An Improved Chemical Delivery Apparatus for Use in Intermittent-flow Bioassays

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Flow-through systems (diluters) represent a distinct improvement over static systems for determining the toxicological and physiological effects of pesticides in aquatic organisms. Many of the intermittent-flow toxicant systems that have been devised incorporate a host of movable components, some of which are expensive or are delicate and difficult to construct. The delicate assemblies especially necessitate frequent and time-consuming cleaning, adjustment, and repair in order to maintain dependability of operation.

BURKE and FERGUSON (1) reviewed many of these problems and proposed a simplified method of toxicant delivery. McALLISTER et al. (2) devised a metering device which contained no moving parts and delivered small volumes of toxicant accurately. The Mariotte bottle is commonly used as a toxicant reservoir. However, it is difficult to fill and may require insulation where ambient temperatures vary considerably.

The chemical delivery apparatus used for our chronic studies does not require the use of a Mariotte bottle and is designed to replace the troublesome metering devices on existing intermittent-flow systems without altering their basic function. It is easily adapted for use on diluters previously described (3, 4), is free of moving parts, and may be constructed quickly of materials available in a well equipped laboratory. Delivery of small or large volumes of toxicant is made possible merely by a variation in the volume of the device.

Construction of Metering Device

The continuous-flow apparatus described in Figure 1 alleviates many of the problems and inconveniences of the Mariotte system and may be constructed to fit the needs of the investigator. Double-strength glass and flint glass tubing are used throughout the system, and an inert form of aquarium or glass cement (Dow-Corning 781 Building Sealant[1/]) is used to join glass surfaces. Latex rubber tubing is used to join the various components and is replaced when a different toxicant is used or when it becomes brittle from exposure to solvents.

1/ Reference to a product does not imply recommendation to the exclusion of others that may be suitable.

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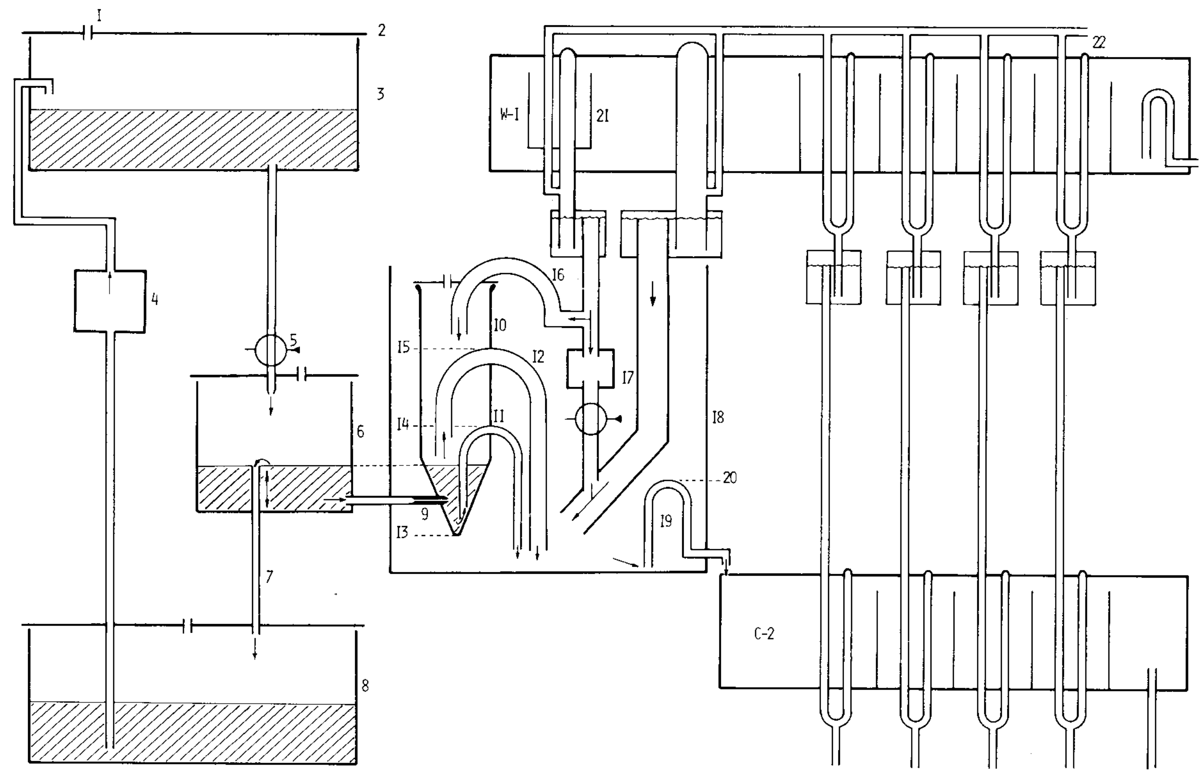

FIGURE I. METERING DEVICE AND CONTINUOUS-FLOW TOXICANT SUPPLY IN RELATION TO THE CONVENTIONAL FLOW-THROUGH APPARATUS (NOT TO SCALE). LEGEND: I, VENT; 2, LID; 3, TOXICANT HEAD TANK; 4, PERISTALTIC PUMP; 5, GROUND-GLASS STOPCOCK; 6, STANDPIPE RESERVOIR; 7, STANDPIPE; 8, LOWER STORAGE RESERVOIR; 9, CAPILLARY TUBE; I0, CENTRIFUGE TUBE; II, I2, SIPHONS I, 2; I3, I4, I5, TOXICANT LEVELS; I6, WATER REGULATOR TUBE; I7, WATER REGULATOR; I8, MIXING BOX; I9, SIPHON 3; 20, LIQUID LEVEL AT WHICH SIPHON 3 IS INITIATED; 2I, WATER REGULATION CHAMBER; 22, VACUUM MANIFOLD; W-I, C-2, WATER AND CHEMICAL CELLS, AS IN MOUNT AND BRUNGS (4).

The metering device consists basically of a 15-ml conical centrifuge tube that is fitted with two siphons and a capillary tube. The hole for the capillary tube is carefully drilled in the bottom of the centrifuge tube (placement is not critical) with a hand grinder (Dremel Moto-tool[2/], fitted with a No. 953 emery wheel point). The tapered end of a length of capillary tube (0.75 mm I.D., 25 mm long) is inserted into this hole (Figure 1). The length of the capillary tube and its bore diameter depend on the amount of toxicant and water which will be siphoned out and on the cycle rate of the diluter. For faster cycle rates a larger bore may be necessary. A longer siphoning (cycle) time may necessitate a smaller bore diameter or greater length of tube to prevent the entry of additional toxicant or the back entry of water during operation. Additional lengths of capillary tubing may be closely connected in series with latex rubber tubing when necessary.

There are two methods by which the siphons can be constructed and installed. One-piece glass siphons are preferable, because they ensure more efficient and trouble-free operation. These can be installed rather easily by cutting the centrifuge tube in two at the indicated levels with the aid of a Pistorius abrasive cut-off saw[3/]. The saw then is used to grind U-shaped depressions in the cut edges to facilitate placement of the siphons. After the siphons are in position, the cut edges of the centrifuge tube are rejoined with Dow-Corning 781 to form a watertight union.

If a Pistorius saw is not available, the siphon holes must be drilled in the centrifuge tube. The lower hole must be placed at a height in excess of the maximum volume of toxicant to be delivered during each cycle. The height of the upper hole is not of critical importance. Each siphon is constructed from two pieces of glass tubing that are joined by a length of latex rubber hose which is pulled through a carefully sized hole to effect a tight seal. The disadvantages of this type of siphon are that the rubber tubing deteriorates with use and on occasions the siphon may be blocked by formation of a bubble at the juncture of the glass and rubber tubes.

2, 3/ Reference to a product does not imply recommendation to the exclusion of others that may be suitable.

Regardless of the type of siphon used, the ID of the lower siphon should be 3 to 4 mm and that of the upper siphon should be 5 mm or more (depending on the diameter and slope of the bottom of the centrifuge tube). The second siphon, which extends some distance into the centrifuge tube, permits rapid emptying of the device and is of great advantage if a fast diluter cycle time is anticipated.

Operation of Metering Device

1. Toxicant enters the empty metering device through the capillary tube at its base. The graduations on the centrifuge tube serve as a reference point for calibration of the metering device. The insertion of the capillary and glass tubes alters the scribed values of the tube, therefore, these alterations must be allowed for during calibration.

2. The toxicant rises to a constant level in the metering device. This level is determined by the level in the reservoir of the continuous-flow toxicant source (Figure 1). This level must be less than the distance from 13 to 14 of Figure 1, otherwise the device will automatically siphon and deliver stock concentrations of toxicant before cyclic water properly triggers its operation. Toxicant level also may be altered simply by raising or lowering the entire metering device independently of a toxicant supply.

3. A small volume of water cyclically enters the end of the metering device through the water regulation tube. A siphon inserted into a water regulation chamber (Figure 1) located within the water cell W-1 and connected to the common vacuum manifold may be used as a source of this water. The water flow is of short duration and should be only that amount which will initiate both siphons. The desired volume is obtained by adjustment of the ground-glass stopcock attached to the water regulator. This regulator also prevents water seepage following the cycling of the metering device, since any additional water between cycles alters the volume of toxicant delivered at the next cycle. The cyclic water quickly raises the liquid level in the metering device to level 15 (Figure 1). Both siphons are initiated at this time and all of the liquid is quickly removed from the device. Siphon 2 is optional, but it speeds emptying of the device. Early and abrupt emptying of the contents of the metering device into the mixing box ensures more uniform blending of diluent and toxicant before their introduction into the chemical cells. A balance must be made between an adequate water supply and the rapid emptying of the metering device. The flow resistance offered by the walls of the capillary tube prevents the entry of additional toxicant as the device is emptying.

4. Siphon and overflow waters join and mingle with diluent waters in the mixing box before distribution to the diluter chemical cell 2 (4).

Operation of Continuous-flow Toxicant Supply Apparatus

1. The toxicant head tank is designed to deliver fluid volumes at a constant rate slightly in excess of that used by the metering device (Figure 1).

2. A ground-glass valve provides for precise adjustment of the drip rate into the standpipe reservoir.

3. The standpipe height is adjusted to secure the desired volume within the metering device.

4. The excess toxicant overflows the sharp margins of the standpipe, thus maintaining a constant level in the standpipe reservoir tank. This level is reflected in the metering device.

5. The excess toxicant overflows into a lower storage reservoir of appropriate size and is returned at intervals by means of a peristaltic pump.

6. Toxicant should start to flow into the lower storage reservoir before the resumption of the next cycle to ensure proper filling of the metering device.

Evaluation of Performance

McALLISTER *et al.* (2) used a dye dilution technique to compare the consistency of delivery of their metering device with that of MOUNT and BRUNGS (4) and found that the coefficients of variation for the two devices were 2.4 and 6.6, respectively. By using the same technique, we determined that the coefficient of variation of our device is 1.46.

The advantages of this toxicant delivery system are: (1) freedom from moving components, (2) simplicity of construction, (3) low cost, (4) accuracy, and (5) minimal maintenance.

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