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Intermittent Flow System for Population Toxicity Studies Demonstrated with *Daphnia* and Copper

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Until the introduction of continuous-flow procedures, the physical aspects of testing the toxicity of chemicals and aqueous effluents to aquatic organisms had been of minor consideration. Today's devices ranging from pneumatic systems to electric pumps (Smith et al. 1977; De Foe 1975), all have some drawback or other but many of them are reduced to a minimum by the use of the proportional diluter (Mount and Brungs 1967; Benoit et al. 1981), which is a well-established and reliable dosing apparatus. However, the Mount-Brungs diluter cannot be used for testing volatile chemicals, nor does it allow simultaneous dosing of a constant food suspension and several toxicant concentrations, which are important conditions for population toxicity studies with small invertebrates like the crustacean Daphnia magna. These restrictions are removed by the use of electric pumps, solenoids and time relays. The system described here provides for the delivery of 250 mL every 5 min to 6 h with no perceptible current-induced effects on the test organisms; it allows the automatic supply of known concentrations of food at each dilution cycle as well as the testing of volatile chemicals. The system has operated for almost 3 years and has proven to be reliable, accurate and easy to maintain. In order to illustrate its usefulness in tests with Daphnia populations, the toxicity of copper was tested. Other examples are given in Van Leeuwen et al. (1985; 1986; 1987).

MATERIALS AND METHODS

The system described was built to supply a variable flow of water (0.1 to 10 L/h), six toxicant concentrations and a control. It consists of a 500-L heavy duty polyethylene storage tank for lake water, a water delivery pump, a 30-L all-glass constant-level head tank with an overflow to the storage tank (12mm ID silicone tubing), a food supply device consisting of a magnetic stirrer

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and a peristaltic pump, a glass flow-splitter (53x10x20 cm) consisting of seven 1-L volume compartments, a level-sensor (Vegator 256, Schiltach, FRG), a six-channel syringe pump (Braun, Melsungen, FRG) with all-glass 50-mL syringes and teflon tubing to deliver toxicant solutions. This perfusor allows an injection volume of 96 µL/h to 390 mL/h. The daphnids are exposed in seven glass test vessels of 20-L each which consist of outer and inner containers. The outer containers are connected to a constant-level drain at the rear of the apparatus by means of flexible silicone tubing. The 12-L inner containers (30 cm x 23 cm ID) are divided into four compartments with nylon mesh screens covering the bottoms to prevent escape of daphnids. On top of the test vessels are 10-cm long glass flow-splitting cylinders (5 cm ID) mounted on glass bases of 30x30 cm. The cylinders are drained through four 5-cm capillaries (glass, 3 mm ID) to control the flow to the test compartments. An overall view of the apparatus is shown in Figure 1. The solenoids applied (Gemü, Ingelfingen-Criesbach, FRG) open when energized. They have wetted PVC parts and soft valves (Viton) which ensure tight closure. The low line pressure allows the use of large bore seats (10-12 mm ID). The operation of the system is controlled by an adjustable automatic reset interval timer (Schleicher, Dassel, FRG; Figure 2). This electromechanical relay can be manually set to provide a cycle range from 0.2 sec to 60 h. In practice only 5-min to 6-h intervals are used which provide flow rates of 20 L/h to 4 L/day. In the event of power source failure the relays return to their starting positions to ensure safe operation conditions.

Water, algae and toxicant are delivered intermittently. During a renewal-cycle water is pumped from the reservoir tank to the constant-level head tank which is connected to the flow-splitter by means of the water supply solenoid. When the solenoid is activated water flows into the flow-splitter where it is vigorously mixed with the algal suspension at the inlet. The compartments of the flow-splitter are subsequently filled. The first compartment fills and then overflows into the second compartment, etc. until the last compartment is filled and the water level reaches the upper level sensor thus cutting off the electricity supply to the water supply pump, the water supply solenoid and the peristaltic pump for the food suspension. At the same time the solenoids under the flow-splitter are activated and open. The perfusor is also activated. This pump is connected to a timer set for 30 sec in order to stop the delivery of the toxicant solutions before the flow-splitter compartments have emptied.

The injected toxicant solutions are transported by a teflon tube to an Y-shaped glass mixing tube where the turbulence of the incoming water ensures adequate mixing. The flow-splitting cylinders receive the 1-L volumes from the mixing tube and deliver 250 mL to each of the four replicate compartments. After the water level has reached the lower level sensor in the flow-splitter the solenoids are no longer activated and close again. In all experiments with D.magna the automatic reset interval timer was set for a 90-min interval.

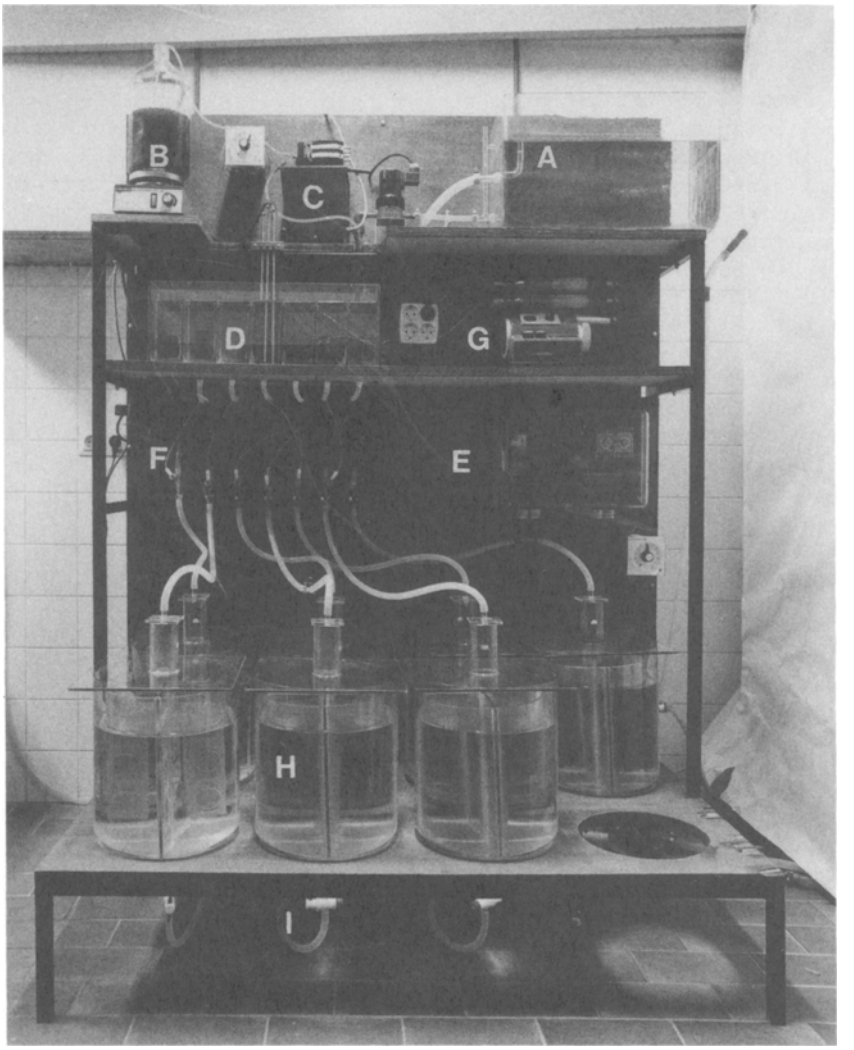

Figure 1. Intermittent-flow system for population toxicity studies. Main components: A, constant-level head tank; B, storage vessel for algae and magnetic stirrer,; C, peristaltic pump; D, flow-splitter with level sensor; E, electronic circuit with reset timers; F, solenoids and mixing tubes; G, syringe pump; H, test vessel which consists of an outer and an inner container; the container is divided into four compartments; I, drain (partly visible).

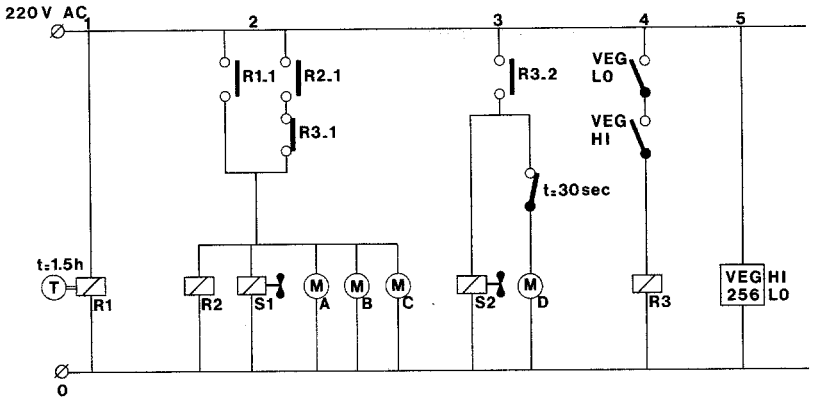

Figure 2. Basic wiring diagram of timer circuit, solenoids, pumps and level sensor. Legend: A, water supply pump; B, magnetic stirrer; C, air pump (optional); D, syringe pump; R, relay; S_1, water supply solenoid; S_2, cell-solenoid (7x); T, timer; VEG, Vegator level sensor.

The experiments with D. magna were carried out in a constant-temperature room of 20 ± 0.5°C with a 12-h photoperiod. The test medium used was 50-µm filtered, UV-sterilized Lake IJssel water with a pH of 8.1 and a hardness of approximately 225 mg/L (as $CaCO_3$). The toxicity of copper ($CuCl_2$, Merck; chemical purity >99%) was tested both with cohorts in semistatic 21-day experiments and in population toxicity experiments under flow-through conditions. The semistatic experiments were started with neonates, randomly distributed into cohorts of 10 animals each in five toxicant concentrations and a control. The number of surviving females and the number of neonates produced were recorded daily, with new neonates discarded from the test vessels after counting. After 3 weeks, the experiments were terminated and the carapace length was determined using an ocular micrometer. Population dynamics of D. magna under different levels of copper stress were studied in the intermittent-flow system described above. The test was initiated with exponentially growing populations of 20 daphnids composed of cohorts of different ages. The total number of daphnids in each test compartment (biomass) was counted at regular intervals. The effects of copper on the population dynamics of D. magna were calculated by means of a parametric model developed by Kooyman et al. (1983). The experimental procedures have been described in full detail in previous papers (Van Leeuwen et al. 1985; 1986; 1987). The copper concentrations were analyzed by atomic absorption spectrophotometry. All results are based on actual copper concentrations.

RESULTS AND DISCUSSION

From existing literature it appears that the objections to the use of moving parts, in particular electric pumps, have led to unnecessary complications in the construction of flow-through systems. Introduction of mechanical and electric devices has simplified such systems. The reliability of the system described in this paper proved to be high. No problems occurred during the almost 3 years of testing. One reason for this may be its low renewal frequency, i.e., its long passive and short active periods, which keeps mechanical wear and tear low. Due to its durability and simple construction the maintenance demands are negligible. During the evaluation period of the system the mean coefficient of variation (C.V.) of concentrations of 10 compounds, including copper (cf. Van Leeuwen et al. 1987), was 10.2%. The lowest C.V. was 1.57% (for sulphuric acid expressed as pH). The highest C.V. was found for pentachlorobenzene (20%). These C.V.s represent diluter error and analytical error. The C.V.s for lipophilic, water-insoluble compounds such as pentachlorobenzene probably also include errors due to sorption. The mean C.V. in the test with copper was 11%. Verification of the densities of the algal suspensions in the compartments of the flow-splitter box showed that the variation among these compartments was well below 5%.

The results of the life-table experiments with daphnids are shown in Table 1. The main cause for the reduced intrinsic rate of natural increase (r_m) at a total copper concentration of 110 µg/L was increased mortality rather than reduced reproduction. This is in line with previous observations (Van Leeuwen et al. 1987). The 21-d LC_{50} and its 99% confidence limits (C.L.) was 69 (37-110) µg/L. As reported earlier (Van Leeuwen et al. 1985; 1987) the present study also confirms that individual growth can be a sensitive index of toxic stress. The carapace length of daphnids was significantly reduced at a copper concentration of 37 µg/L.

Table 1. Demographic information for Daphnia magna from 21-day semistatic experiments at various concentrations of copper.

Concentration (µg/L)	Survival (%)	$r_m \pm SE$[a] (day^{-1})	Carapace length±SE (mm)
2.6	96	0.335±0.022	3.91±0.13
3.9	92	0.341±0.009	3.92±0.12
6.1	94	0.348±0.015	3.87±0.16
12.6	100	0.335±0.015	3.87±0.16
36.8	100	0.354±0.009	3.59±0.15[b]
110	0[b]	-[b]	-

[a] Intrinsic rate of natural increase
[b] Lowest concentration significantly different ($\alpha < 0.01$) from the control

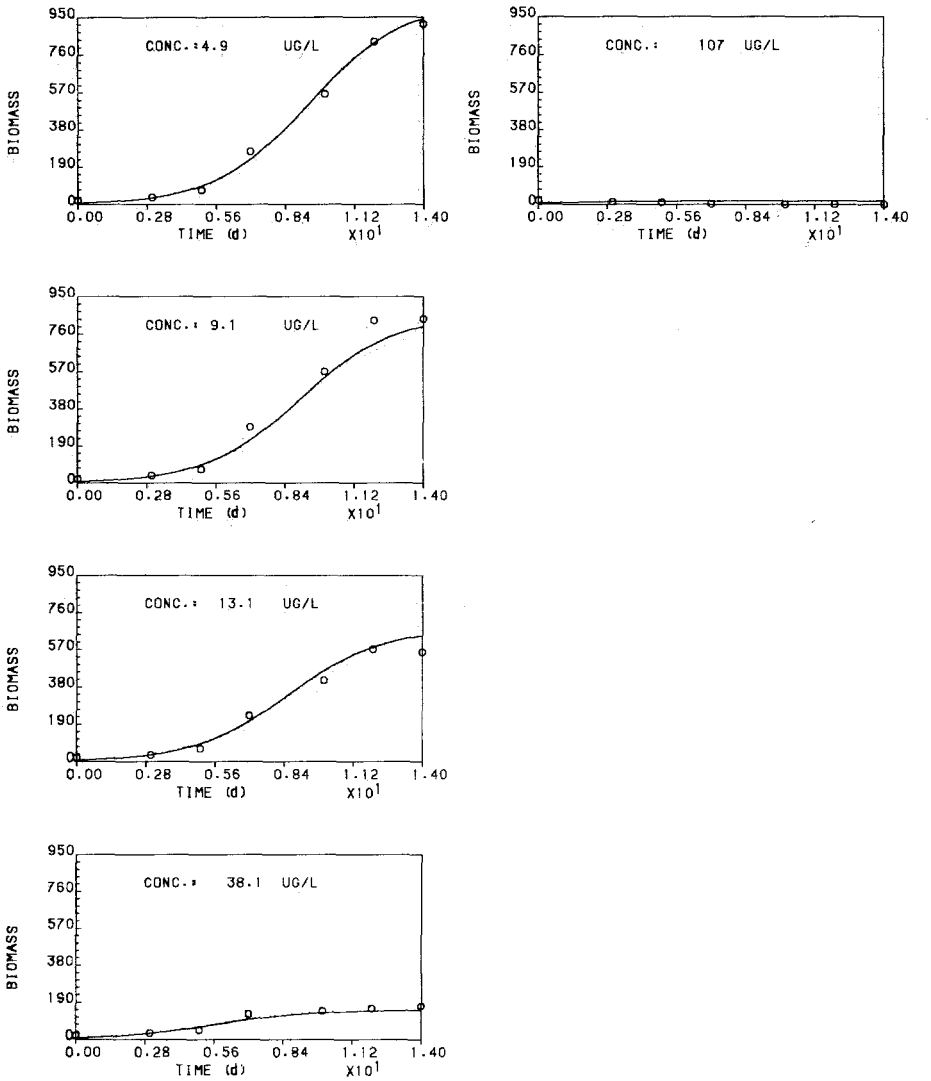

Figure 3. Effects of copper in a 14-day experiment with Daphnia magna populations. Circles represent the observed and lines the expected values based on model calculations. Biomass represents the mean number of daphnids of four replicate (3-L) compartments.

In the population experiment copper-induced effects were observed at much lower concentrations. Population growth, i.e., the upper numerical limit or yield, was clearly reduced at a concentration of 9.1 µg/L (Figure 3). The 21-day EC_{50} for this parameter was 16.1 µg/L. Its 95% C.L., was 12.2-21.3 µg/L. The 21-day EC_{10} was 5.9 µg/L. These low effect concentrations are probably caused by the fact that in population toxicity studies daphnids become additionally stressed by food-limitation. However, changes in the speciation of copper due to high densities of the Daphnia populations by the end of the test may be an important factor as well. Daphnids are known to be in close contact with their environment due to their relatively high filtration rates (approximately 0.1 L per individual of 3 mm at 20°C in 24 h; Burns 1969). These results confirm Winner's conclusion that copper is toxic in hard water (Winner 1985). The reason for this may be that in hard water Cu^{2+} is displaced from binding sites on humic substances by Ca^{2+}, Mg^{2+}, or both (Winner 1986).

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