Wat. Res. Vol. 21, No. 10, pp. 1249–1257, 1987
Printed in Great Britain.

0043-1354/87 $3.00 + 0.00

A MICROCOMPUTER-INTERFACED CONTINUOUS FLOW TOXICITY TEST SYSTEM

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(*Received October 1986*)

Abstract—While continuous-flow tests for toxicity evaluation are preferable over static tests, their use has been limited due to problems associated with their operation. Fluctuations in toxicant concentrations during exposure periods requires frequent analyses and represent a drawback in conventional diluter systems. To reduce toxicant fluctuations and to maintain suitable water quality during long-term test periods, a low cost microcomputer-interfaced monitoring system (MIMS) was installed on a Benoit type serial diluter. The system monitored flow rates of test solutions and measured a number of water quality parameters.

The MIMS system provided up-to-date information on whether the test was progressing well and indicated when diluter maintenance was needed. The MIMS interfaced diluter system performed well in long-term continuous-flow tests with minimal disruption and eliminated experimental failure.

Key words—aquatic bioassays, continuous-flow tests, fish bioassays, microcomputer, biomonitoring, toxicant analysis

INTRODUCTION

Continuous-flow toxicity tests have many advantages over static tests by providing constant levels of toxicant solution and a controlled environment for the test organisms. Hence, many "Standard Methods" recommend this form of testing as a norm for exposing fish, macroinvertebrates, and amphibians (ASTM, 1980; APHA, 1985; U.S. EPA, 1985). In recent years, flow-through exposures have been employed in short-term acute tests, extended period lethal tests over a 96 h period, long-term sublethal tests, early life stage (ELS) tests, and bioaccumulation tests (Buikema *et al.*, 1982; Rand and Petrocelli, 1985; Mount, 1979; Bishop and Maki, 1980).

With extended exposure time in continuous-flow tests, the possibility of experimental failure is increased, mainly due to malfunctions of the toxicant delivery system. Numerous toxicant delivery systems have been developed and modified over the last two decades. However, some physical limitations are inherent, even in diluters designed to deliver several concentrations of test solutions over long-term test periods. Fluctuating toxicant levels caused by diluter malfunction were the most frequently observed problem. Since toxicant concentrations can not be determined instantly for most chemical compounds, the variation in toxicant level is not immediately noticed. Frequently, this problem leads to unreliable data or even experimental failure. Other factors such as toxicant and dilution water over-flow, dry-up, a sudden change in water quality, and a breakdown in the test water delivery lines may also result in test failure. It is not always possible or practical to repeat the experiment, since costs for the long-term flow-through test are considerable.

Many of the problems encountered in long-term continuous-flow tests can be resolved by closely observing the test system during the experiment. However, continuous surveillance of the test system by the experimentor is not always possible. To circumvent this, a monitoring method for the continuous-flow toxicity test system was developed. The following contains a general description of a microcomputer-interfaced monitoring system (MIMS) and its operational principles. The performance of this system is evaluated in terms of data accuracy and associated costs.

SYSTEM HARDWARE DESCRIPTION

Installation of microcomputer-interfaced monitoring system (MIMS)

The Benoit diluter system, a compact serial diluter developed at the U.S. EPA Duluth Laboratory (Benoit *et al.*, 1982), was selected for a MIMS installation because of its small size and its wide applicability. As suggested in the construction manual of the system (Benoit *et al.*, 1981), the diluter was designed to deliver five different concentrations of toxicant and one control at a flow rate of approx. 3500 ml min^{-1}. A total of 24 exposure chambers measuring 9.5 W × 20.0 L × 14.0 H cm provided four replicates

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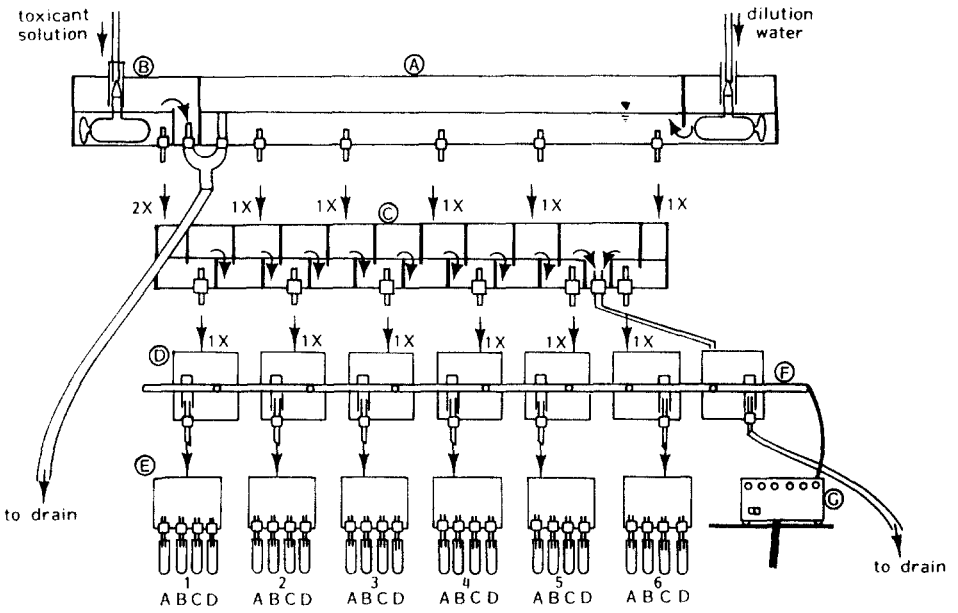

Fig. 1. Schematic of Benoit mini-diluter and flow-monitoring system. Ⓐ Constant head dilution water; Ⓑ constant head toxicant; Ⓒ dilution cell; Ⓓ flow booster cell; Ⓔ flow splitter cell; Ⓕ i.r. emitters–detectors; Ⓖ flow monitoring box.

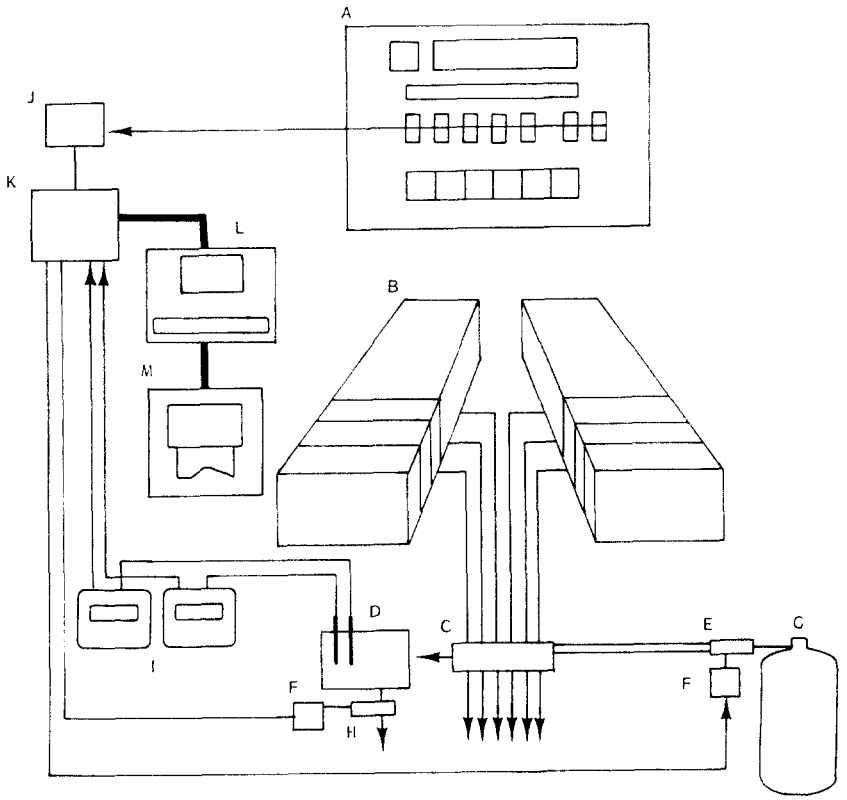

Fig. 2. Schematic of continuous-flow toxicity test system with microcomputer-interfaced monitoring system (MIMS). A—diluter board; B—exposure chambers; C—6-position rotary valve; D—sampling chamber; E—pneumatic solenoid valve; F—BSR modules; G—compressed air tank; H—discharge solenoid valve, I—transducers; J—flow-monitoring box; K—ADC-1 data acquisition and control system; L—microcomputer; M—printer.

for each concentration. Stock toxicant solution was delivered to the constant head cell by a peristaltic pump and the dilution water was supplied to the diluter by gravity (Fig. 1). The MIMS was designed to carry out two principal tasks: (1) to monitor test solution flow rate through each concentration channel and (2) to monitor water quality variables in the exposure chambers. The MIMS system consists of a microcomputer, a flow rate detection unit, a water quality sampling chamber, and an electronic signal processor (Fig. 2).

Diluter modification

Before the MIMS was attached, minor modifications were made to accommodate electronic sensors in the flow booster cells to improve the accuracy of test solution delivery volume from the flow splitter cells to the exposure chambers. First, the siphon tubing within the flow booster cells was altered to prevent irregular discharges of water due to entrapped air bubbles between the inner and outer tubes of the siphons (Fig. 3). To alleviate this problem, the flow booster cells were made larger than the original design. In addition to the six flow booster cells originally suggested, another cell was installed at the end to receive discharge water from the dilution cell (Fig. 1). Thus, the amounts of toxicant and dilution water transferred to the diluter were equal to the amount of the water discharged to the seven flow booster cells.

Secondly, the original capillary tubes (1.5 mm i.d.) of the flow splitter cells were replaced with tips of borosilicate glass Pasteur pipets, since slightly unequal distribution of water from each cell to the four replicate exposure chambers was frequently observed (Fig. 3). They were much thinner than the standard capillary tubes, while the internal diameter remained the same. The open space between the capillary tubing and the distribution tube prevented the entrapment of air inside the distribution tube and hence facilitated adequate gravity flow. No further problems were experienced in delivering test solution to the exposure chambers with these changes.

Test solution flow-monitoring device

Previous experience with the Benoit system in our laboratory revealed fluctuating toxicant concentrations during the test period (Meier, 1983). These variable concentrations were mainly due to clogging of capillary tubes by attached microorganisms and deposition of scale. Other causes of toxicant fluctuation were associated with the interruptions of

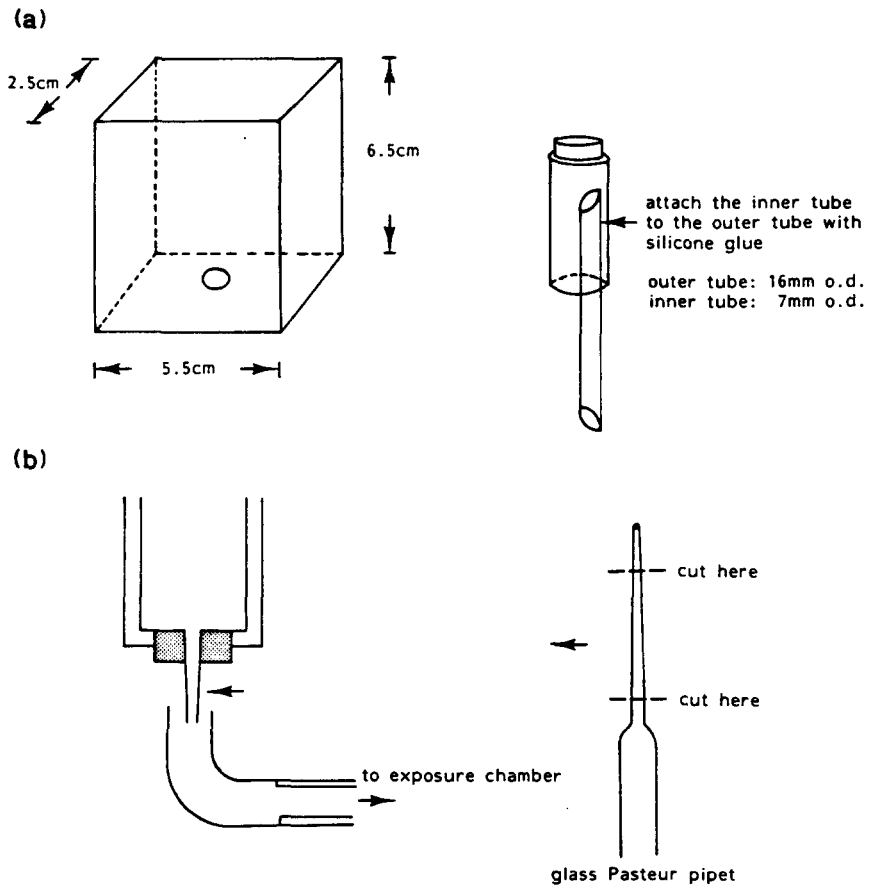

Fig. 3. Modification of Benoit mini-diluter components. (a) Flow booster cell with siphon; (b) flow splitter cell.

toxicant and dilution water delivery due to malfunctions of pumps, electrical power failures, and dry-up of toxicant tank.

Instantaneous detection of toxicant level is the most ideal confirmation that the dilution system is working properly. However, it is not feasible in most tests because of the difficulty in the chemical analysis of toxicant. Therefore, an alternative method of monitoring toxicant concentrations was devised in this approach.

The principle of the flow monitoring device is based on the assumption that as long as the amount of toxicant and dilution water delivered to the diluter was constant and the flow rates through the seven flow booster cells remained stable, the toxicant concentrations delivered to the exposure chambers should remain the same (Fig. 1). Fluctuations caused by the incomplete mixing in the dilution cell were considered minor.

Since consistent replacement of test water in the flow booster cell occurred after improving the siphon system as described, the rate of fill-up and discharge of the test water was monitored as a parameter of the amount of flow for each concentration. It should be noted that the booster cell modification is not necessary for normal operation. A pair of i.r. (infrared red) emitters and detectors (Radio Shack Catalog 276–142) were installed in front and in back of each flow booster cell (Fig. 4). The i.r. light emitted was absorbed by the test water when the flow booster cell was filled and thus no light was detected by the detector. However, when the test water was discharged, the sensor detected i.r. light and the flow monitoring box amplified the electric signal (Fig. 1). A typical example of the analog signal, the voltage change, generated by the flow monitoring box is shown in Fig. 5. By monitoring this voltage change through an A/D converter the microcomputer counted the rate of replacement volumes of test water in each booster cell. Higher or lower frequency of voltage changes in one or more channels indicated abnormal diluter function.

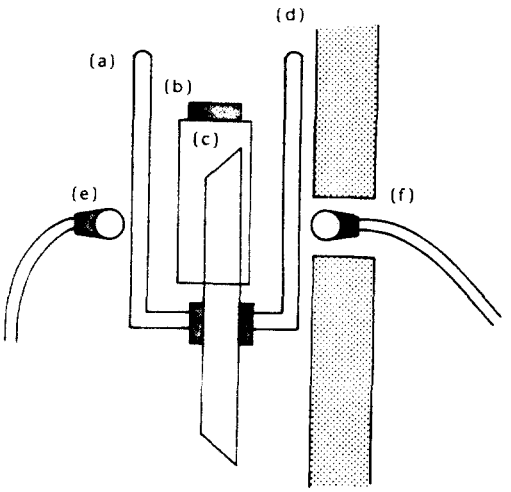

Fig. 4. Schematic of infrared (i.r.) emitter and detector installation. (a) Flow booster cell; (b) outer siphon tube; (c) inner siphon tube; (d) diluter board; (e) i.r. emitter; (f) i.r. detector.

The amount of flow delivered through each flow booster cell was determined by multiplying the working volume of the cell by the number of replacements in a given time period. The total volume of test water delivered to the diluter, e.g. toxicant and the dilution water, was the sum of the amount of flow in seven cells; six test water cells and one discharging water cell.

Test solution sampling chamber

A test solution sampling chamber containing sensing probes for temperature, dissolved oxygen, conductivity, and pH was designed to receive automatically the discharges from the chambers (Fig. 6). Discharge water from each exposure chamber was collected by common lines serving each of the six different toxicant concentrations. Each line entered the six-position rotary selection valve (Rheodyne, Anspec Catalog H2420) which alternately fed the test water from a set of exposure chambers through a line

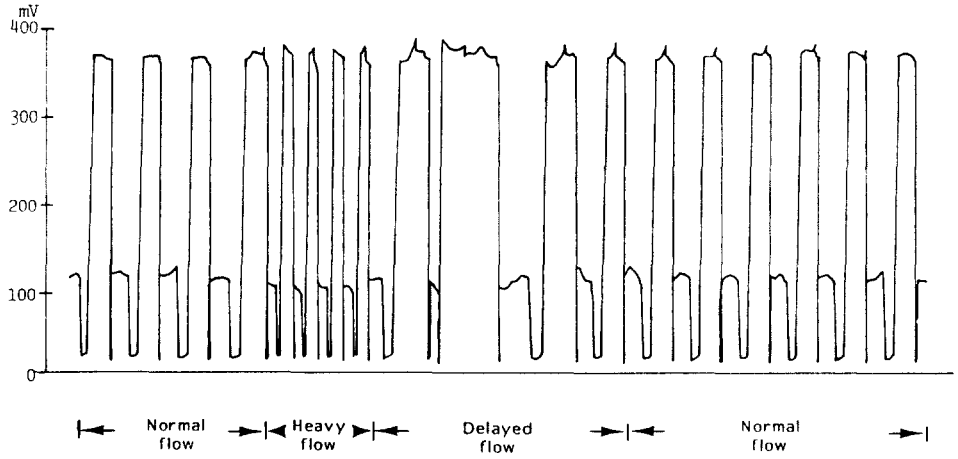

Fig. 5. Example of voltage signals generated from monitoring box.

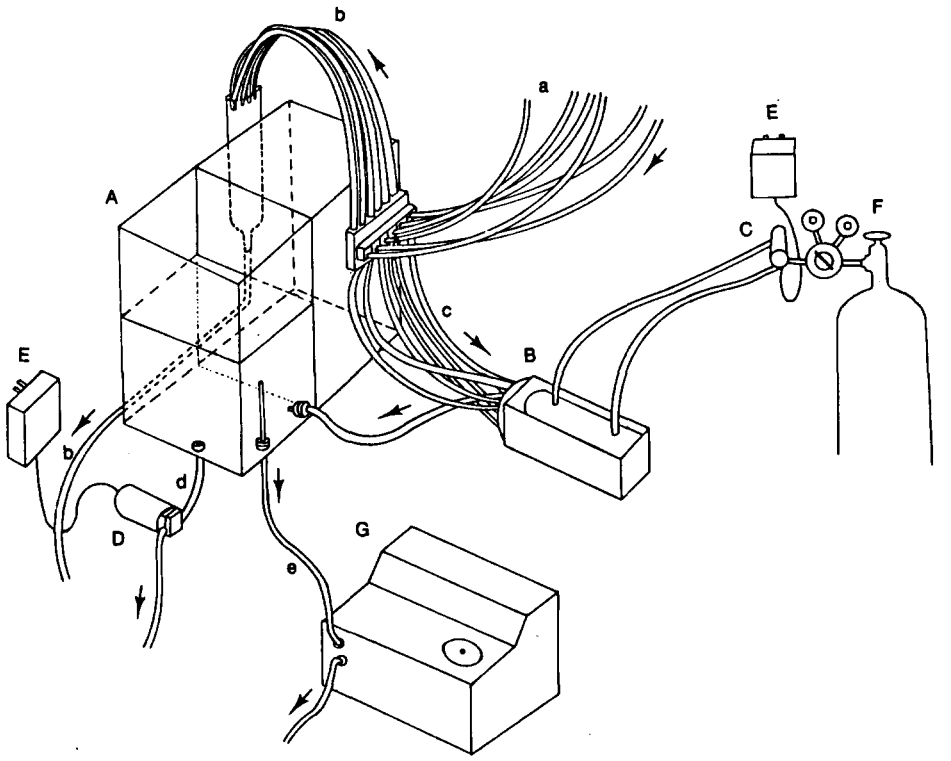

Fig. 6. Schematic of test solution sampling box located under the exposure chambers. A—Sampling chamber; B—6-position rotary valve; C—pneumatic solenoid valve; D—discharge solenoid valve; E—BSR modules; F—compressed air tank; G—fluorometer; a—common lines from the exposure chambers; b—drainage; c—inlet lines to the sampling chamber; d—sample water drainage; e—additional sample collection line.

into the sampling chamber. The valve position was changed at a designated time interval by the computer. The switching of the input lines was conducted by turning on and off a pneumatic solenoid valve (Anspec Catalog F1930).

A solenoid valve was installed on the outlet line of the sampling chamber to control the water discharge. This valve and the pneumatic solenoid valve were controlled by generating the signals at the ADC-1 data acquisition and control system (see "Microcomputer interfacing"). The signals were transmitted over AC wiring to BSR modules through which the valves were connected to the power line. In this design, the sampling chamber was situated beneath the exposure chambers and was filled through gravity flow (Fig. 2). The electrical signals generated by the instruments were taken from their recorder output and connected to the A/D channels of the ADC-1. Other devices which require the transfer of samples to a detection unit, e.g. a fluorometer or a spectrophotometer, may be incorporated by connecting delivery tubes from the sampling chamber (Fig. 6).

Microcomputer interfacing

A Radio Shack Model 100 microcomputer in conjunction with an ADC-1 data acquisition and control system were used as a data processing and control device. The Model 100 is a lap size, low-cost microcomputer with several built-in features such as BASIC, a real time clock, a modem, and an RS-232C interface. The basic requirements for a microcomputer in this test system are: (1) an RS-232C interface which makes it possible to communicate between the computer and ADC-1 system; (2) a real-time clock to collect data and control devices according to the preprogrammed schedule; (3) a data storage capability such as a disk drive; and (4) the availability of peripherals like a printer. In this research, a printer was connected to the Model 100 and all data were printed out.

The ADC-1 data acquisition and control system (Remote Measurement Systems Inc., Seattle, Wash.) is a low cost, multichannel A/D converter with the following features: (1) 16 analog channels with input range of ± 0.4096 V and a resolution of 100 μV; (2) 4 digital input channels; (3) remote control of the devices via signals transmitted over AC wiring to BSR modules: and (4) an RS-232C communication port. Again, other commercial or custom-made multichannel A/D converters could be used in place of the ADC-1.

Seven lines from the flow monitoring box were connected to the ADC-1 analog input channels, numbers 1–7, respectively. The lines from a pH meter and a DO meter were connected to the analog channel 8 and 9. Additional instruments could be

connected to the channels 10 through 16. Since the analog input voltage range is limited to +0.4096 V in the ADC-1, the signals from the flow monitoring box and the instruments were conditioned for this range. Two BSR modules (Radio Shack Catalog 61-2684) were installed between the power lines of the solenoid valves and the wall sockets for controlling them. The microcomputer and the ADC-1 were connected using an RS-232C communication cable.

OPERATIONAL SOFTWARE

Data processing system check

Four test programs were run prior to an actual experiment to evaluate the data collection system. These were: (1) proper signal generation from the flow monitoring device; (2) turning on and off the solenoid valves through the BSR modules; (3) generation of signals from the instrument; and (4) the communication between the microcomputer and the ADC-1.

Three BASIC programs were developed for startup tests. FRTE.BA (Flow-Rate TEst.BAsic), when executed, took signals from the designated analog input channel on the ACD-1 and displayed the plot of input voltage vs time on the computer screen. If the plot was not similar to the curves shown in the Fig. 5, it was modified by manually adjusting the potentiometers on the circuit inside the flow monitoring box. BSRCN.BA (BSR CoNtrol) turned on and off two solenoid valves at designated time intervals.

Another program, SIRGN.BA (SIgnal ReGressioN), checked the signals from the instruments at different nominal values (e.g.) at pH 5.0, 6.0, 7.0, and 8.0 when a pH meter was used) and calculated the slope and the intercept of the plotted regression line, nominal values vs input voltage. Values saved in a RAM file, REGRN.DO, were recalled by the main operation program, SYSMO.BA (SYStem MOnitoring) every day over the duration of the toxicity test.

Long-term system monitoring

The main program, SYSMO.BA, could be started at any time of the day, but it was designated to stop execution at 10:00 a.m. the next day. Regular maintenance work was carried out at that time. Printer output was examined for operational problems, such as changes of flow or in water quality, that may have occurred overnight. As soon as these tasks were completed, the main program was restarted.

The data collection process was controlled by a real time schedule (Fig. 7). The operational program was designed to monitor the flow rates as well as the water quality every 30 min, assigning 4 min to each channel. Within this time interval, the program would initiate the change of test water in the sampling chamber from one exposure concentration to the next by briefly turning on and off a pneumatic solenoid valve. After 3 min, the time necessary to fill the sampling chamber, the system recorded water quality data. Meanwhile, the system also monitored the flow booster cells and counted the frequency of test water replacement through the cells. Finally, the computer opened the drain valve of the sampling chamber and the test water was replaced with exposure water of the next replicate chambers. At the end of the cycle there was a 2 min recess period which was devoted to data processing, data storage into the RAM file, print out of a summary of flow rates and water quality, and a screen display of the operational results.

If there were a sudden rate change in the flow booster cell(s), over ±5% of predetermined levels, the computer would generate an alarm and display the cause of problem(s) until it was manually corrected. The alarm system would also be set off when a considerable change in water quality was detected.

At the end of every 30 min, the data on flow rates and water quality were stored in the data file under

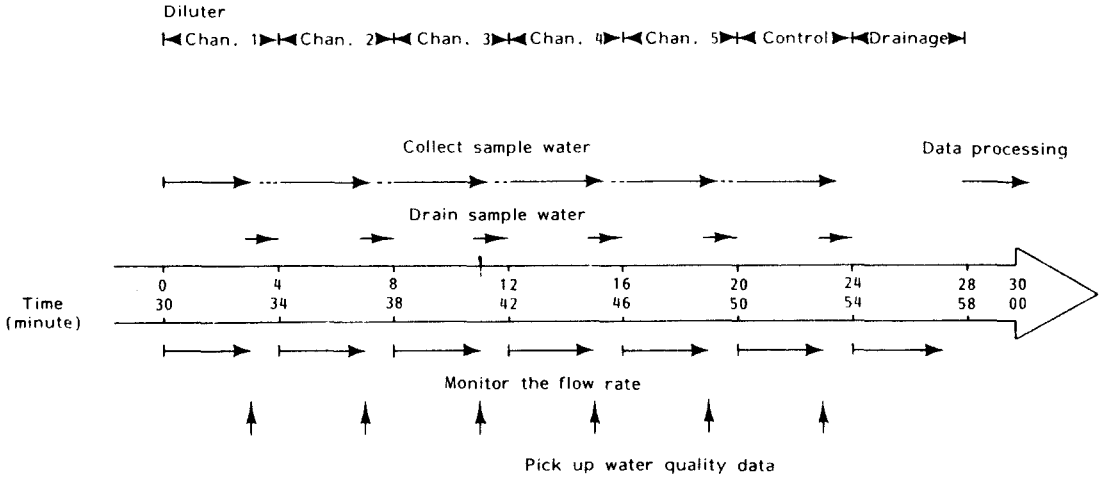

Fig. 7. Computer executed monitoring of real time schedule of continuous-flow toxicity test system.

a different file name each day. For example, the data of 11 February were stored in the file "D0211.DO".

RESULTS AND DISCUSSION

Toxicant level fluctuations

Prior to the installment of the MIMS on the Benoit system, a 2-week trial test was carried out to determine the fluctuations of toxicant concentration on this diluter system by using sodium chloride. During the test period, toxicant tank conductivity was set at 6800 μmhos and five different concentrations were measured daily from the exposure chambers with a YSI model 33 conductivity meter. Routine maintenance of the diluter system was carried out every morning after the samples were taken. Cleaning of diluter flow tubes was performed more often due to capillary tube clogging which was attributed to the hardness of the dilution water.

Although careful maintenance was practised, large fluctuations of toxicant were observed during the test period (Fig. 8). The overall mean SD of five channels was about 15%. However, the lower concentration channels, demonstrated a wider fluctuation because of inappropriate mixing in the dilution cell combined with the effect of capillary tube cloggings. For comparison, the fluctuation of toxicant concentration data published by Spehar *et al.* (1982) who also used a Benoit diluter for long-term testing, were similar to these results and hence appeared to be normal for this type of diluter design.

The new diluter system which incorporated the MIMS was evaluated by carrying out four 2-week long tests by using diquat (1:1′-ethylene-2:2′-dipyridium dibromide) and fathead minnows *Pimephales promelas*. The MIMS worked well throughout the whole test period and no system malfunction was observed. Maintenance of the mini-diluter was performed only when the computer generated an alarm.

To evaluate the fluctuation of toxicant levels in this system, test samples were taken daily from the exposure chambers during two separate 14-day experiments and analyzed at a 310 nm using a Beckman Acta III spectrophotometer (Summer, 1980). Results of toxicant fluctuations for this system were plotted (Fig. 9). The overall mean SDs of the toxicant in these experiments were 8.3 and 3.95% respectively. The variations of diquat levels in these long-term experiments were considerably lower (6.1% mean deviation) than that of sodium chloride levels obtained without the MIMS (15.0%). Even though these two tests are not directly comparable, they indicate that a MIMS system identified problems early and allowed timely corrections.

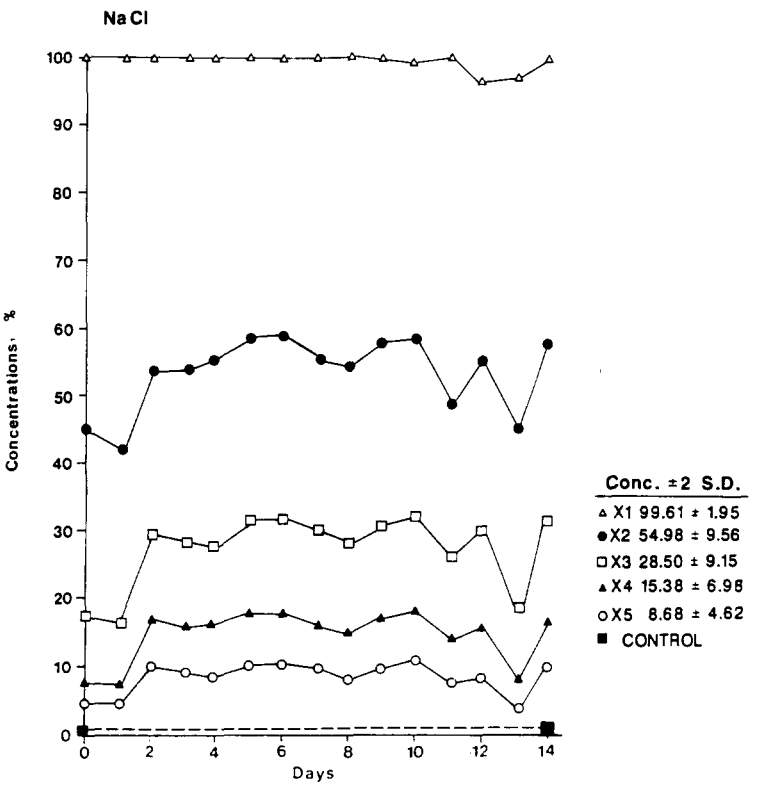

Fig. 8. Fluctuations in NaCl concentrations obtained from Benoit diluter system without microcomputer-interfaced monitoring system (MIMS). NaCl concentrations represent the mean of 14 observations ±2 SD.

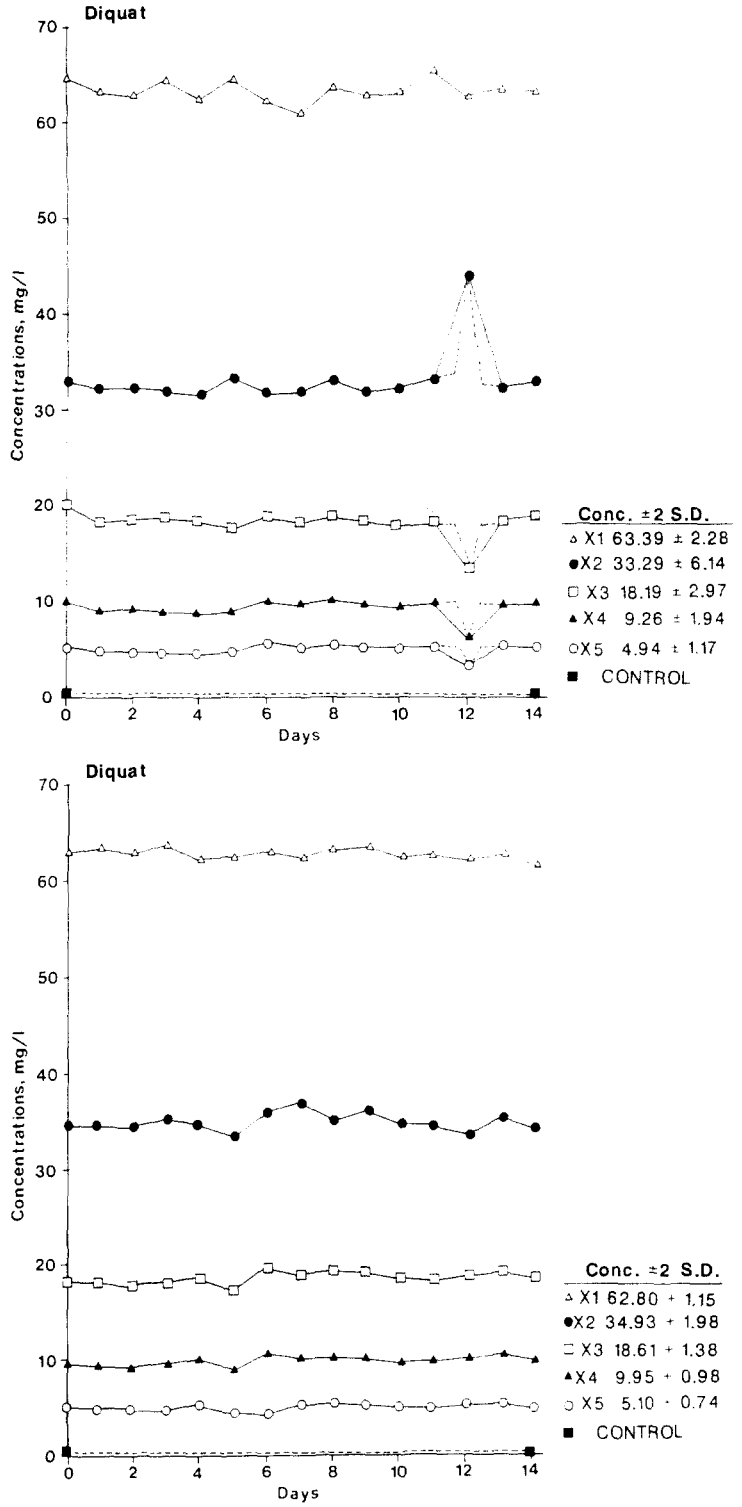

Fig. 9. Fluctuation of diquat concentrations in Benoit diluter system with microcomputer-interfaced monitoring system. Diquat concentrations represent mean of 14 observations ±2 SD.

Water quality parameters

The fluctuations in DO, pH, and temperature were minimal and the MIMS system monitored them successfully throughout the test period. This system also provides the capabilities to adjust various water quality parameters that would assure reliable toxicity data under certain situations. For example, variable pH in an industrial effluent could be adjusted and maintained before it is delivered to the diluter. Under

this condition, a pH meter and controller could be installed on the toxicant tank and connected to the microcomputer. By using a BSR module as a on-off switch for the pH controller, it would be possible to regulate toxicant pH during the exposure period.

Early detection of diluter malfunction

One major advantage of the MIMS system is that it provides up-to-date information on diluter system performance during long-term experiments. Hence, the experimentor would know whether the toxicity test was progressing well and/or when maintenance was needed. By timely correction of malfunctions within the test system, one can prevent the failure of experiments in most cases.

Furthermore, based on the computer output the decision on whether to terminate the ongoing test or not can be made, if an abrupt change of toxicant concentration had occurred. For example, a major diluter malfunction was detected during one experiment (see Fig. 9, Test A day 12). In this case, the information generated by the microcomputer revealed that the dilution water supply to the diluter was inadequate during the night. Since the time of occurrence was recorded by the computer, the duration of diluter malfunction was estimated to be 4 h. Based on this information, it was concluded that the possible effect of the changing toxicant concentration on fish would be negligible. Thus, it was decided to continue the experiment.

CONCLUSION AND SUMMARY

For the last two decades, investigators developed numerous types of dilution systems to improve the quality of toxicity testing. Even to date, many inherent problems associated with the diluter systems exist. None of the designs at present insure long-term constant delivery of toxicant, unless frequent and careful maintenance are provided. The delivery of several concentrations of toxicant at relatively low flow rates for long periods of time would be the basic requirement of the diluter. However, this is not obtainable by merely improving the design of the diluter configuration with reasonable costs. Therefore, no reliable and trouble-free continuous-flow test system can be constructed if one depends on the dilution mechanism alone.

The MIMS system explained here resolves most of the problems. It provides continuously up-to-date information on diluter system performance in long-term tests. The most important output generated by the computer are the actual flow rates of test solution through different toxicant level channels. This output relays information on whether the toxicity test was progressing well and/or when diluter maintenance was needed. Based on this information, long-term continuous-flow toxicity tests can be carried out with a high probability of not only completing the tests, but also generating good quality toxicological data.

We have estimated that a long-term toxicity test can be performed with this system at approx. 20% of labor compared to existing systems. The cost for installing a MIMS system on an existing Benoit diluter is <$1500 which includes the cost of the microcomputer. Hence, this system is cost effective. Since toxicant solutions are provided with less fluctuation and water quality is monitored, less frequent sampling is needed. Therefore labor required for routine diluter maintenance and toxicant analyses is reduced. The chances of test failure are lessened, because of the constant availability of test system performance information.

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