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Influence of Benzene on the Phytoplankton and on *Daphnia pulex* in Compartments of an Experimental Pond

J. P. LAY, W. SCHAUERTE, L. PEICHL, W. KLEIN, AND F. KORTE

Gesellschaft für Strahlen- und Umweltforschung mbH München, Institut für Oekologische Chemie, Ingolstaedter Landstrasse 1, Neuherberg D-8042, Federal Republic of Germany

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Benzene, with initial concentrations of 100 and 50 mg per liter, was dosed in duplicates into four compartments of a small pond. The decrease of chemical concentration in the water was exponential with a mean half-life of 4.7 ± 0.9 days. Following benzene application, the phytoplankton density and diversity slightly increased relative to the controls. Both concentrations were lethal for the daphnids present. During 24-hr *in vitro* tests with *Daphnia pulex* (initial benzene concentrations <50 mg per liter), a direct correlation between mobility and decreasing chemical concentration was observed.

INTRODUCTION

The experiment demonstrates the effects of effective concentrations of an environmental chemical upon plankton groups in a complex, naturally derived ecosystem.

Although more and more data are becoming available for the accumulation and toxicity of chemicals in isolated species, the relation between a pollutant and its ecological consequence remains largely unknown. Therefore, primary and secondary effects upon planktonic organisms and upon physicochemical parameters. following dosages of 50 and 100 mg benzene per liter were investigated. In addition to the outdoor experiments, a 24-hr short-term test demonstrated the direct toxicity to *Daphnia pulex* at several lower benzene concentrations. A further aim was to compare the results of our field experiment with the data obtained from laboratory toxicity studies using short-term tests with monocultures in an artificial aquatic system.

The present outdoor study with benzene was performed under comparable methodological conditions as the experiments described with pentachlorophenol, 2,4,6-trichlorophenol (Schauerte *et al.*, 1982), and trichlorethylene (Lay *et al.*, 1984).

Benzene was selected as a model substance because of its environmental relevance: production amount ($>10^7$ tons/year, Stephenson, 1977), its estimated amount released into the environment (10^5 tons/year), its great mobility, and the direct as well as indirect toxicity. The toxicological effects of benzene on man, including mutagenicity and carcinogenicity, are well investigated (Forth *et al.*, 1975). Data indicating the ecotoxicological effects are sparse: the LD_{50} for *Carrassius auratus* is 46 mg/liter and for *Brachydanio rerio* it is 24 mg/liter. The toxicity to *D. magna* Straus is described with 38 and 18.7 mg/liter, respectively (EC_{50}) (Schmidt-Bleek *et al.*, 1982), for *D. magna* it is 250 mg/liter (24 hr LD_{50}, Le Blanc, 1980), and for *D. pulex* it is 305 mg/liter (48 hr LD_{50}, Canton and Adema, 1978).

For the implementation of this experiment, a small, natural pond was subdivided into discrete compartments by the aid of tubes. The physicochemistry of water, the sediment structure, and the plankton species assemblage were similar at the beginning

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of the experiment. The low dose of benzene was selected on the basis of laboratory EC_{50} data for daphnids. In addition, the high dosage was tested because a high volatility, a possible adsorption to sediment, and a fast biotic/abiotic degradation of this chemical was expected.

MATERIALS AND METHODS

In autumn 1981, a small pond (3 × 4 × 1 m) rich in microfauna and -flora was selected for ecotoxicological investigations and was compartimentalized by opaque PVC tubes (50 cm diameter) into seven compartments with a mean volume of 196 liters each. The tubes were pressed into the sediment to isolate the compartments. Three compartments served as controls and two compartments each were used for low and high dosing of benzene. Two weeks following installation, the chemical application was carried out by gently stirring in benzene-saturated aqueous solutions, corresponding to the intended concentrations of 50 and 100 mg benzene per liter. For a period of 3 weeks following dosing, the toxicological effects of benzene were observed by measuring the physicochemical parameters and by sampling the biota at increasing time intervals.

Physicochemical Parameters

The actual concentration of benzene in the water of the compartments was monitored by GLC (model Fractovap 2101 AC, Carlo Erba, Italy), equipped with a FID. The column was packed with 0.1% SP-1000 (Supelco). The carrier gas, N_2, had a flow rate of 30 ml/min. The oven temperature was 120°C isotherm. The water samples were analyzed by direct injection of 1–2 μl water. The quantitative estimation was performed with an HP 3388 A integrator (Hewlett–Packard). The concentrations of dissolved oxygen and of H^+ (pH), at a 30-cm depth, were measured with portable apparatuses (model Oxi-Digi 88 and pH 90, WTW, West Germany). The hardness of the water was complexometrically defined with the reagent kit Aquamerck (Merck, West Germany).

Biological Parameters

To compensate diurnal fluctuation of biotic parameters such as the vertical movement of zooplankton and phytoplankton, all limnological measurements and sampling of biota were performed between 11 AM and 1 PM.

Counting of daphnids in the compartment experiment. For counting these organisms, samples were taken by the aid of an 80-cm-long, 1.5 liter device at three different areas in the compartment. The daphnids were filtered over a 180-μm sieve, fixed with formaldehyde, and then counted by stereomicroscopy.

Counting of daphnids in the 24-hr short-term test. In addition to the long-term outdoor experiments, short-term *in vitro* tests were performed. Their purpose was to reveal the toxic effects of benzene on daphnids, in concentrations less than the lethal concentrations found in the outdoor compartments (100 and 50 mg/liter).

For the *in vitro* tests, daphnids were collected from the water of a nearby untreated pond. Test populations of 30 daphnids each were introduced to six 600-ml beakers (test set). Two of these beakers contained water samples from the low-dosed benzene compartments, two from the high-dosed benzene compartments, and two from the control compartments. In total, three sets of *in vitro* tests were performed: the first

using compartment samples taken 8 days after dosing, and the second and third using compartment samples taken 9 and 10 days after dosing, respectively. Each respective day reflected the different toxic effects (EC_{50}) of benzene on the mobility of daphnids due to the varying concentrations and volatility rates. The times of EC_{50} observation were t = 0.25, 0.5, 0.75, 1, 1.5, 2, and 24 hr.

The EC_{50} (mobility) values for the varying benzene concentrations were measured in the following way:

(1). Two populations of 30 daphnids each were introduced to the two control beakers.

(2). For these beakers, the average maximum travelling height to which 100% of the daphnids swam, was measured. The mean height was then transferred to the other four dosed beakers and labeled as the "no effect level."

(3). Daphnid test populations (30 each) were then introduced to the four dosed beakers.

(4). At times t = 0, 2, and 24 hr the decreasing benzene concentration levels in these beakers was measured and recorded.

(5). At the seven varying time intervals listed above, the maximum average height to which 50% of the daphnids reached were recorded. These values were calculated as a percentage of the maximum "no effect level" obtained from the controls. In this manner, the degree of daphnid immobility was revealed. The bottom of each beaker represented 0% mobility (e.g., total immobility) and the "no effect level" represented 100% mobility (zero immobility).

(6). Graphs were then constructed to correlate the decrease in benzene concentration with the increase in daphnid mobility (see Figs. 6A and B).

RESULTS

Physicochemical Parameters

Concentration decrease of benzene in the water. The maximum initial concentrations of benzene in both *high dosed* compartments were 100 mg per liter.

During the 24-day period following dosing, the reduction in benzene (Fig. 1A) was

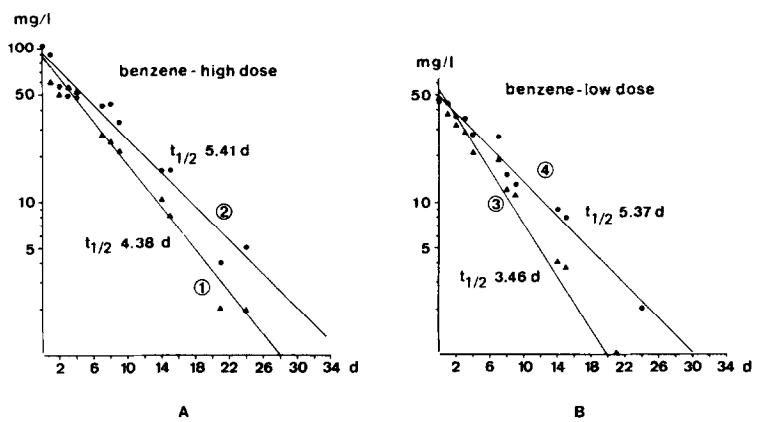

FIG. 1. Concentration decrease of benzene with time. (A) High-dosed compartments 1 and 2; (B) low-dosed compartments 3 and 4.

different for the two compartments. The benzene decrease can be described by the function $y = e^{(4.476-0.159 \cdot x)}$ for compartment 1 and with $y = e^{(4.534-0.127 \cdot x)}$ for compartment 2. The correlation coefficients $R = 0.98$ ($n = 12$) were equal for both compartments. The calculation of significant differences in the following text always refers to a 95% confidence limit ($P < 0.05$). The t test for calculation of the difference between the slopes of both functions showed a significant difference $t(20) = 2.49$ in the course of the two linear regressions in the semilogarithmic graph. The calculated half-lives were 4.4 and 5.4 days for compartments 1 and 2, respectively.

The actual initial concentrations of benzene in the two *lower applicated compartments* (3 and 4) were 48 and 45 mg/liter, respectively (Fig. 1B). The exponential benzene decrease can be seen from the functions $y = e^{(3.995-0.199 \cdot x)}$ ($n = 12$) for compartment 3 and $y = e^{(3.895-0.128 \cdot x)}$ ($n = 11$) for compartment 4. The correlation coefficients of $R = 0.98$ were identical for linear regressions 3 and 4. The difference in the concentration decrease between compartments 3 and 4 was significant according to the t test ($t(28) = 5.26$).

Comparing the slopes of functions 1 and 3, the difference was significant ($t(19) = 2.77$). No significant difference was found between functions 2 and 4 ($t(19) = 0.09$).

The mean half-life decrease for benzene in all four compartments was calculated with 4.7 ± 0.9 days.

Temperature, pH, water hardness, and O_2 concentration. At the time of benzene application the following values characterized the physicochemical state of the water in the compartments: temperature 12°C; pH 6.2; O_2 concentration 1.1–1.6 mg/liter; water hardness 2.86 m mol/liter. Following benzene application these parameters were periodically measured. However, only significant changes in the O_2 concentration was observed (Fig. 2).

For the three control compartments a mean O_2 concentration of 1.9 mg/liter was measured on Day 0, which decreased to about 0.7 mg/liter on Day 4 of the experiment.

During the same period comparable O_2 concentration decreases were observed in both the high- and the low-benzene-dosed compartments. Thus, up to Day 4, no correlation between benzene application and O_2 concentration was apparent.

Following Day 4 of the experiment, the O_2 concentration increased in all compartments with a difference in trend between controls and treated water. The course of O_2 concentration increase in the controls can be described by the function $y = -0.428 + 0.737 \cdot \ln x$ ($R = 0.97$; $t_R(3) = 6.78$); in the benzene high-dosed water by $y = -0.248 + 0.328 \cdot \ln x$ ($R = 0.70$; $t_R(3) = 1.70$) and for the low-dosed compartment by $y = 0.063 + 0.263 \cdot \ln x$ ($R = 0.47$; $t_R(3) = 0.91$). These regressions were graphically indicated by the dotted lines in Fig. 2. In addition, the slopes of these functions were compared by the aid of the t test: controls vs high-dose benzene, $t_s(6) = 1.95$ ($P < 0.10$); controls vs low-dose benzene, $t_s(6) = 1.54$ ($P < 0.20$); high-dose vs low-dose benzene, $t_s(6) = 0.06$.

Phytoplankton

A total af 32 phytoplankton taxa were analyzed during the entire test period. Chlorophyta and Euglenophyta consitituted >90% of all algae observed (Table 1). The total number of individuals (abundance) counted is given as means per day from the controls, high- and low-dosed compartments (Fig. 3).

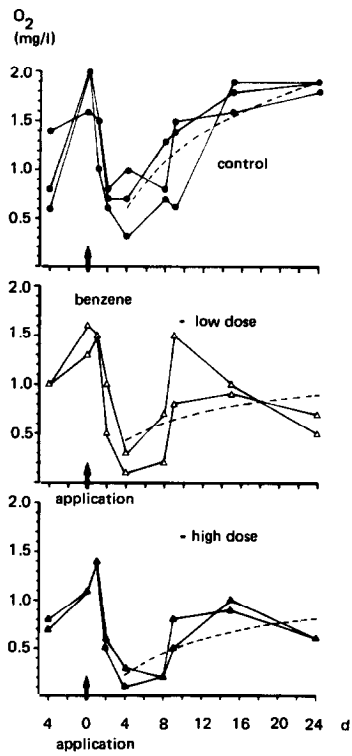

FIG. 2. Dynamics of dissolved oxygen concentration in control, low- and high-benzene-dosed compartments. Dotted lines were drawn from the calculated regressions.

Abundance. In the water of the control compartments the phytoplankton density increased on an average of 1705 individuals from the pre- to the postapplication period (values calculated as means of the mean difference of each compartment). The benzene-dosed compartments, however, showed an increase in the number of algae

TABLE 1

IDENTIFIED PHYTOPLANKTON TAXA

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

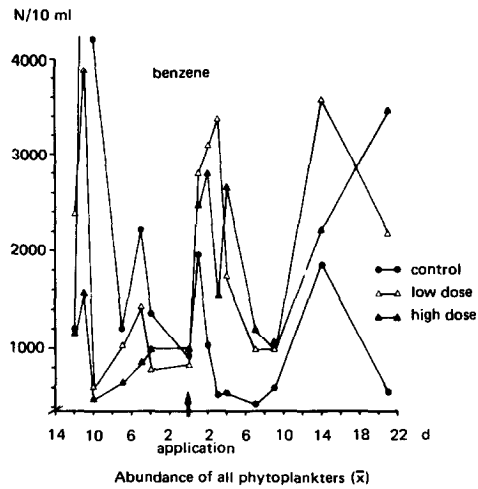

FIG. 3. Mean number of phytoplankton organisms as counted from 10-ml samples of controls, low- and high-dosed compartments.

by 779 individuals in the low-dosed and 1202 in the high-dosed ones. Because of the great variations in the density of algae and the small sample sizes, inferential statistical analysis could not verify the significance of the trends of increase of individuals.

Diversity. Comparing the mean number of taxa of the pretreatment with the post-treatment period, the changes were as follows (method of calculation analogous to the abundance): control compartments, decrease by 1.02; low-dosed compartments, increase by 2.50; high-dosed compartments, increase by 2.86.

The *t* test of these mean differences revealed the following *P* values: controls vs low-dosed compartments, 0.025; controls vs high-dosed compartments, 0.052; and low-dosed vs high-dosed compartments, 0.870.

The number of counted taxonomies (diversity) during the pre- and postapplication period in controls, high- and low-dosed compartments is compiled in Table 2.

Zooplankton

Outdoor toxicity of benzene to D. pulex. The self-dynamic of *D. pulex* in three control compartments is illustrated in Fig. 4. The density of daphnids is subjected to very large deviations within these control communities.

Initial concentrations of 50 and 100 mg benzene per liter were immediately lethal for the daphnid populations present (Fig. 5). After Day 22, few organisms recurred in the low-dosed compartments. Following the die-off of all daphnids in the outdoor system, no further information about the toxicity of benzene at concentrations less than 50 mg per liter could be obtained. For this reason, the *in vitro* tests were conducted.

In vitro short-term toxicity/mobility tests with D. pulex. The concentration decreases of benzene in the four water samples (taken 8, 9, and 10 days following chemical dosing) and the corresponding curves of *D. pulex* mobility are shown in Figs. 6A and B, respectively, As seen from each curve of all diagrams, the mobility of daphnids increased to a 100% value with decreasing benzene cocentrations during the 24-hr test procedure.

TABLE 2

SPECIES RICHNESS (DIVERSITY) OF PHYTOPLANKTON, SEPARATED FOR EACH COMPARTMENT INVESTIGATED

Day of investigation	Control compartments			Benzene-treated compartments: Low dose		High dose	
12[a]	2	5	6	6	4	7	1
11	6	2	6	5	5	5	2
10	5	3	7	4	3	3	2
7	5	7	6	7	3	4	5
5	5	4	4	7	5	6	4
4	5	7	4	4	3	7	6
1	4	8	8	8	6	9	4
1[b]	4	5	5	9	7	5	9
2	5	5	4	9	7	8	8
3	2	2	7	9	7	8	8
4	4	1	5	5	7	8	10
7	3	4	4	9	6	8	4
9	2	6	3	10	3	4	6
14	2	5	10	13	4	9	8
21	3	4	5	11	4	7	10

[a] Preapplication period.
[b] Postapplication period.

DISCUSSION

The effective, initial concentrations of benzene measured in all compartments were in the range of the theoretical aimed values. This was due to the relatively good water solubility of benzene. Therefore, in contrast to many chlorinated benzenes, no significant bioaccumulation or adsorption to inorganic particles of the compartment system occurred.

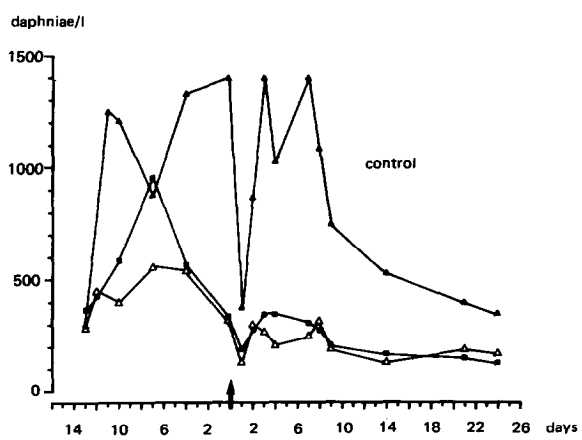

FIG. 4. Dynamics of *Daphnia pulex* in the three control compartments over the entire investigation period.

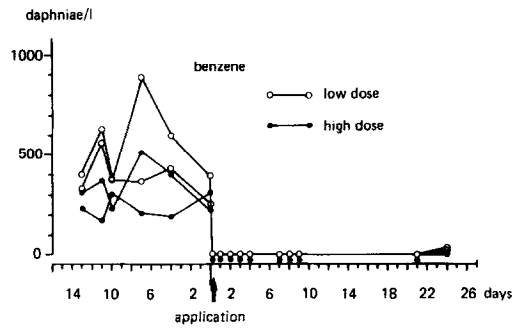

FIG. 5. Dynamics of *Daphnia pulex* during the 14 days preinvestigation and 24 days postapplication period in the four treated compartments.

The course of benzene concentration decrease in the compartments was independent of the initial concentrations, as revealed in Fig. 1 by a comparison of the four regression lines. No consistent trends can be observed. With regard to the dynamics of oxygen concentration in all compartments, a significant differentiated development between control and chemically treated compartments was not observed. The difference in the mean density of algae between the controls on one hand and between high- and low-dosed compartments on the other (postapplication period) described a trend which could not be assessed by statistical analysis. The analysis of phytoplankton diversity (Table 2) was based only on the spectrum of species listed in Table 1. The results of the diversity investigation were equivalent to those obtained from the abundance, and proved to be of statistical significance.

The mean higher algae density as well as the species richness following benzene dosing, in contrast to the controls, might suggest a positive effect due to benzene 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 the stimulation effect of benzene. The constant density of daphnids in the control compartments indicates that the low oxygen levels did not affect the population dynamics.

The three short-term tests on mobility of daphnids, using unexposed *D. pulex* and benzene-treated water from the compartments 8, 9, and 10 days after application, provided additional information to the outdoor experiment. The methodology used, however, should not be compared to standardized laboratory-toxicity tests. Because of the varying test conditions in the outdoor field, such as changing weather (overnight), varying composition of compartment water, and randomly sampled daphnid populations for each of the three tests, the reproducibility of the test presented is limited. However, this test did provide further information about the dose-dependent effects of benzene on daphnids, after these populations had been eliminated by the high initial dosages of benzene in the compartments. As expected, the tests indicated a proportional relationship between dapphnid mobility and decreasing benzene concentration (Fig. 6).

The benzene concentration decrease in the test beakers was much faster than that in the experimental compartments. This can be attributed to the different physicochemical parameters of the two test systems: water volumes, exposed surface areas, light intensities, and temperature values.

The data represented in Fig. 6 indicate a recreation of daphnids at <5 mg benzene per liter. At about 50 mg benzene per liter the mobility declines close to zero which

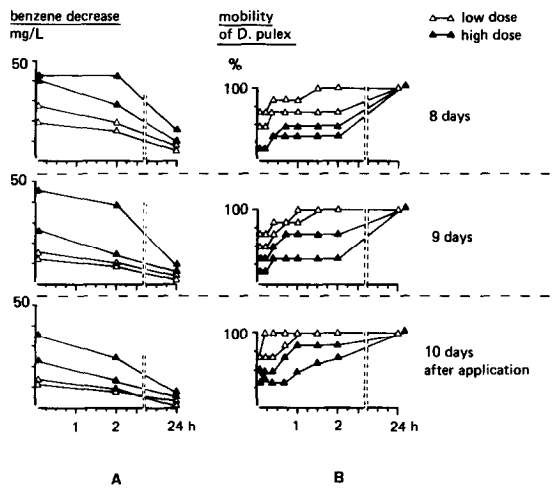

FIG. 6. Twenty-four hour short-term tests. (A) Benzene concentration decrease in the beakers, containing water from the contaminated compartments on Days 8, 9, and 10 after application. (B) Increase of daphnid's mobility with time in the test vessels.

is in good agreement with the observed effects in the low-dosed compartments at 50 mg initial benzene dosage. The mobility data obtained from these 24-hr short-term tests, as a function of time and chemical concentrations, can scarcely be correlated with literature data on the LC_{50} values for daphniae species from laboratory tests: Canton and Adema (1978) published a LC_{50} of 305 mg benzene per liter in 48 hr for *D. pulex* and 373 mg/liter for *D. cucullata.* Le Blanc (1980) found LC_{50} values for *D. magna* of 250 and 200 mg per liter in 24 hr and 48 hr, respectively. His data about a no discernable effect level of <13 mg per liter is in the same order of magnitude as our findings. Because of the nonharmonized test methods, however, the conflicting results should not be further discussed in this context.

The results from this experiment clearly show that benzene can harm the population structure of a freshwater outdoor system. From an environmental point of view, the elimination of only one essential link of the food chain, as demonstrated for the daphnids, is a serious interference. It causes subsequent secondary effects, such as increases in phytoplankton counts. The possibilities of contaminating an aquatic system with benzene are manifold due to the broad spectrum of its industrial application (e. g., use as solvent, constituent of fuels). Furthermore, benzene possesses ideal physicochemical properties as revealed by its good water solubility and high volatility rate. Therefore, it is easily transported via air to outdoor water systems which in turn serve as possible sinks.

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