use of six opaque PVC-tubes (1.25 m long, 50 cm diameter). They were pressed up to 25 cm into the clayey sediment resulting in a mean water volume of about 200 litres, and thus avoided water- and bioexchange with the surrounding. By this method comparable starting conditions were achieved for replication and controls. Three tubes each were used for a single chemical treatment, the remaining three serving as controls.

The investigation period covered two weeks pre-application and three weeks post-application phases. The application of 1,2,4-Trichlorobenzene (TCB) was performed once by gently stirring aliquots of a water-saturated solution of TCB into the tubes. The intended chemical concentrations were 250 $\mu g \cdot l^{-1}$. Sampling of biota and measurements of the physico-chemical properties of the water were carried out between 11.00 h and 13.00 h to avoid wide diurnal fluctuations of the limnological parameters such as the vertical movement of phyto- and zooplankton and changes in oxygen concentrations.
Water samples (500 ml) were extracted with n-pentane 10:1 for TCB-residue analysis. The actual TCB-concentration of the extracts was determined by GLC (model Fractovap 2300, ECD, Carlo Erba, Italy). Column (1.1 m) was packed with 5 % OV 101, carrier gas was N_2 (40 ml $\cdot$ min^{-1}), oven temperature 120°C isotherm. An integrator (model HP 3388 A, Hewlett Packard) was used for quantitative analysis.
Oxygen, pH and temperature were measured in 30 cm water depth by portable apparatus (Oxy-Digi 88, pH 90, WTW, Germany). Water hardness was defined colourimetrically by Aquamerck® reagent kit (Merck, Germany).
Sampling of daphnids was carried out with a sampler of 80 cm length and 1.5 litre volume. Three samples were taken from each tube, filtered over a 180 µm sieve, fixed with formol and counted by stereomicroscopy. The filtered water was returned to each tube to avoid nutrient and phytoplankton depletion.
Two phytoplankton samples of 20 ml each were taken from 20 cm depth of each tube and were immediately fixed by "Lugols-solution". Ten millilitre sub-samples were placed into microplankton meters and counted according to the Utermöhl method (Utermöhl 1958) by phase-contrast microscopy.

RESULTS AND DISCUSSION

The physico-chemical measurements revealed no significant difference between controls and treated tubes and between pre- and post-application periods, with the exception of O_2 concentration. Mean water temperature was 12 $\pm$ 1°C, water hardness was 16 $\pm$ 2° (German degrees) (= 2.86 m mol $CaCO_3$ per litre), pH was 6.3 $\pm$ 0.3 in the pre- and 6.5 $\pm$ 0.6 in the post-application phase.

The dynamics of dissolved oxygen in water of each tube investgated, is shown in fig. 1. No difference can be seen up to day 4 after chemical dosing. The mean course of O_2-concentration from day 4 until 24 can be described by the linear regressions: $y = - 0.53 + 0.062\ x$; $R = 0.82$, $t_R = 5.16$ ($n = 15$) for the controls, and $y = - 0.12 + 0.101\ x$; $R = 0.94$, $t_R = 10.32$ ($n = 13$) for the treated tubes. Calculation of the t-value for both slopes was $t = 2.50$ (26 d.f.) and $p < 0.05$.

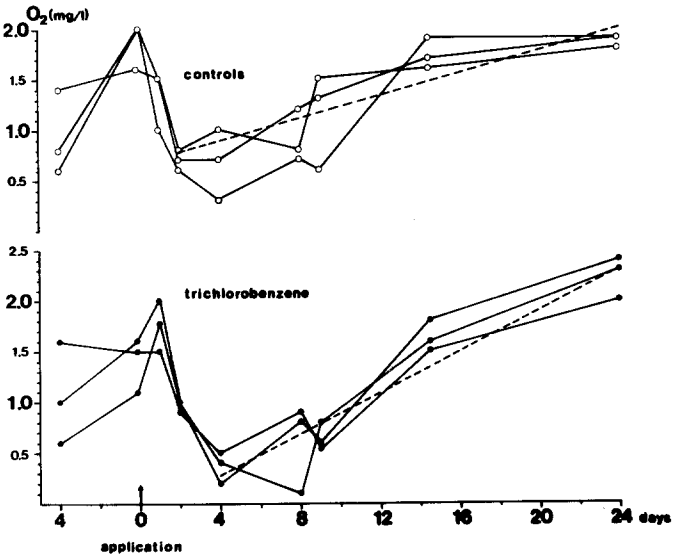

Figure 1. Course of dissolved oxygen concentrations in the water of each tube.

The concentration decrease of 1,2,4-Trichlorobenzene in the water of the three water-bodies applied, is illustrated in fig. 2. Besides the individual measuring points the corresponding linear regression lines (marked as a, b, c including the resulting of these = d) were compiled for the calculation of the half-lives. The correlations of R (for lines a, b, c) were highly significant ($p < 0.001$). As straight line b is significantly different from a and c, a fourth regression d was calculated and drawn on the basis of all measurements. The function of d is: $y = 215 - 7.84\ x$; $R = - 0.93$, $t_R = 12.65$ (25 d.f.), $p < 0.05$; $t_{1/2} = 13.7 \pm 2.5$ d. The percentual deviation from the max. initial concentrations (a, b, c) is 4.9 %, from the half-lives it is 18.2 %.

The decrease of TCB-concentrations in the three tubes was monitored until a detection limit of about 40 ppb.

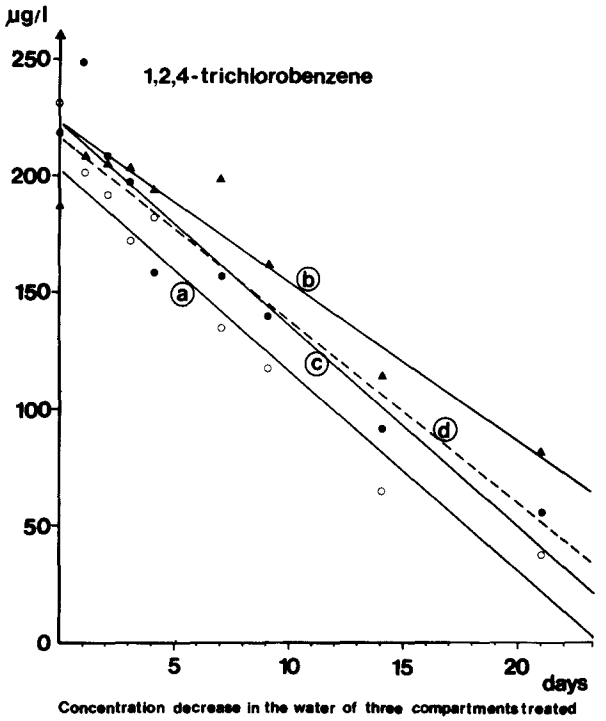

Figure 2. Concentration decrease of 1,2,4-Trichlorobenzene in the water of the three tubes.

The functions are for

a : $y = 203 - 8.63 \cdot x$; $R = 0.98$, $t_{1/2} = 11.8$ d;
b : $y = 221 - 6.73 \cdot x$; $R = 0.97$, $t_{1/2} = 16.5$ d;
c : $y = 222 - 8.62 \cdot x$; $R = 0.95$, $t_{1/2} = 12.9$ d.

The theoretical, initial concentrations aimed at, were 250 ppb. As to be seen from fig. 2, the mean max. concentration was 215 $\pm$ 10.7 ppb. It is assumed that about 10 % of TCB applied was quickly adsorbed to sediment and other particulated matter (biota), except in the sampling tube represented by line c (fig. 2), where an effective concentration of 250 ppb was analyzed 24 hrs following dosing. The study of accumulation potential of chlorinated hydrocarbons to the sediment of this and neighbouring ponds was the objective of a previous outdoor study (Schauerte et al. 1982b). The soil sorption coefficient (K_{OC}) of TCB was calculated to be 2042 (Calamari et al. 1983).
From all phytoplankton samples taken, a total of 31 species/genus were counted (tab. 1). Chloro- and Euglenophyta constituted more than 90 % of all algae observed.

Table 1. Identified taxa during the entire experimental period.

Cryptophyta	Chrysophyta	Chlorophyta	Cyanophyta	Euglenophyta
Cryptomonas	Navicula	Chlorella vulgaris	Chroococcus limneticus	Euglena pisciformis
Chilomonas	Gomphonema constrictum	Oocystis	Oscillatoria	Euglena acus
Chroomonas	Stauroneis anceps	Chlamydomonas	Microcystis	Trachelomonas hispida
	Synedra ulna	Carteria		Trachelomonas volvocina
	Nitzschia acicularis	Stichococcus bacillaris		Notosolenus apocamptus
	Eunotia arcus	Pleurococcus vulgaris		
	Cymbella helvetica	Scenedesmus quadricauda		
	Characiopsis	Siderocellis ornata		
		Ulothrix moniliformis		
		Selenastrum bibraianum		
		Closterium ehrenbergii		
		Spirogyra		

The mean phytoplankton density is illustrated in fig. 3.

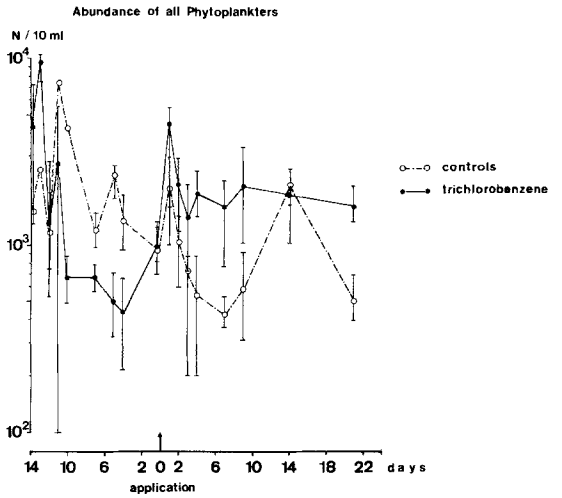

Figure 3. Mean phytoplankton abundance.

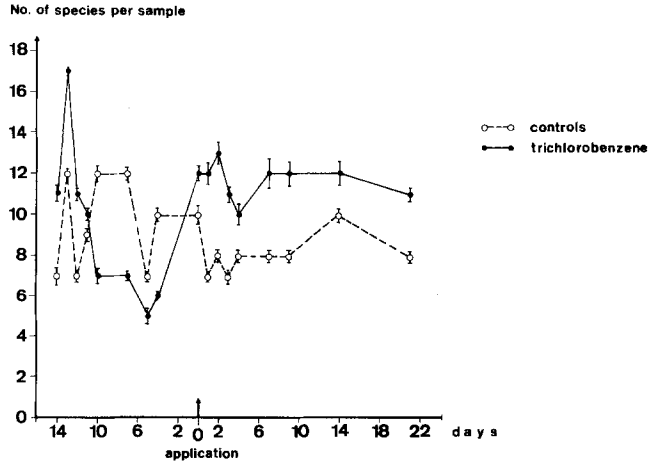

Figure 4. Mean phytoplankton diversity.

During the pre-application-phase there were great intra- and interspecific deviations in the number of algae among the tubes provided for application and the controls (see. fig. 3). The mean number counted was significantly different for controls and TCB treatment provided tubes on day 7, 5, and 4 of this investigation period ($p < 0.05$).

The phytoplankton diversity (species richness) is shown in fig. 4 as mean values per day of investigation. During pre-treatment this sum-parameter varied between the single tubes, but no significant differences were shown. Following TCB-application a mean higher diversity as well as higher mean numbers of algae were counted during 22 days of observation. Due to relative high standard deviations of the values between control and TCB-treated tubes, the differences remained insignificant. From the slight increase in diversity and abundance in the treated tubes, no primary, chemical caused intoxication to the phytoplankton community can be concluded. The slight increase in the two parameters might suggest a positive effect due to TCB-dosing. However, these increases should be interpreted as a secondary effect, where algal blooms were a result of grazer (e.g. daphnids) mortality and not a result of a stimulation effect of TCB.

As shown in fig. 5 the chemical was highly toxic to the daphnids population present causing a significant reduction. The mean number of daphnids counted in the treated tubes was less than 10 % of the controls during post-application period. A regeneration phase seemed to begin on day 21, when the TCB-concentration in the water was 50 - 100 µg per litre. The relative constant density of daphnids in the controls indicated that the low oxygen concentration did not effect the population dynamics.

The toxicity to daphnids found in this outdoor study showed a great discrepancy to laboratory toxicity data. Literature values of laboratory experiments for *Daphnia magna* (48 hrs EC_{50}) were described in the range of 1.2 to 21 mg per litre (Umweltbundesamt 1982; Bringmann and Kühn 1982). A minimum of 1.2 mg TCB was found to be the immobilization concentration for *D. magna* (24 hrs IC_{50}) (Calamari et al. 1983). From this pond experiment, a 90 - 100 % mortality of *D. pulex* was observed at initial TCB-concentrations of 250 µg per litre. These differences can either be interpretated by the different daphnid species tested or by additional toxic effects to the populations in the pond caused by chronic uptake of TCB-contaminated food. From bioaccumulation tests with ^{14}C-TCB and the green alga *Chlorella fusca* as test organism, accumulation factors of 250 (wet weight) have been obtained (Geyer et al. 1984). This result indicates the relative strong lipophilic character of the chemical and

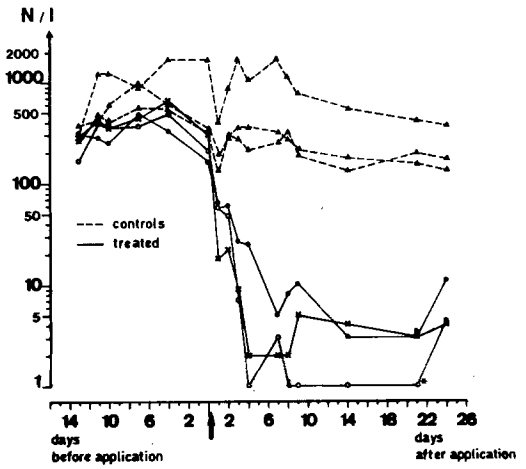

Figure 5: Dynamics of daphnids in controls and treated tubes.

a possible high accumulation potential for the plankton in the pond system. A further influencing factor for the higher toxicity to daphnids in the outdoor experiment could be the longer half-life of TCB in the water. In contrast to laboratory volatility data ($t_{1/2}$ = 6.5 hrs at 20°C, Umweltforschungsplan 1981) we found significant lower evaporation/disappearance rates of TCB in the pond ($t_{1/2}$ = 11.8 - 16.5 d at 12 ± 1°C). This phenomenon could have induced a toxic long-term effect to daphnids at low TCB-concentrations in the contaminated water.

Although the results of the present study are applicable only for the effective fresh-water-system, some general conclusions were obtained in agreement with data from further experiments with 2,4,6-trichlorophenol, pentachlorophenol, tri- and tetrachloroethylene, and benzene. As to the physico-chemical behaviour of the test chemicals, we always found a lower water solubility and a marked reduced volatility from water phase in the field experiments compared with laboratory testing.

Furthermore there was always a significant higher sensitivity of the daphnids to the pollutants as compared with the EC_{50} laboratory values. Regardless of the sometimes different species (D. magna - D. Pulex) tested, the effective toxic concentrations were lower up to a factor of hundred as compared with literature. These

findings should be considered in the context of predicting the hazard of chemicals from screening tests to the "real" environment.

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