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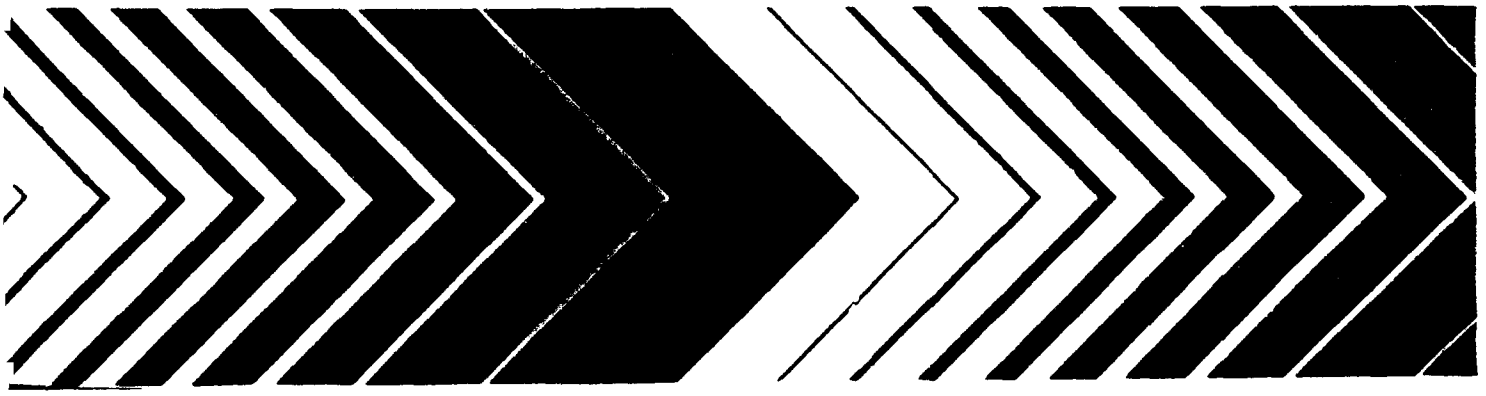
Manual for Construction and Operation of Toxicity-Testing Proportional Diluters



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MANUAL FOR CONSTRUCTION AND OPERATION OF TOXICITY-TESTING PROPORTIONAL DILUTERS

by

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ABSTRACT

This paper presents a discussion of the testing procedures using proportional diluters. The construction, calibration, and operation of the equipment is explained, and trouble shooting techniques necessary for successful use of such equipment are given.

A bibliography includes many related published materials that are not discussed in the text but which should be useful to the reader. Included are numerous citations on physical toxicity testing methods, but papers on statistics or biological test procedures are not included.

CONTENTS

FIGURES

TABLES

ACKNOWLEDGMENTS

We wish to thank those present and former associates who have, over the years, provided suggestions for design changes, operation, and other features of proportional diluters. Without such positive influence this report would be no improvement on the original 1967 publication.

SECTION 1

INTRODUCTION

The physical aspects or characteristics of testing toxicity of chemicals and aqueous effluents with aquatic organisms were minor considerations before the initiation of continuous-flow procedures. Initial recommendations called for the use of "round (cylindrical) glass jars 9 to 12 inches in diameter, 10 to 12 inches high, of good quality clear glass 1/8 to 1/4 inch thick and with a capacity of 2 1/2 to 5 gallons" (Hart et al., 1945). Equipment for temperature control and oxygenation completed the procedures. Similar recommendations persisted (Doudoroff et al., 1951) until more details were presented by Henderson and Tarzwell (1957) that included a floor plan of a proposed toxicity-testing laboratory for static procedures with industrial effluents.

A serial-dilution apparatus was described by Mount and Warner (1965) that delivered continuously a series of different concentrations of a material dissolved in water. The proportional diluter, to be discussed in detail throughout this report, was developed shortly thereafter (Mount and Brungs, 1967). This system is simpler to build and operate than the serial diluter and its use is more flexible. These systems and others (see Bibliography) helped initiate the conversion to continuous-flow testing procedures to alleviate some of the toxicity-testing problems inherent in the static procedures.

The use of continuous-flow techniques received added impetus with the addition of general procedures to the 13th edition of "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association et al., 1971). Only static tests had been described in earlier editions. After 1971, numerous detailed toxicity test procedures were developed that used or required continuous-flow techniques. The Environmental Research Laboratory-Duluth (1971, 1972a, 1972b) recommended chronic toxicity test procedures for the fathead minnow (Pimephales promelas), the brook trout (Salvelinus fontinalis), and the flagfish (Jordanella floridae). The U.S. Environmental Protection Agency (1973) published methods that included, among others, those just cited for the fathead minnow and the brook trout. The European Inland Fisheries Advisory Commission (1975) in its discussion of fish toxicity-testing procedures concluded that a continuous-flow procedure was ideal for obtaining reproducible results for a wide variety of substances. The Committee on Methods for Toxicity Tests with Aquatic Organisms of the U.S. Environmental Protection Agency (1975) published both static and continuous-flow methods for toxicity tests with aquatic organisms. The American Society for Testing and Materials (1976) is drafting standard procedures for static

and flow-through toxicity tests for chemicals and aqueous effluents. Methods for both static and continuous-flow toxicity tests are included in the 14th edition of "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association et al., 1976). This latest edition has been expanded to contain procedures to test algal productivity, phytoplankton, zooplankton, scleractinian coral, marine polychaete annelids, crustaceans, aquatic insects, and molluscs.

As interest increases in the need for continuous-flow toxicity testing, we believe that this manual will expedite the generation of data with which to better protect the aquatic ecosystem.

SECTION 2

DILUTER OPERATION - GENERAL

The purpose of the proportional diluter (Mount and Brungs, 1967) is simply to provide several different mixtures, or proportions, of a toxic solution with dilution water. Such diluters typically provide five different toxicant concentrations and a control, which is dilution water. The automatic and consistent production of dilutions permits the conduct of continuous-flow toxicity tests with aquatic organisms.

The following description and reference to Figure 1 will clarify the basic components of an operational diluter and their function. Dilution water enters the upper left of the top row of glass cells, called the W, or water, cells. During operation these W cells will empty to mix with water from the lower C, or chemical, cells. The left W cell will empty into the M, or mixing cell, and cause the introduction of a small volume of the toxic solution. The two volumes mix before overflowing into the third level of cells, the FS cells. When the W cells empty, they cause the matching C cells to empty by venturi action. Different proportions or volumes of dilution and toxic-solution waters are mixed to provide the different toxicant concentrations to be tested. These mixtures enter the fourth level of cells, the FS or flow-splitting cells, that divide each concentration into equal volumes for distribution to the replicate test chambers containing the organisms being exposed.

A more detailed discussion of the diluter operation will follow after the basic components and construction details have been described.

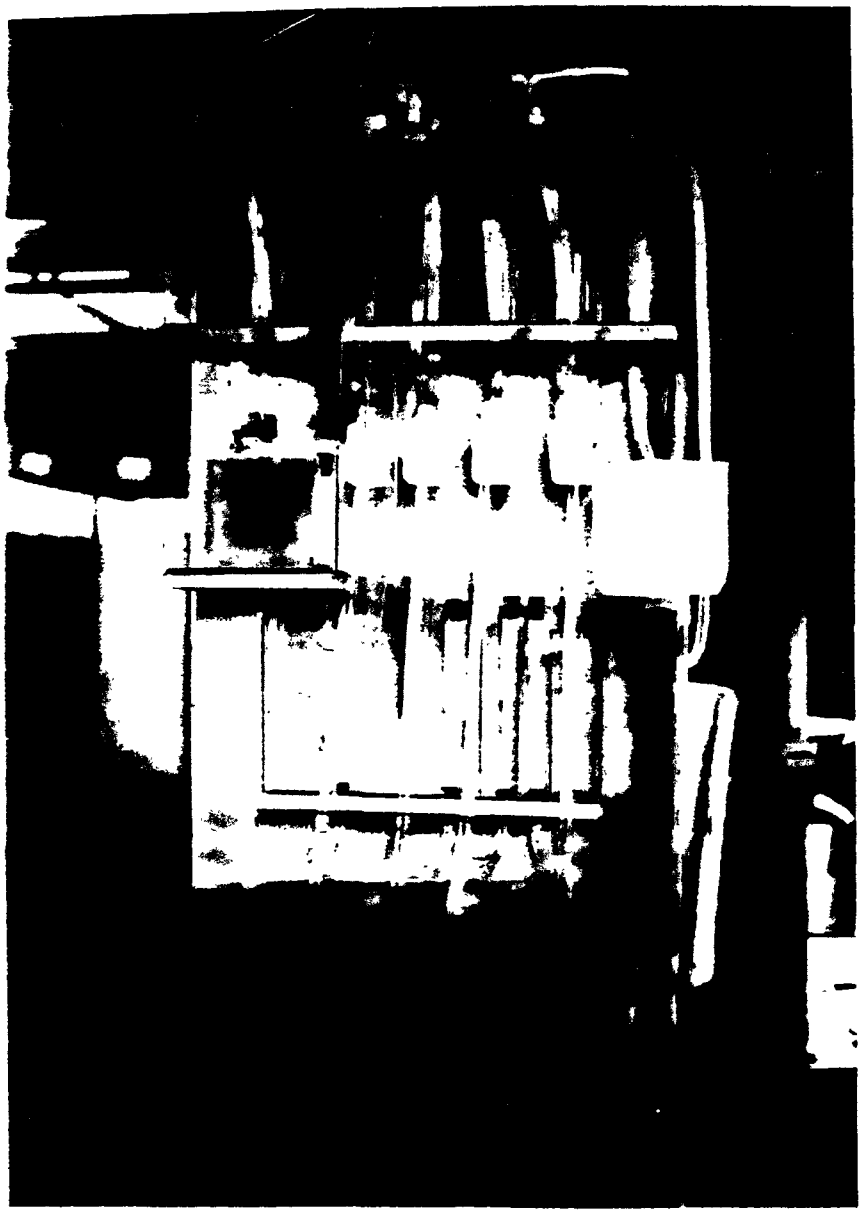


Figure 1. Completed mounted diluter.

SECTION 3

DILUTER CONSTRUCTION

EQUIPMENT

Diluter construction is greatly simplified and much improved by having the proper equipment, such as sharp glass cutters, a glass cutting table, a glass saw, a set of glass drills, which are used on a standard heavy duty drill press, and a power stopper borer.

Sharp glass cutters are needed to obtain straight, smooth cuts to prevent leaks. An optional piece of equipment is the glass cutting board, similar to those used by hardware stores to cut window panes. One style is available from Fletcher Terry Co., Bristol, Conn. 06010. A large flat surface and a good straight edge may be substituted. The glass saw is used for cutting glass tubing and is generally useful for a variety of cutting purposes. It is used to make cut ends on tubing, both square and angled, as required during diluter construction. A rolling table model, such as the Model C manufactured by Pistorius Machine Co., Hicksville, New York 11801, is desirable, but if only diluter and other glass tubing is to be cut, their Model CC12, which has a tilting table, is satisfactory. The glass drills are necessary to drill holes in the various glass cells and are listed as diamond impregnated tube drills in the catalog of Sommer and Maca, Glass Machinery Co., 5501 W. Ogden Ave., Chicago, Ill. 60650. These drills are relatively expensive, but enable the diluter builder to also drill drain holes in aquaria and test chambers. The drill press can be of any type, but should be sturdy and vibration free. Hand-held drills may be used, but breakage is increased.

The boring of stoppers for various parts of a diluter is time consuming, and a power stopper-borer, such as that manufactured by E. H. Sargent Co. (Model No. S-232DT), is very useful. Some glass bending is necessary, and if a glass shop is not available, an air-blast-type burner, such as that manufactured by Fisher Scientific Co., is very convenient. This burner enables the operator to apply sufficient heat to the tubing to allow uniform bending. If much glass work is to be accomplished, a ribbon burner of at least 6 inches flame length is also very useful.

Accurate rulers and steel tapes, a micrometer for inside and outside measurement, felt marking pens, and a sufficiently large work area to prevent moving of assembled parts during construction and assembly also save time and increase efficiency.

DILUTER-BOARD CONSTRUCTION

We'll begin our detailed procedures with the simplest part of the diluter, the board and shelves on which the glass parts for the 2-liter diluter will be placed.

The pieces are most precisely cut on a table saw, but hand-held saws may be used as necessary. Although metric measurements are used in this report, some of the materials used in the construction of a diluter are not yet available commercially in metric dimensions (see Table 1 for conversions). All pieces should be cut from 2-cm exterior plywood and assembled as shown in Figure 2. The holes necessary to permit exit of the tubing from the bottom of the various chambers should be marked accurately with the drilled glass bottom (to be discussed later) as a pattern. They should be larger than the holes in the glass to permit manipulation of the stoppers or plastic tubing. The bracing also must be placed so as not to interfere with the glass tubing, but at the same time to prevent sagging in the shelf.

The board size shown and the placement of the shelves do not need to be exact, but the W and C cell shelves should slope down to the right about 1.3-1.5 cm.

We have modified these dimensions in various ways where space, especially height, is a limiting factor. The dimensions shown have been found to be useful with regard to cleaning, calibration, and construction ease.

GLASS CUTTING

The primary skill needed to be successful in building a diluter is the ability to cut glass with straight edges and parallel sides. A commercial glass cutting board, if well maintained, is particularly good for long cuts. A second technique is to use a large flat sturdy table and a heavy ruler or other straight edge to guide the cutter. This latter technique is faster and more versatile once mastered. All pieces should be cut with minimum tolerance. After cutting, all edges should be dulled with a stone or fine-grit sand paper to prevent hand cuts. The pieces should be cleaned by washing in a detergent solution and then rinsed thoroughly and dried. Removal of grime is necessary to ensure good glue adhesion. Glass should be double strength, but the "B" or second grade is satisfactory. Flint glass tubing is preferred to pyrex because the lower melting point of the former makes bending and cutting the glass easier.

The dimensions for all the flat glass and tubing used to construct a diluter are listed in Table 2; a list of accessory parts is given in Table 3. Figures 3 and 4 contain layouts to guide the builder toward an efficient use of 30.5- by 61-cm glass sheets, a readily available and handy size to use. These layouts will ensure that the proper number of pieces are available before assembly begins. The crosshatched areas are waste. The 2-cm drain holes should be cut before assembly as shown in Figures 3 and 4. The exact location of each hole in the W, C, and FS cells should be marked so that they are centered between the cell dividers or cell ends (in the case of the FS cells).

TABLE 1. METRIC CONVERSION TABLE

Inches	Centimeters
48	122
24	61
16	40.6
14	35.5
12	30.5
11	28.0
10	25.4
8	20.3
6	15.2
5	12.7
4	10.2
3	7.6
2	5.1
1	2.54
0.75	1.91
0.5	1.27
0.25	0.64

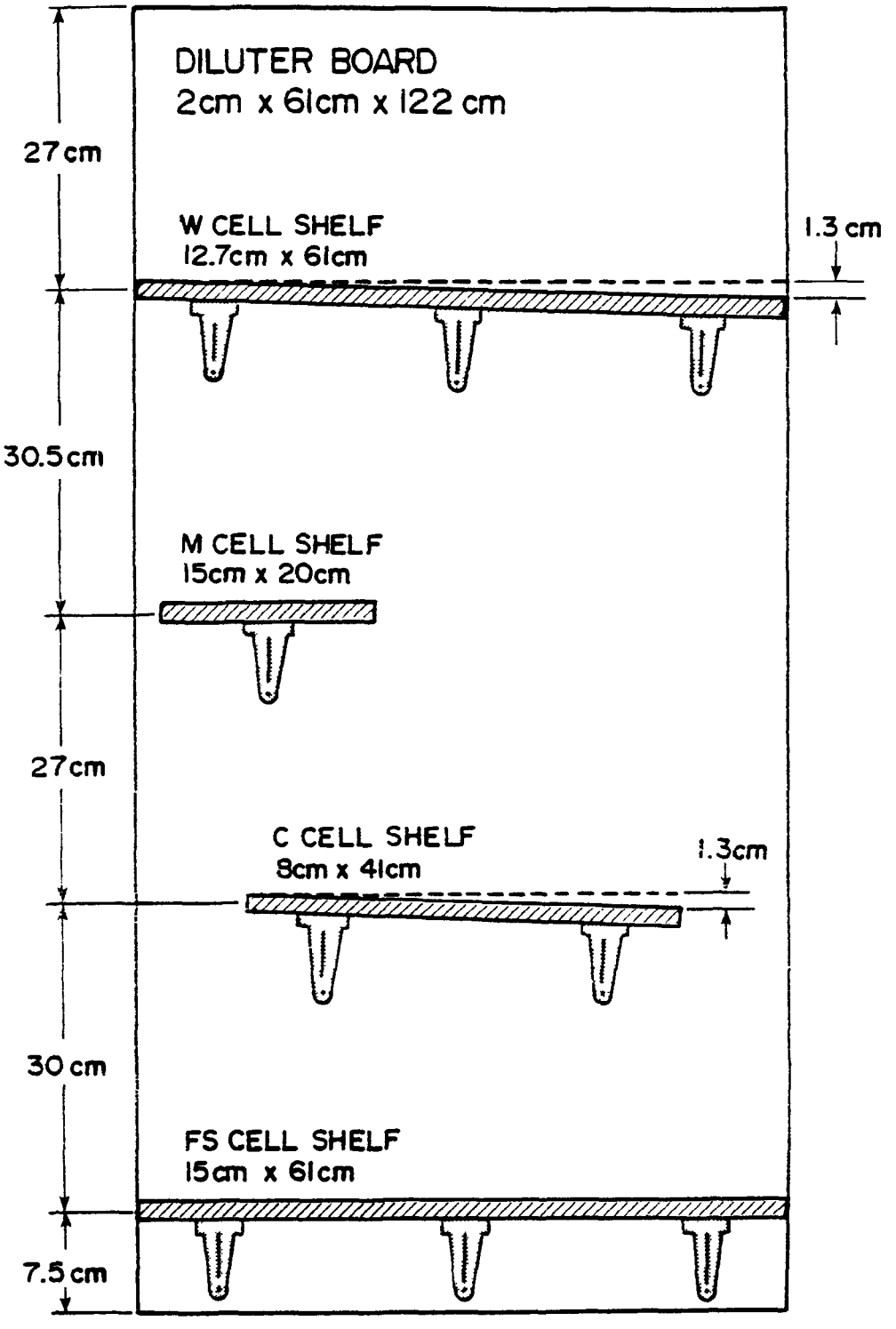


Figure 2. Diluter board with shelves mounted.

TABLE 2. DIMENSIONS OF PRINCIPAL GLASS PARTS FOR THE 2-LITER DILUTER

W Cells	1 bottom	12.7 cm X 61 cm	5 in X 24 in
	2 sides	26.7 cm X 61 cm	11 in X 24 in
	2 ends	12.7 cm X 27 cm	5 in X 11 in
	5 dividers	11.8 cm X 24.1 cm	4 3/4 in X 10 in
C Cells	1 bottom	7.0 cm X 40.6 cm	3 in X 16 in
	2 sides	21.6 cm X 40.6 cm	9 in X 16 in
	2 ends	7.0 cm X 21.6 cm	3 in X 9 in
	4 dividers	6.0 cm X 20 cm	2 1/2 in X 8 in
M Cells	1 bottom	21.6 cm X 18.7 cm	6 in X 8 in
	2 sides	21.6 cm X 20.3 cm	6 in X 8 in
	2 ends	17.8 cm X 21.6 cm	6 in X 6 in
FS Cells	6 bottoms	10.2 cm X 21.6 cm	4 in X 6 in
	12 sides	10.2 cm X 17.8 cm	4 in X 7 in
	12 ends	15.2 cm X 17.8 cm	6 in X 7 in

Siphon tube length	(W-1 through W-6)	-	30.5 cm
	(C-2 through C-5)	-	25.4 cm
	(M)	-	20.3 cm
	(valve bucket)	-	dependent upon bucket dimensions
	(FS)	-	17.8 cm
Siphon tube diameter	(W-1)	-	16 mm OD
	(W-2 through W-6)	-	12 mm OD
	(C-2 through C-5)	-	14 mm OD
	(M)	-	12 mm OD
	(valve bucket)	-	10 mm OD
	(FS)	-	10 mm OD
Siphon tube U's glass	(W-2 through W-5)	-	10 mm 3/8 in OD
	(C-2 through C-5)	-	12 mm 1/2 in OD
Venturi and siphon tube T's connecting tubes (flint glass)	(Water blocks to C siphon U's)		12 mm
	(C siphon T's to FS cells)		15 mm
Tygon tubing	(miscellaneous)	-	as needed to connect glass tubing
Siphon sleeve diameter	(all)	ID area to be at least double of OD area of siphon tube as $A = \pi r^2$ - satisfactory sizes below	
Outer siphon tubes; size and length			
W1	40 mm		18.5 cm
W2	18 mm		11.8 cm
W3	18 mm		19.8 cm
W4	18 mm		21.2 cm
W5	18 mm		variable depending on cycle time
M			
C1	18 mm		19 cm
C2	18 mm		11.4 cm
C3	18 mm		11.5 cm
C4	18 mm		10.8 cm
C5	18 mm		7.7 cm
FS 12 each	14 mm		13 cm

TABLE 3. ACCESSORY PARTS NEEDED TO CONSTRUCT A DILUTER

Item	Dimension	Number
Glass tubing	8-mm	1 1-m length
	10-mm	6 1-m lengths
	12-mm	5 1-m lengths
	14-mm	2 1-m lengths
	16-mm	1 1-m length
	18-mm	4 1-m lengths
	25-mm	1 1-m length (or pvc pipe of same size)
Glass T's	10-mm (3/8-inch)	6
Glass U's	10-mm (3/8-inch)	4
Tygon Y's or glass	12-mm (1/2-inch)	5
T's as available	10-mm (3/8-inch)	4
Glass sheets	30.5- X 61- X 0.3-cm	11
Tygon tubing	8-mm (5/16-inch)	10 ft
	10-mm (3/8-inch)	10 ft
Box of tissues		1
Neoprene stoppers	For 19-mm (3/4-inch) holes	3 dozen
	Size 11	2
	Size 8	1
Polyethylene plastic bottles	125-ml	5
	150-ml	1
Plastic container	1-qt	1
Glass cutter		1
Glass glue		2 tubes
Plastic syringes	10-ml	as needed
	20-ml	"
	30-ml	"
Microswitch (Burgess CT2 KRA2 is satisfactory)		1
Solenoid valve, stainless steel (Valcor 15P19C8-4 is satisfactory)		1

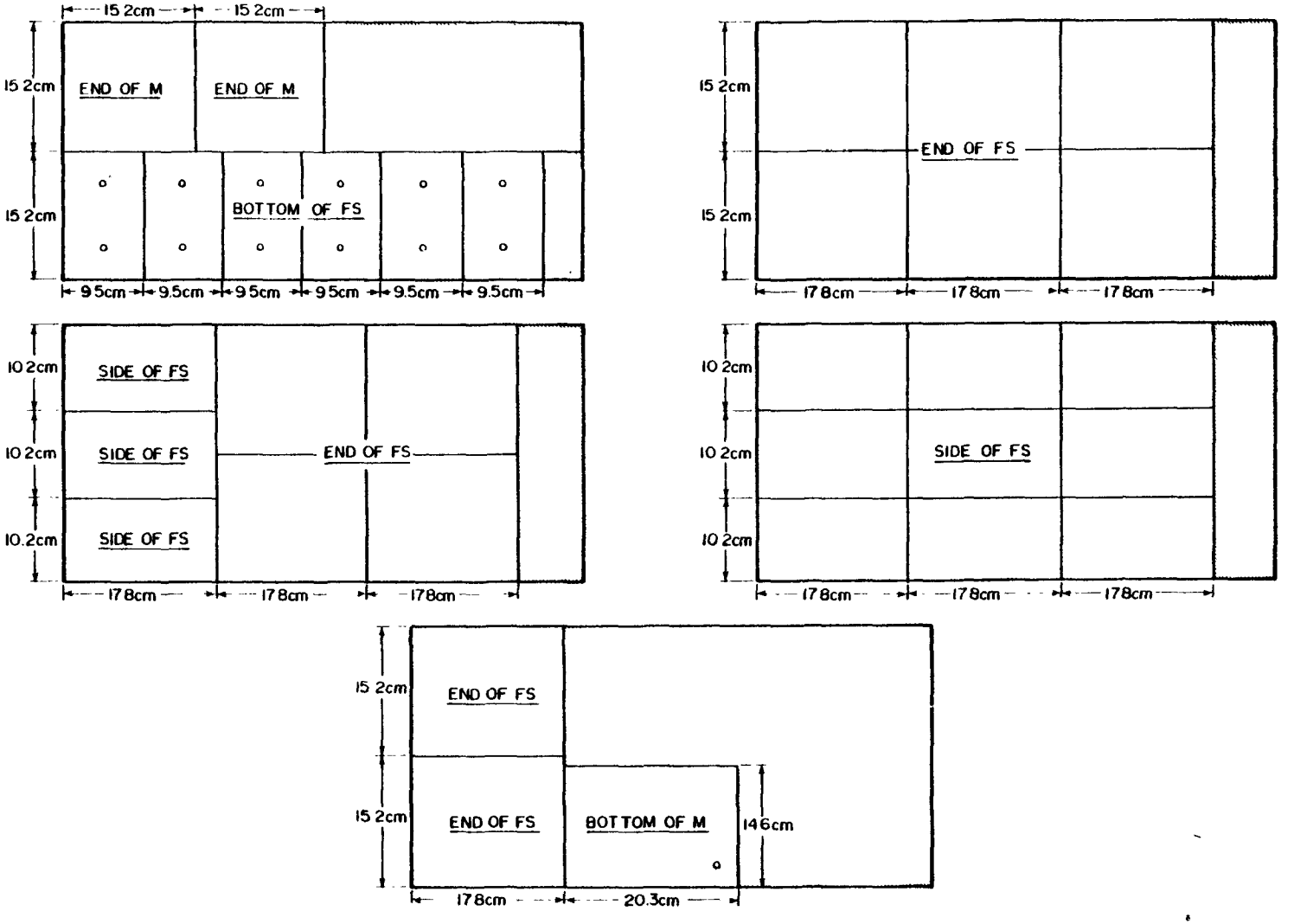


Figure 3. Glass-cutting layout.

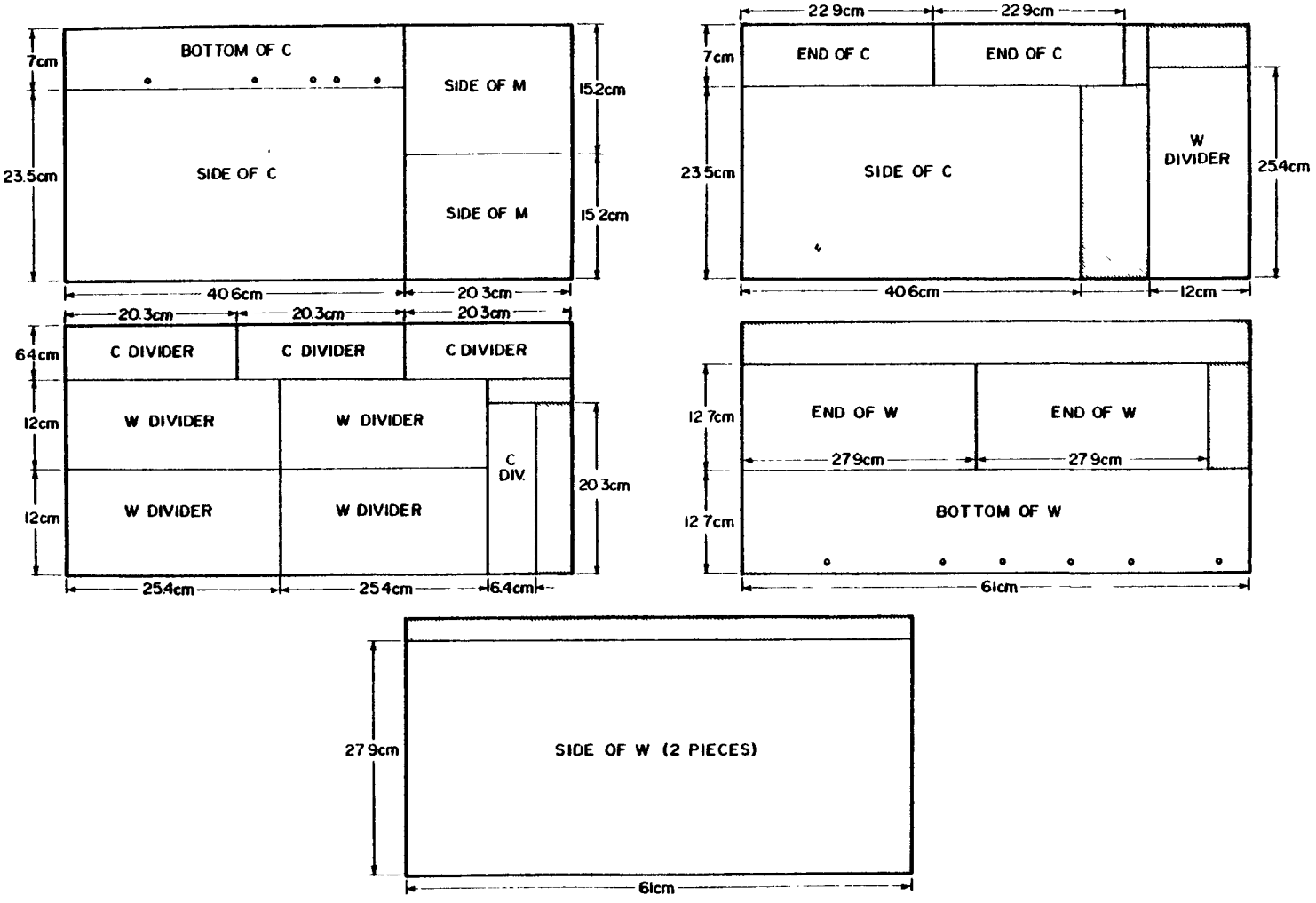


Figure 4. Glass-cutting layout.

After the holes are drilled in the cell bottoms, they can be used as templates to mark the position of the holes to be drilled in the wooden shelves of the diluter board. These latter holes should be larger than those in the glass.

The most critical cutting is for the W and C dividers since leakage will occur between individual cells if the width of the dividers varies.

GLASS ASSEMBLY

The most important construction material is the silicone sealant or glass glue. Dow Corning Glass and Ceramic Cement and General Electric Corporation RTV are both satisfactory. Glues that are listed as dish-water safe are preferable so that cleaning the assembled diluter with hot water will not cause the joints to fail. Disposable 10- or 15-ml plastic syringes with enlarged bores in the tips for faster application are useful for applying a thin bead of glue as needed and can be used with one hand (Figure 5). Application with the original collapsible tube requires two hands to maintain a steady and constant flow of glue from the tube to the edges of the glass. If the bead of glue is too thin, any irregularities in glass cutting will not be filled by glue and will leak.

Assembly should begin with the M and FS cells, since they are the simplest in terms of number of pieces. Waxed or other paper is placed on the table top to catch any excess glue. The paper can be removed easily after the glue has dried.

Before placing glue on the glass edges, follow the assembly procedures in a dry run. For the M and FS cells, glue is placed on all four edges of the bottom before it is placed on the waxed paper. Next, one of the two longer pieces (the right and left side of the FS cell or the front and back of the M cell) is held and glue placed on the two end edges. This piece of glass is placed against the bottom and propped in a vertical position. The opposing piece is glued and placed similarly. The remaining two pieces are placed against the glued edges already in place. Slight pressure at all glued joints distributes the glue, helps prevent leaks, and places the glass surfaces in closer contact. To ensure against leaks, a pencil eraser or rounded wooden dowel may be used to spread the freshly applied, excess glue along each seam. Use care to avoid moving the glass.

The fronts, backs, and bottoms of the W and C cells should first be placed as shown in Figure 6 and the lines drawn as indicated to show the location of the cell dividers during assembly. A wax pencil or felt pen can be used. It is important to remember during assembly, however, that these marked surfaces should be on the outside of the cells so that glue adhesion is not affected by these lines.

The W and C cell assembly procedures are identical. The initial difference between this procedure and that used for the M and FS cells is that the W and C cells are built beginning with the back piece placed on the table top.

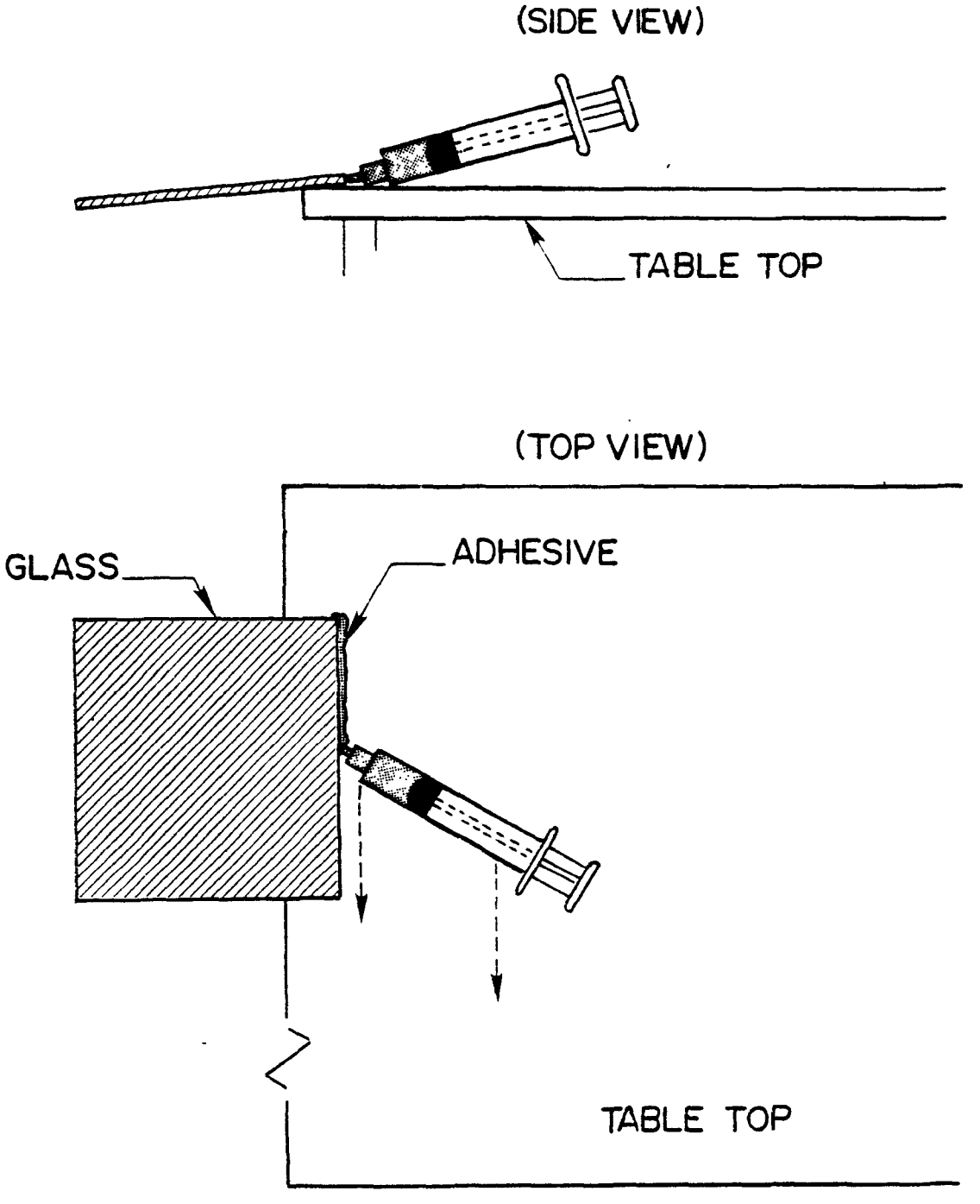


Figure 5. Glue-application system. Syringe tip to be bored out to approximately 4 mm to give sufficient bead size.

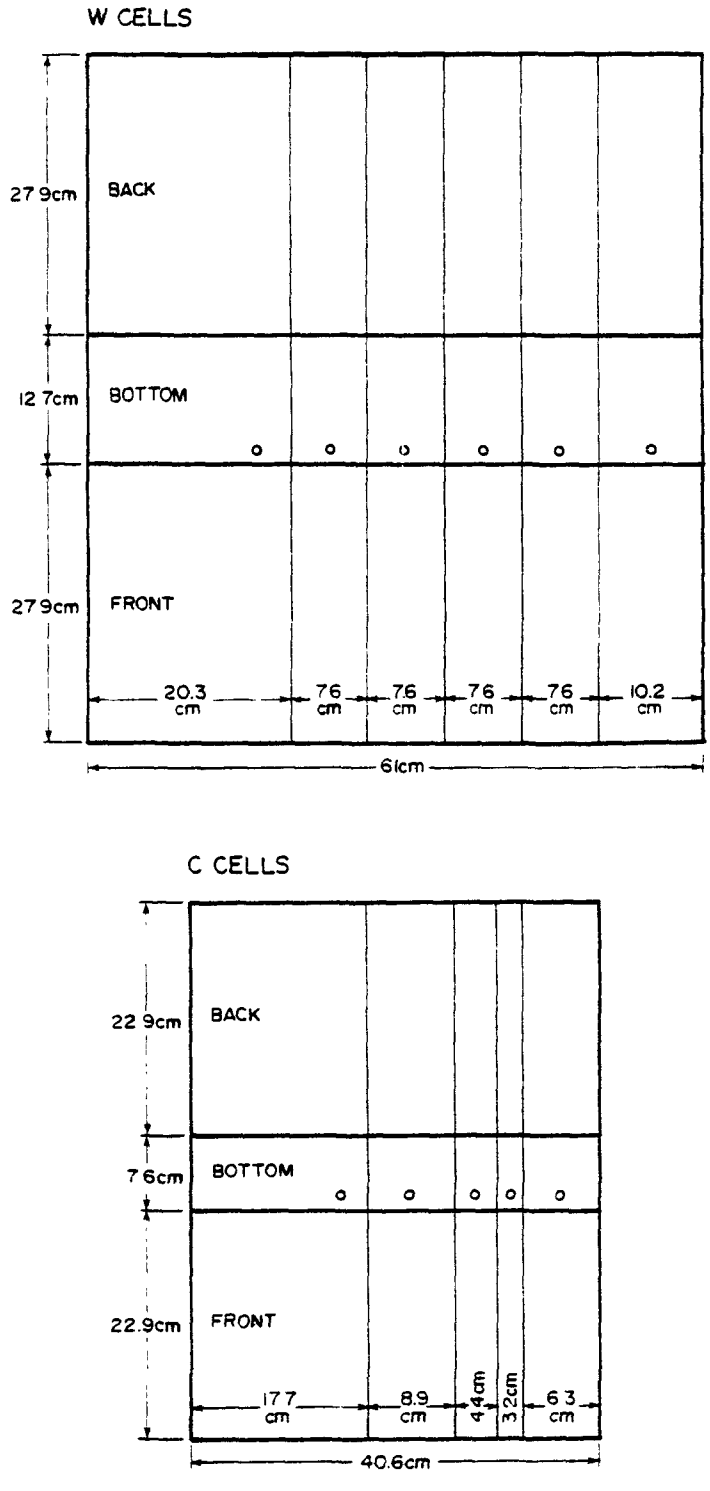


Figure 6. Gluing layout for W and C cells.

A bead of glue is placed on the bottom edge and ends of the back piece of the W or C cell, and it is placed on the paper covering the table top. Glue is placed on each end of the cell bottom, which is then placed against the large edge and supported as shown in Figure 7. If the angle between the back side and bottom is kept at slightly more than 90°, the first divider can be placed more easily. Beads of glue are placed on three edges of the first divider; none is added to the top edge. This divider is placed on edge on one of the lines on the back side of the cell so that the two bottom edges are in line (Figure 7). The bottom of the cell is rotated up to a 90° angle with the back so that the bottom edge of the divider matches the line drawn on the cell bottom. Continue to support the bottom with a block. Glue is added to three edges of each of the remaining dividers and each is carefully placed in position. The next step is to place a bead of glue on the bottom edge and ends of the front side. This edge is placed in the corner formed by the bottom of the dividers and the bottom of the cell. The front side is then lowered to rest on the dividers so that those edges match the lines drawn on the front side. If difficulty is encountered with multiple gluing of the W and C cell dividers, each may be glued on one short to long side only and set individually, leaving a 2-hr setting period between each. After these are dry, glue may be applied to the long exposed edge of each divider. The long edge of the eventual front is also glued, and the front is placed on the glued edges as above. The ends can now be put in place without glue since they will contact previously glued edges. As with the M and FS cells, a pencil eraser or wooden dowel can be used to smooth the glue before drying to help prevent leaks if this is necessary. With practice, this procedure may not be necessary as the glue beads should then be placed evenly enough and thick enough to avoid leaks.

After all cells have dried overnight, they should be tested for leaks after plugging the drilled holes. The exposed edges should be smoothed with a file or sandpaper to eliminate cuts. All cells can now be placed on the diluter board in the approximate positions shown in Figure 8.

Other assembly procedures may become more desirable after experience has been gained. The above details should be acceptable for beginners.

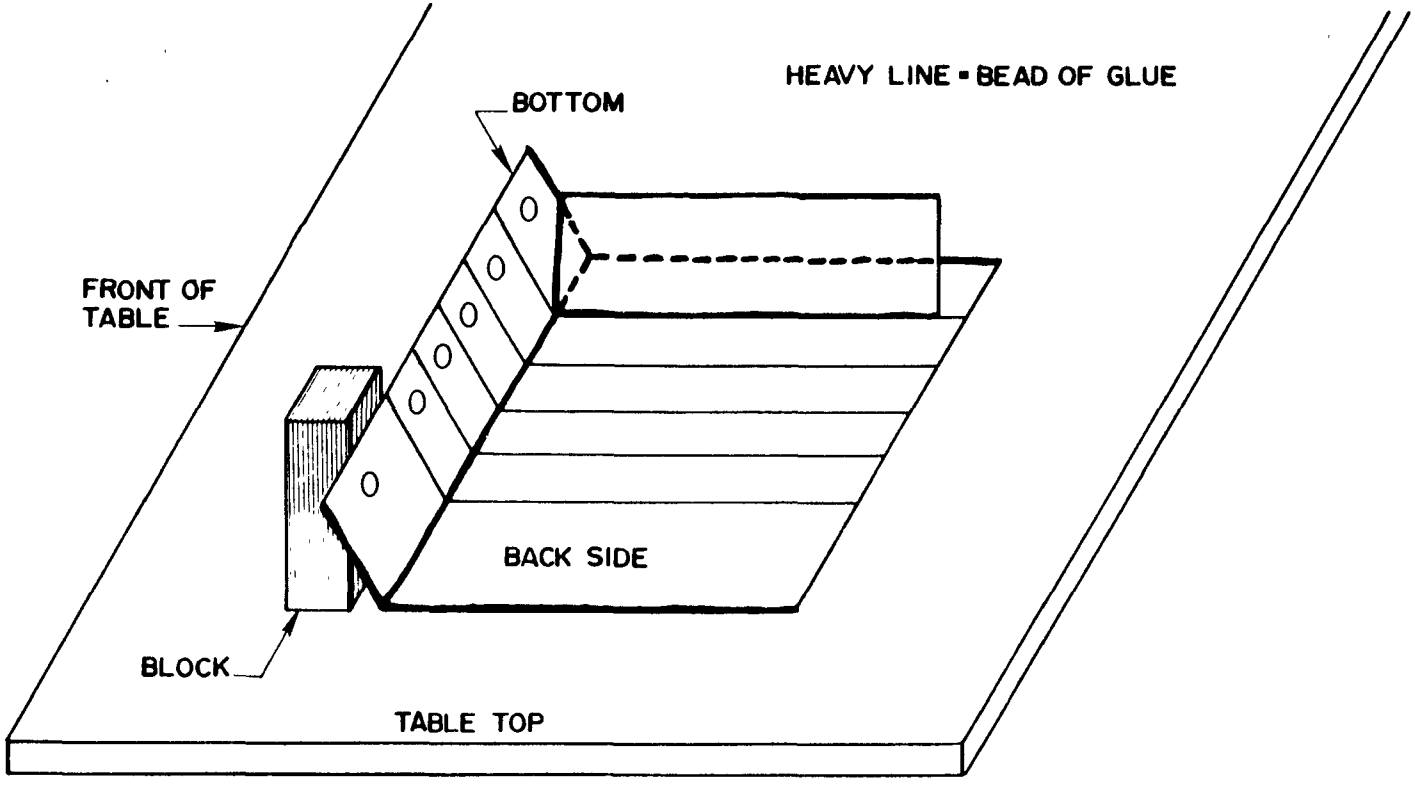


Figure 7. Gluing procedure for W cells.

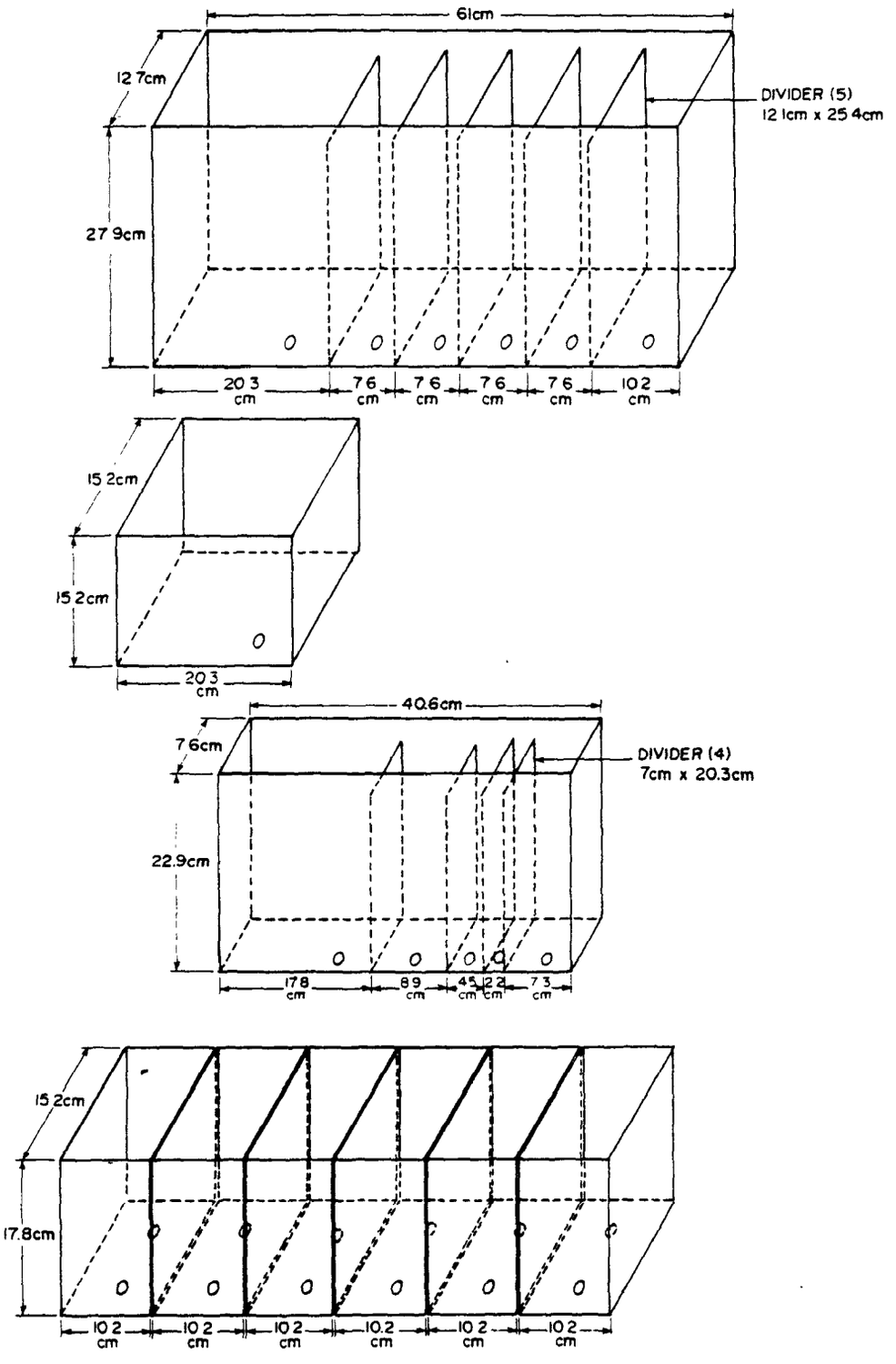


Figure 8. Final assembly plan for all cells.

SECTION 4

DILUTER OPERATION

MECHANISMS OF ACTION

The operation of the proportional diluter (Figures 1, 9, and 10) depends on the venturi action of water flowing past a tube which is part of a closed air system. The principal is the reverse of that of a carburetor in which incoming air sucks gasoline from the carburetor jets. As water flows through the W cells and overflows by siphon by siphon W-6 into the valve bucket, the weight of the valve bucket increases and operates the microswitch, which causes the valve bucket to come to rest on the bucket stop. This closes the diluent water valve allowing the water levels in the W cells to drain down to the tops of the dividers. As the valve bucket fills, its siphon begins to empty through the venturi T (Figure 10). As water passes the opening of the vacuum line, air is removed resulting in a reduction in air pressure in the vacuum line. This water from W-6 passes through the venturi T and to FS-6 as the control test water. The vacuum line connects to the siphon U's in cells W-1 through W-5 (Figure 9). Each siphon U is covered on one end by water in its corresponding W cell and on the other end by water in the water block. Consequently, the air volume within the vacuum line is closed to the atmosphere at the time the vacuum is applied by the venturi action of the venturi T. As the air pressure decreases in the vacuum line, water is pushed into each end of each siphon tube in cells W-1 through W-5 by the atmospheric pressure, which exceeds the pressure in the vacuum line. As the water enters the top of the siphon tube a siphon begins, which empties these five cells. The W-1 cell empties into the M cell where the toxic solution is added, resulting in the highest toxicant test concentration. Cells W-2 through W-5 empty through the C cell venturis from cells C-2 through C-5. Again, the pressure inside the C siphon tubes is reduced, and those cells containing the toxic solution are emptied into the FS cells together with the dilution water from the corresponding W cells. Since the W-1 cell contains the greatest volume of water, the remaining W and C cells should be empty before the M cell containing the water from W-1 and the added toxic solution overflows to refill the C cells. (If the M cell begins to empty before the siphons are broken in W-2 and C-2, the water from the M cell will be siphoned out through the C-2 siphon.) Cells C-2 through C-5 are filled, and the remaining 2 liters of the highest toxicant test concentration water enters C-1 to flow to FS-1. All the tubes emptying into and from the M cell and into the valve bucket and into the FS cells should be cut at about a 45° angle so that the tube drains completely after siphoning.

The selection of the tubing sizes controls the flow rate of water from W-6 through the venturi T to FS-6 so that the valve bucket contains water long enough to keep the dilution-water flow from entering W-1 before W-1 has

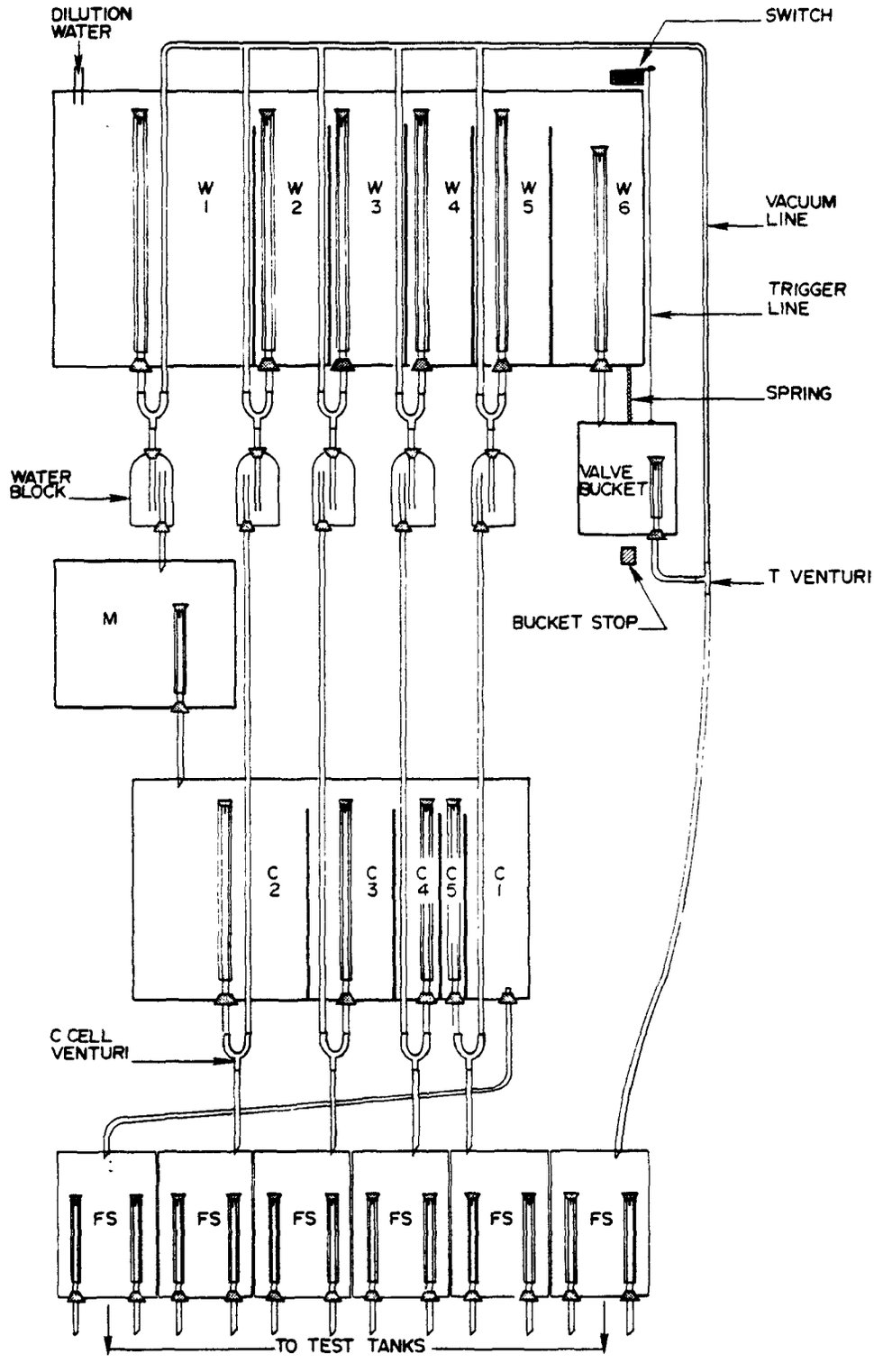


Figure 9. Tubing and accessory plan for assembled diluter.

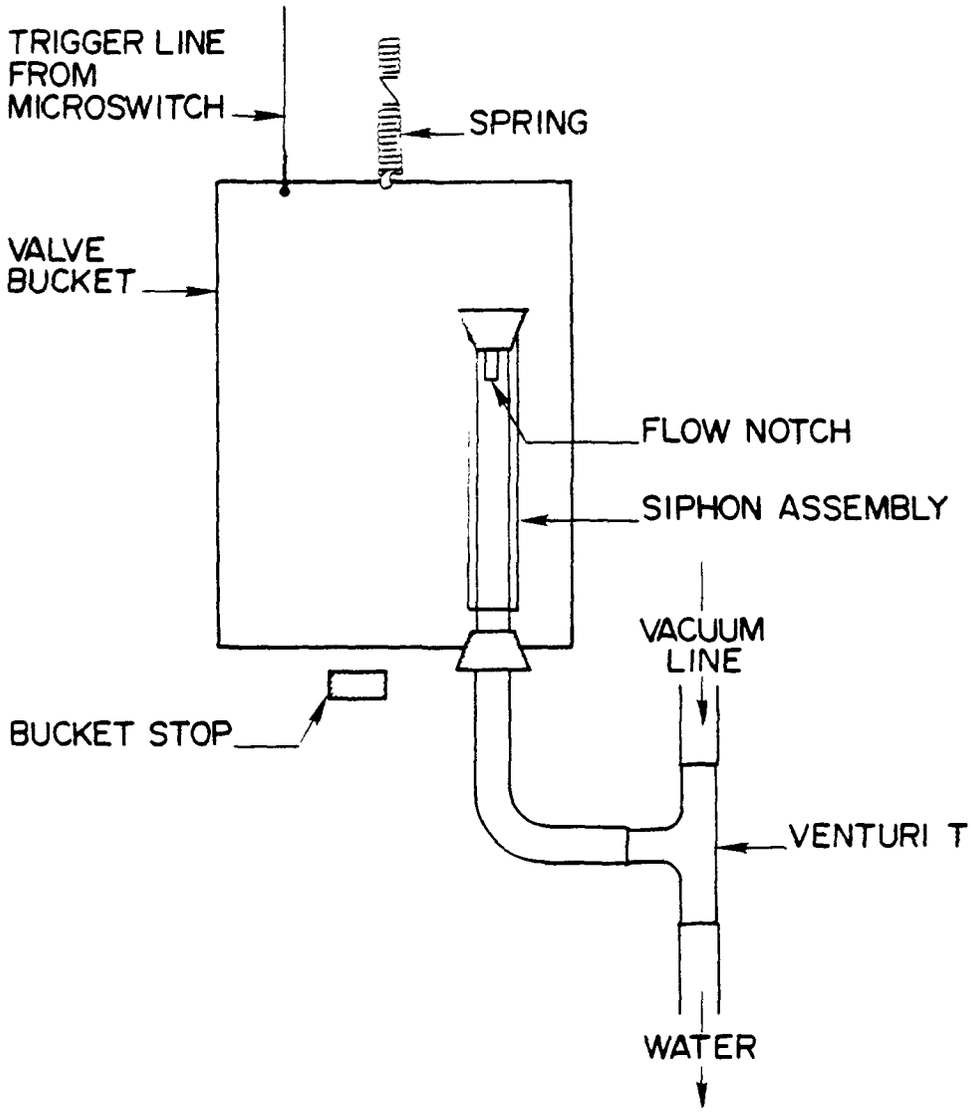


Figure 10. Valve bucket assembly.

completely emptied into the M cell. If dilution-water flow begins before the W-1 siphon is broken, water will continuously flow directly through W-1 preventing the filling of the remaining W cells and subsequent proper functioning of the diluter.

When most of the control test water from W-6 has flowed out of the valve bucket allowing it to rise from the bucket stop and causing the microswitch to open the dilution-water valve, the diluter should be in the following mode: (1) All W cells are empty and their siphons broken; and (2) the M cell has emptied to fill cells C-2 through C-5. The cycle will begin again at this time.

FLOW RATE REQUIREMENTS

The flow rate of the dilution water determines the length of each cycle of operation, and this flow rate is adjusted to obtain the desired flow to the test chambers. If duplicate test chambers are used, as this design allows, 1 liter of test solution will enter each test chamber each cycle from the 2-liter diluter. The total test solution for the diluter is 12 liters per cycle. If each test chamber contains 20 liters and the experimental design requires 10 volume exchanges per day, a total of 200 liters is necessary for each chamber each day. Consequently, 200 diluter cycles per day are required; this is just over 7 min per cycle (1,440 min ÷ 200). The dilution-water flow rate is then set to provide 12 liters of water plus the rest of the cycle in 7 min.

Coordination between flow rate and volume is necessary for the diluter to function. The flow rate of dilution water and the height and size of the siphon cell W-6 must be such that the amount of water flowing between the time the valve bucket turns off the dilution water and cell W-6 empties is equal to the delivery volume (2 liters) of one cycle to the rest of the pairs of cells. The size of the bucket and the height of the siphon must be such that the water begins to flow out of the valve bucket just before the water stops flowing from cell W-6. The line from the valve bucket must be just small enough so that all of the other operations of the cycle will be completed before it empties. A smaller line will work, but it will lengthen the cycle time needlessly. The venturi T must be sized so that it will draw a stream of bubbles from the vacuum line during the flow from the valve bucket. The siphon in the M cell must be adjusted so that nearly all of the water from the W-1 cell has entered before the siphon starts the flow into cell C-2. This siphon should be rather large so that after the flow starts it will empty fast and not delay the cycle time. The last consideration for smooth operation is at the venturi below the C cells. In this case, the size of the pipe from the W cells must be such that a good vacuum is formed. The main consideration here is that the tube from the W cells is not too large causing the water to back up into the C cell outlet tubing rather than forming the necessary vacuum.

ACCESSORY PARTS

By now the reader should have an adequate general understanding of the proportional diluter and should be able to begin the connections between the various W, M, C, and FS cells. The principal accessories are the valve bucket, venturi T, microswitch, dilution-water valve, water blocks, siphon tubes, neoprene stoppers, and miscellaneous glass and plastic fittings and tubes (Table 3). The toxicant-metering devices will be discussed in a later section.

DILUTER ASSEMBLY

First cut the required five tubes for cells W-2 through W-5 31.5 cm (1 ft) long from 10-mm OD glass tubing. Notch one end of each tube about 12 mm deep with the glass saw; remove about one-half of the wall. All tubes should be notched approximately the same.

Bore stoppers to fit the tubes, and install into bored holes in W cells. In chambers W-2 through W-5 the bottom of the tube notch must be above the tops of the dividers to prevent premature siphoning. The siphon in cell W-6 is installed with all of the tube below the dividers so it is self initiating.

Cut the required five tubes 25.5 cm long from 10-mm OD tubing for the C cells. Notch and install in cells C-1 through C-4 with notch bottoms above the dividers and in cell C-5 with the complete tube below the dividers.

Cut the one required tube 25.0 cm long from 10-mm OD tubing. Notch and install with all of the tube 25 mm below the top edge of the M cell.

Cut the required 12 tubes 23.0 cm long from 8-mm OD tubing. Notch and install with all of the tubes 25 mm below the top edge of the FC cells. (Note: If notches are not of identical depths, install with the bottom of all notches 35 mm below the top of FC cells.)

Cut the one required tube 34.0 cm long from 18-mm OD tubing; bore a 4-mm hole in the side of the tube 48 mm from one end. Notch the opposite end to install in cell W-1 with the 4-mm side hole at a 45° angle to the divider between cells W-1 and W-2 and just below the bottom of the chamber support. Glue in place with silicone sealant.

Select and remove the tube restrictions from four 14-mm OD glass T's. Y's may be used, but one arm of the Y must be bent to clear the W cell supports.

Select four 10-mm OD glass T's, remove the tube restrictions from the opposite ends, and fasten together with suitable Tygon pieces with all the right-angle outlets in the same plane to make the vacuum manifold. Fasten to the top of the W cells.

Cut 10-mm-diameter center holes in the bottom of four 125-ml plastic bottles. Select and remove the tubing restrictions from four 14-mm OD T's (1/2-inch). Drill a 14-mm hole in neoprene stoppers of suitable size to fit the tops of the 125-ml bottles.

Insert one cut end of a T into each stopper, and add 40 mm of 14-mm (1/2-inch) tubing to each to make a water block.

Cut four 46.0-cm lengths of 12-mm OD glass tubing. Heat the plastic bottles slightly (hot water or careful use of burner or heat gun) and force the tubing into the predrilled hole in each bottle to within 10 mm of the inside top of the bottle.

Make four 90° smooth bends (90 mm around the curve) from 14-mm tubing. Select and remove the tubing construction from two ends at the right angle of 14-mm T's. Connect the T to the C cell siphons with the right angles and appropriate Tygon tubing. Attach the T water-block assembly with suitable Tygon tubing to the standpipes of cells W-2 through W-5 and to the C cell T's.

Mount the valve-bucket stop 32 cm below and the spring support just below the bottom of the W cells. Attach the microswitch to the spring support by using a small block of wood. The spring should have just sufficient power to lift the empty bucket.

Cut both ends from a 250-ml (6-ounce) polyethylene bottle leaving a cylinder 70-75 mm long. Select two size 11 stoppers to fit the ends of the cylinder and bore a 19-mm (3/4-inch) hole in each near one edge. Bore a 14-mm hole in a size 2 stopper, cut a piece of 14-mm glass tubing 90 mm long and insert both into one large stopper. Mount the W-1 cell water block onto the W-1 outlet to just below the 4-mm hole with the second large stopper. Insert a short piece of 4-mm tubing into the hole and connect with suitable tubing to the vacuum manifold.

The preceding assembly instructions result in a diluter system that will function normally. The given dimensions, however, are not in most cases the only ones that will work. The most usual and easiest changes are those required by vertical space limitations found in many laboratories. All of the glass chambers, W, M, C, and FC cells, can be made shorter and deeper from front to rear if necessary. This, of course, makes the cutting of glass more wasteful, but the diluter will function. The simplest procedure is to determine the total height available. Distribute this between the four layers; then, using that dimension vertically, use the given dimension horizontally to determine the depth dimension by dividing these into the volume. All vertical tube lengths must then be adjusted to fit depending upon the amount of change decided upon. One precaution that must be taken is to adjust the siphon lengths carefully as a small vertical dimension change has a large effect because the two horizontal dimensions contribute much more to the volume.

When the diluter has been assembled and all tubing is connected, the equipment should be checked for leaks. When it is leak free, all of the chambers should be fastened to the diluter board with small clips and screws (mirror clips work well) to prevent breakage during cleaning or other manipulation.

The valve bucket (either a cut-off 2-liter bottle or a 2-quart plastic freezer box is satisfactory) is mounted by putting a stainless steel light rod or heavy wire across the bucket as a hanger. The siphon venturi of 10 mm (3/8

inch) is positioned under the bucket (Figure 10). The venturi is prepared by cutting off the tubing restrictions on two right-angle ends of the T and mounting the T with the remaining restriction towards the bucket. The siphon in the bucket is 10-mm tubing 15 cm long, and the outer tube is 15-mm tubing 10 cm long. (If very short or long cycle times are desired, these dimensions must be modified to suit.) The bucket is hung from the upper bracket with a spring and light stainless steel wire. The operating arm of the microswitch is fastened to the bucket hanger by a piece of monofilament line set so that the microswitch is on when the bucket is up and off when the bucket is resting on the bucket stop. (See section on Mechanisms of Action for further explanation.)

Care must be taken to leave enough slack in the exit tube of the valve bucket where it is attached to the venturi T so that the tubing does not hinder the action of the spring. If very cold water is run through the diluter this becomes an important consideration as the tubing becomes very stiff and may cause malfunction of the switch.

SECTION 5

DILUTER CALIBRATION

PRECALIBRATION

Table 4 contains the necessary cell delivery volumes for 50% and 33% dilution ratios for 2-liter diluters with five test concentrations and a control. The easiest procedure for initial precalibration requires that the W-1 through W-5 and C-2 through C-5 cells be filled with an excess that overflows into the W-6 and C-1 cells. A siphon emptying into a graduate cylinder is used to remove the appropriate volume in a cell; the contents of the siphon itself are returned to the cell. A mark is then made at the resultant water level. The siphon sleeve is cut to end at this mark. The core from a neoprene stopper can be used in the top of the siphon sleeve (see Benoit and Puglisi, 1973 in Appendix). Adjustments in the position of this neoprene core in the siphon sleeve allow for final, more precise calibration if necessary.

Calibration of the M cell and valve bucket is different since these containers should be nearly empty after siphoning. The appropriate volume (Table 4) is added, and a mark is made to represent the water level. The top of the siphon tube is set at this level. The siphon sleeve is placed so that it is near the bottom of the container, but not so near as to restrict the water flow. Since the siphon tube is notched at the top, a siphon effect will begin just before the total volume enters the container. The volume of control water entering the valve bucket is dependent upon the flow rate, and any variation in the latter will change this volume. If the valve bucket does not empty completely, the spring may not be strong enough to reset the microswitch.

The top of the W-6 siphon tube is below the top of the cell divider. The siphon sleeve should be set so that less than the required 2-liter volume is needed to start the siphon to accommodate the time lapse between the beginning of the siphon and the turning off of the valve. Also, after dilution water stops entering the W-1 cell, some water will continue to enter W-6 as it drains down from the other W cells. As a start, have the W-6 siphon begin after about 1.5 liters have entered the W-6 cell and adjust if necessary. The reader is referred to Benoit and Puglisi (1973) in the Appendix for details on calibration of the flow-splitting cells.

FINAL CALIBRATION

After precalibration has been completed, the diluter should operate at least overnight before making final adjustments. These final adjustments

TABLE 4. CELL DELIVERY VOLUMES FOR 2-LITER DILUTERS WITH FIVE CONCENTRATIONS AND CONTROL

Item	Quantity	50% dilution volumes (delivery)	33% dilution volumes (delivery)
W cell[a]	1	3,875 ml	2,493.5 ml
	2	1,000 ml	1,667 ml
	3	1,500 ml	1,889 ml
	4	1,750 ml	1,963 ml
	5	1,875 ml	1,987.5 ml
C cell	2	1,000 ml	333 ml
	3	500 ml	111 ml
	4	250 ml	37 ml
	5	125 ml	12.5 ml
M cell		Must hold 4,000 ml with outlet flow beginning at about 3,800 ml to give time delay	Must hold 2,500 ml with outlet flow beginning at about 2,450 ml to give time delay
Flow-splitting cells	6	2,200 ml	2,200 ml

[a]The W-6 (or control) cell should deliver as close to 2,000 ml as possible. Variations in flow rate will cause this volume to vary.

should take place while the diluter is functioning but with the delivery tubes to the test chambers themselves diverted so that any accidents during calibration while tests are ongoing will not adversely affect the test. The delivery volume from each cell should be checked at least twice for proper accuracy.

The W-1 volume is collected by an appropriate graduate cylinder or other container as it enters or leaves the M-1 cell. Necessary adjustments are made based on common sense by moving the neoprene stopper core up or down in the siphon sleeve to increase or decrease, respectively, the volume transferred. By common sense we mean that only the necessary level of accuracy (1 to 5 percent usually) is desirable. Finer adjustment is only time consuming. Cells W-2 through W-5 are calibrated one at a time by disconnecting at the C cell venturi the tube that connects the water block to the C cell venturi. The water from the M cells is then collected when the diluter functions. The corresponding C cell will not empty during this calibration step. After some experience, two W cells can be checked at once. The W-6 volume, as discussed earlier, is flow dependent and should only be calibrated at the intended flow rate. Its delivery volume can be taken at the discharge from the valve bucket or where it enters the control flow-splitting cell. Do not take it before it enters the valve bucket since the valve will not function. Adjustments in the delivery volume are made by the neoprene stopper core in the siphon sleeve of W-6. Once the transfer volumes of cells W-2 through W-5 are fixed, cells C-2 through C-5 are calibrated by collecting the total flow from the paired W and C cells before it enters the flow-splitting cells. The C cell volumes are calculated and adjusted by difference between the total volume of the two cells and that corresponding W cell volume previously determined. The C-1 cell volume is measured as a double check on the calibrations of cells W-1 and C-2 through C-5. Finally, the flow-splitting cells are calibrated after the delivery tubes are replaced to simulate normal operation. The transfer volumes are collected at the test chambers, again to simulate normal operation. These flow-splitting cell volumes should be checked weekly and after any cleaning or other manipulation.

SECTION 6

TOXICANT METERING

The bibliography to this report contains numerous citations for toxicant-metering systems. Any system should be activated only by diluter operation to avoid overuse of the toxicant. We have had significant experimental experience, with three systems, and we will discuss them in the order of their development.

The "dipping-bird" system (Figure 11) was described by Mount and Brungs (1967). Details can be found in the Appendix. This metering device is useful for water-soluble, non-volatile toxicants and some suspended solids. It can be made most easily from a volumetric pipette (Figure 12) of an appropriate size, usually between 2 and 50 ml. The dipping bird is located between the W-1 cell and the M cell. The cup is filled when W-1 empties and the weight and force of water rotates the arm about the pivot to introduce a known and constant volume of stock solution. A small-diameter tube from the side of the W-1 water block above its normal water level should be used to put water in the cup. The force of all the water from cell W-1 could damage the dipping-bird assembly. After cell W-1 is empty, the cup contents empty through the drain hole allowing the arm to return and refill. The adjustable weight is necessary to ensure proper rotation. By adding successive known quantities of water (100 ml) to the Mariotte bottle (2- to 5-gal) it can be calibrated by marking with a diamond point pencil so that the amount of toxicant used each day can be determined. When the amount of toxicant is divided by the number of diluter cycles over a known time period, the mean toxicant-delivery volume per cycle can be determined by calculation. An electric counter wired to the microswitch will provide the number of cycles. Also, the mean cycle time can be determined. For example, over a 24-hr period (1,440 min) there were 576 cycles and approximately 1,210 ml of stock solution were used. The mean cycle time was 2.5 min, and the mean dipping-bird volume was 2.1 ml. This procedure is used for calibration of the dipping bird, and it is required daily to monitor dilution-water flow rate and nominal toxicant concentration.

The McAllister system (McAllister et al., 1972) is described in the original paper. It also requires a Mariotte bottle system. The detail of its relationship to a proportional diluter is shown in Figure 13. This device is more useful for volatile toxicants or toxicants dissolved in a solvent as there is reduced contact with air. Figure 14 shows a modification of the McAllister system by Puglisi (unpublished data) to protect against a malfunction of the Mariotte bottle that would permit the introduction of excessive amounts of the stock solution. The McAllister system can be calibrated and monitored in the same manner as the dipping bird. When constructing this device, the capillary tube should be as small as possible to prevent variations in the addition of the toxic solution if variations in siphoning by cell W-1 occur.

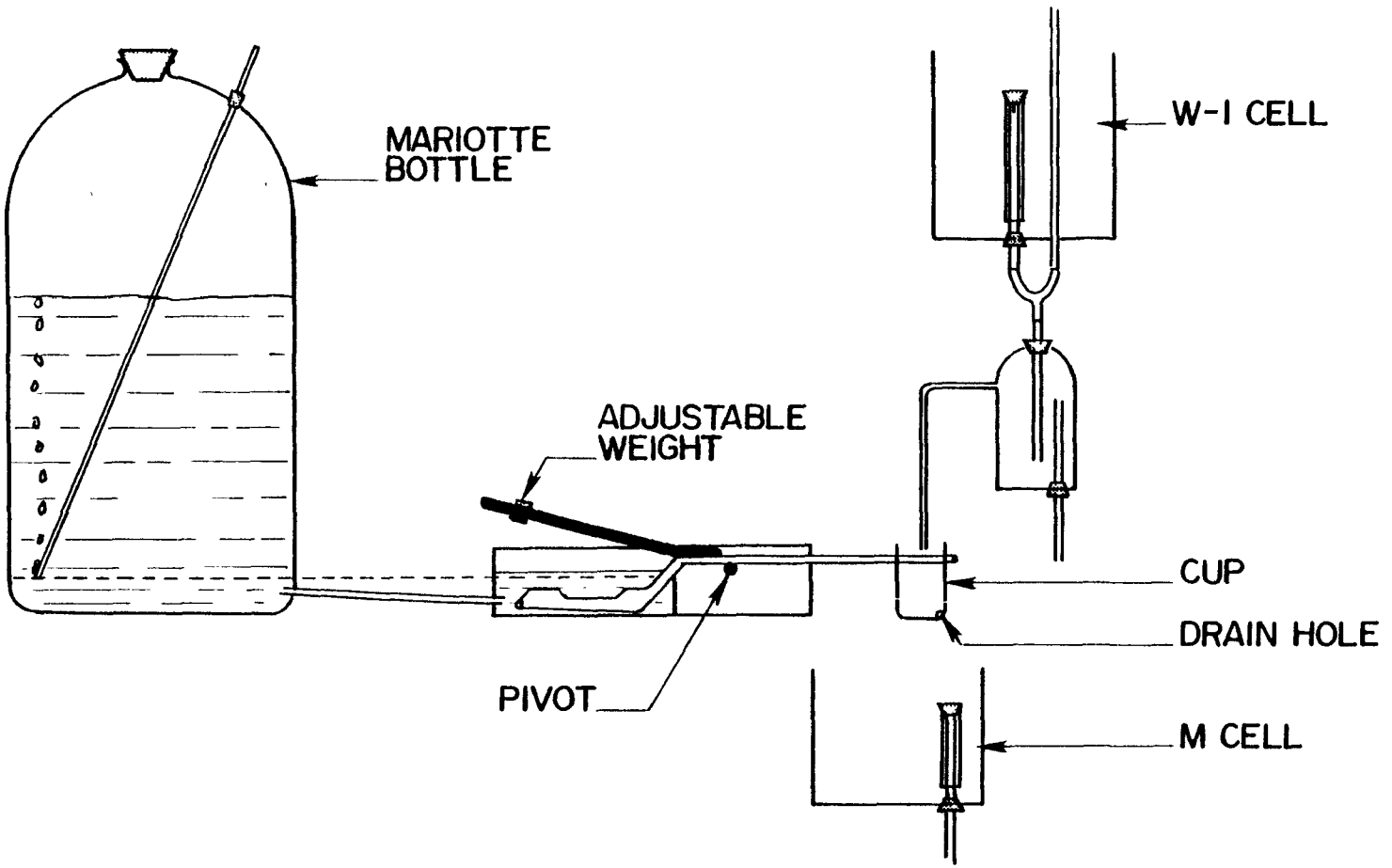
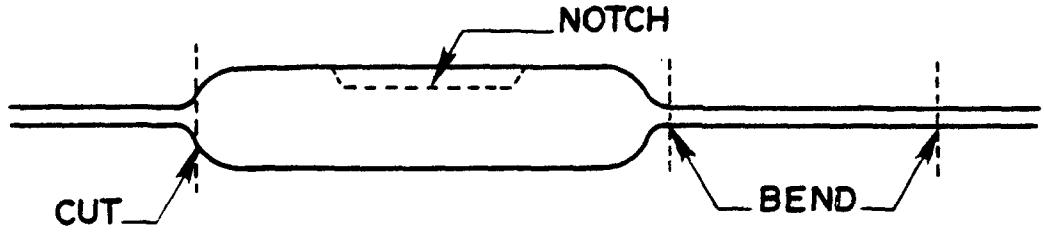
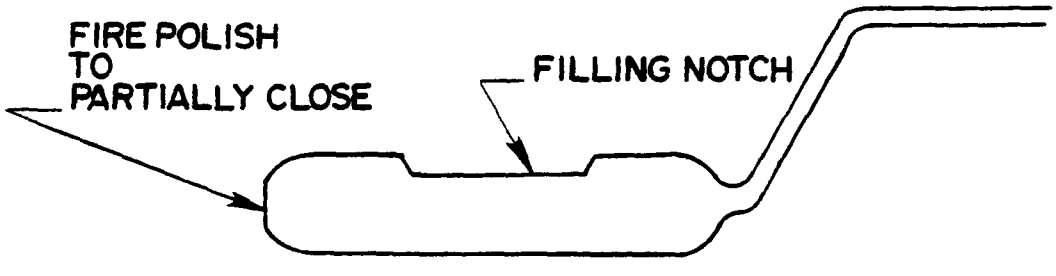


Figure 11. Toxicant metering assembly plan for the "dipping bird" system.



VOLUMETRIC PIPETTE



FINAL PRODUCT

Figure 12. Construction plan for a "dipping bird" toxicant metering system using volumetric pipette.

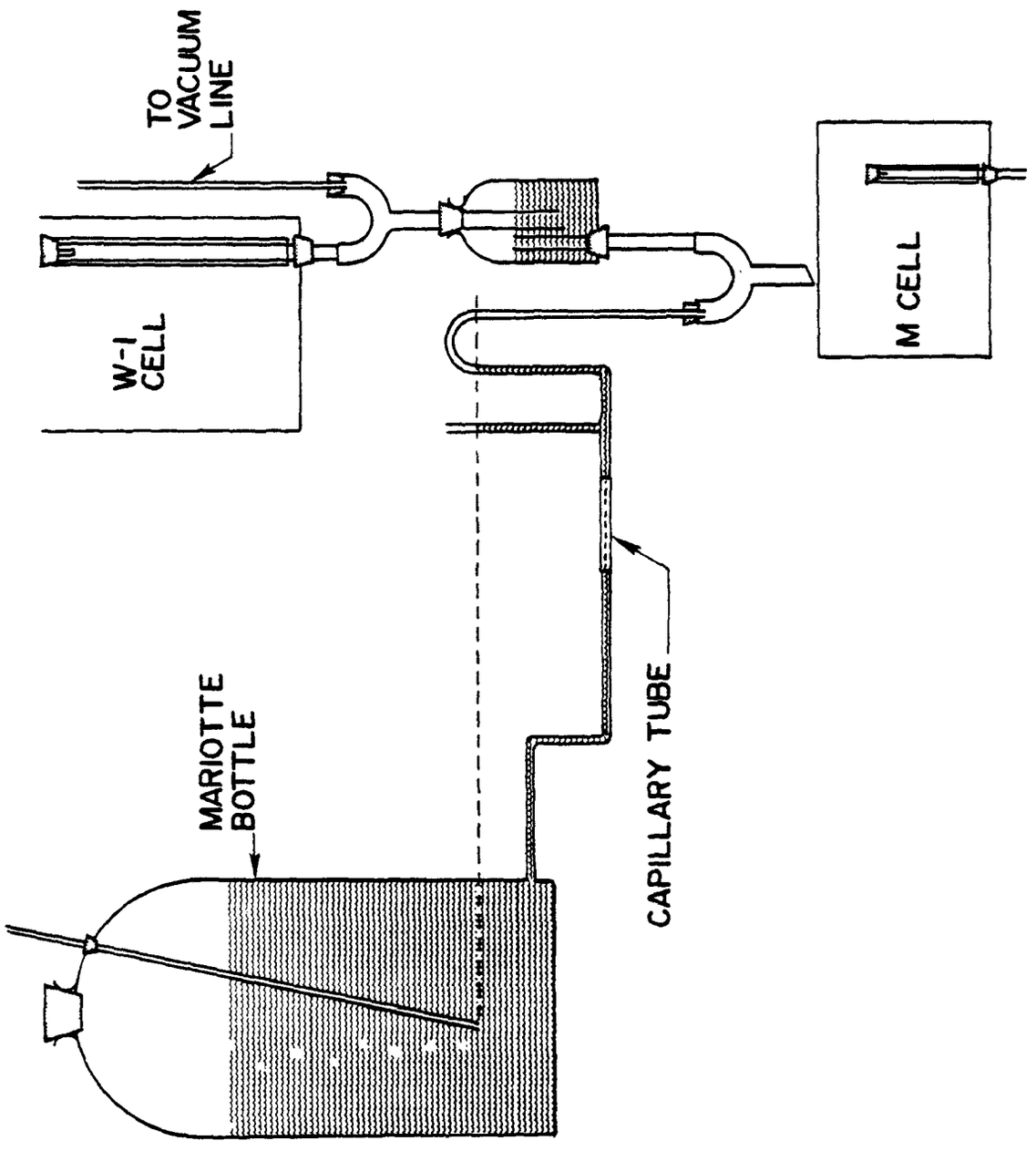


Figure 13. Toxicant-metering assembly using "McAllister system".

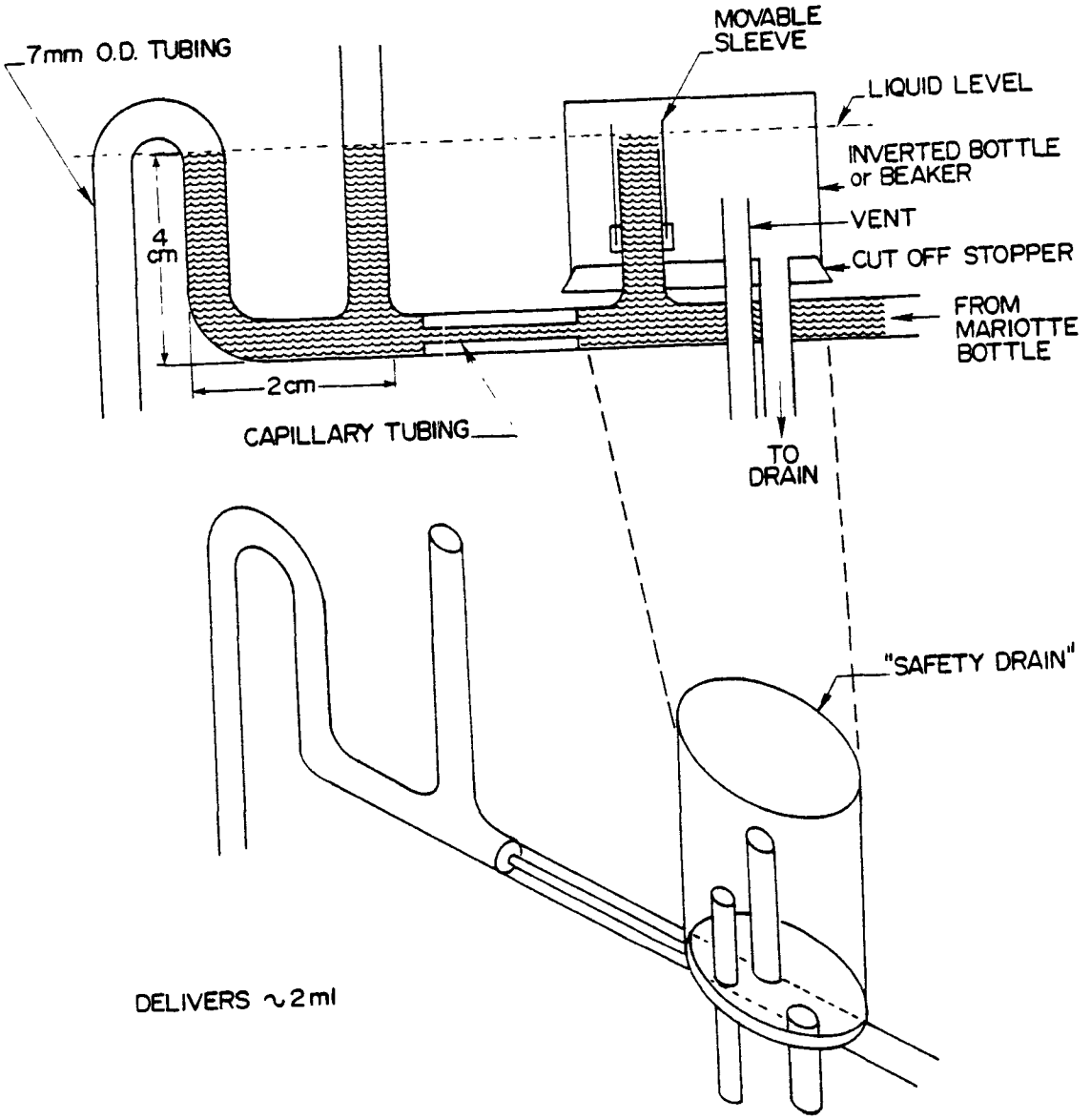


Figure 14. Safety system for the "McAllister toxicant-metering system".

The third system is the multiple syringe injector as described by DeFoe (1975) and included in the Appendix. This system completely eliminates vaporization problems and is useful for highly toxic chemicals dissolved in organic solvents. An additional very useful aspect of this system is the ability to change the dilution factor without recalibration of the diluter. Since each concentration has its own stock bottle, dilution factors can be changed by changing stock-solution concentrations.

Since two of these three systems require a Mariotte bottle, some advice as to their construction and use is necessary. The glass bottle itself should be of 1- to 5-gal capacity and have a narrow mouth for filling. The mouth will be sealed with a neoprene stopper while in use. A small hole should be drilled in the shoulder of the bottle into which will be placed a neoprene stopper with a glass tube (not capillary), the bottom of which will determine the liquid level in the metering system. An additional small hole near the bottom is used for connection to the metering system. As the stock solution initially drains from the bottle, a partial vacuum is produced and air will enter the bottle through the air tube until the air pressure stabilizes. This initial flow may exceed the capacity of the metering system and should not enter the M cell. After stabilization, air will enter the bottle whenever any liquid is removed by diluter operation. Wide variation in room temperature causes variation in the pressure in the bottle and subsequent delivery of the toxic solution. In this case the bottle should be insulated in such a fashion that the calibrations can be read daily for monitoring diluter operation.

SECTION 7

DILUTER MODIFICATIONS

The proportional diluter lends itself to numerous modifications. An experienced person who has built several diluters can usually modify them to adapt to special cases. It is recommended that a regular diluter be made and used first, before modifications are tried. The following directions assume familiarity with the basic diluter. Some of the modifications that we have used are multiple synchronous cycle systems; low toxicity effluent, i.e., 100% effluent; superimposed additions, i.e., turbidity or food added in equal amounts to all concentrations and then a toxicant added and diluted; and equal solvent concentrations with dilution of only the toxicant and including a solvent control.

The use of multiple diluters in synchronous set (Figure 15) is accomplished as follows (Arthur et al., 1975). An extra W cell is added and fitted with an overflow to waste, and the regular sixth or control cell is fitted with a water block and starting siphon. The other cells are the same as in an unmodified diluter. The flow-adjusting valves on the separate diluters are adjusted to fill the W cells just a little faster than the required cycle time. All of the vacuum lines are connected together and are started by a side-arm vacuum aspirator from a water line by having the valve-bucket switch turn on the water as needed. Timing is accomplished by building a separate timing chamber such as shown in Figure 16 (Halligan and Eaton, 1978). The fill time for this upper bucket is slightly longer than that of the W cells. When this chamber cycles into the valve bucket, the supply solenoids are shut off and the vacuum aspirator is activated. The emptying time must be sufficient to allow all diluters to cycle, and when the valve bucket finally empties the cycle is repeated. Equal flows are not necessary once all diluters are started as long as the valve bucket stays down until the last diluter completes its cycle. We have successfully operated three 2-liter and three 1-liter diluters synchronously for nearly a year (Arthur et al., 1975).

In many instances it will be necessary to determine the toxicity of industrial and municipal wastes or other materials that may not be extremely toxic. Mariotte bottles or similar stock solution supplies are inadequate when toxicity is measured in percentage instead of milligrams per liter. The details for a modified diluter whose highest test concentration is 100% effluent are shown in Figure 17. Only a few changes need to be made on a standard diluter to convert it to an effluent-testing diluter.

1) The vacuum tube to cell W-1 is clamped so that the cell cannot empty

2) The M cell and chemical-metering device may be removed.

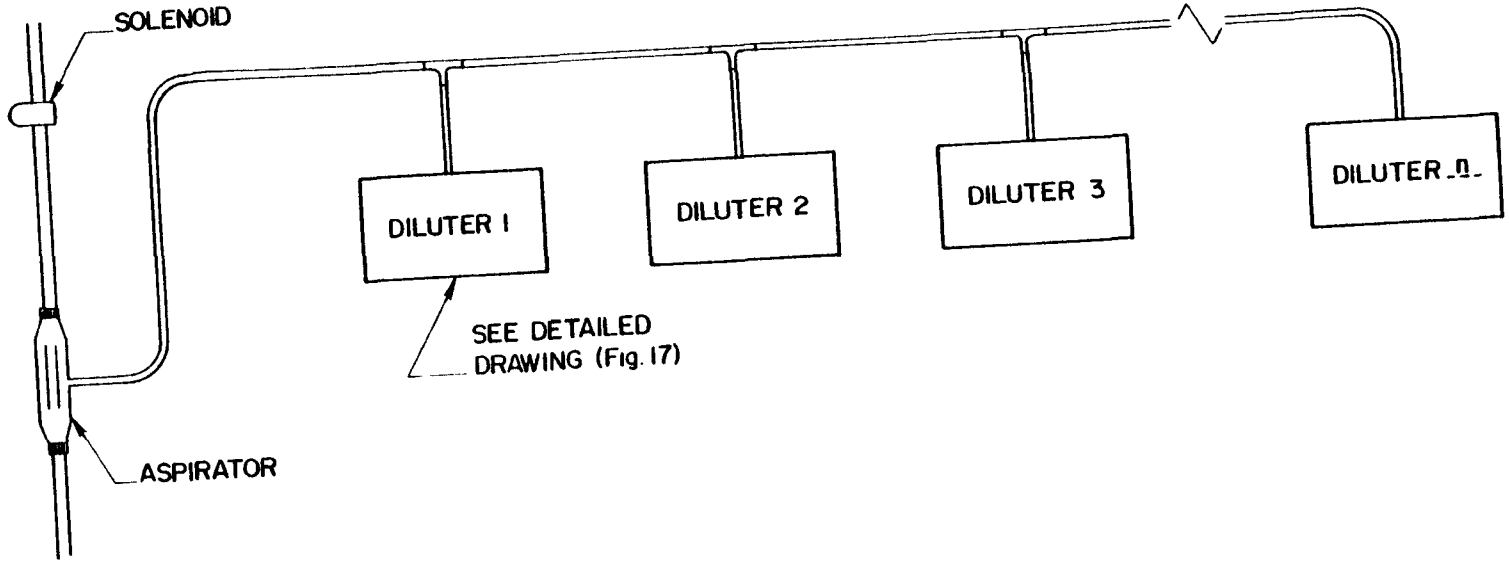


Figure 15. General plan for synchronous multiple diluter system.

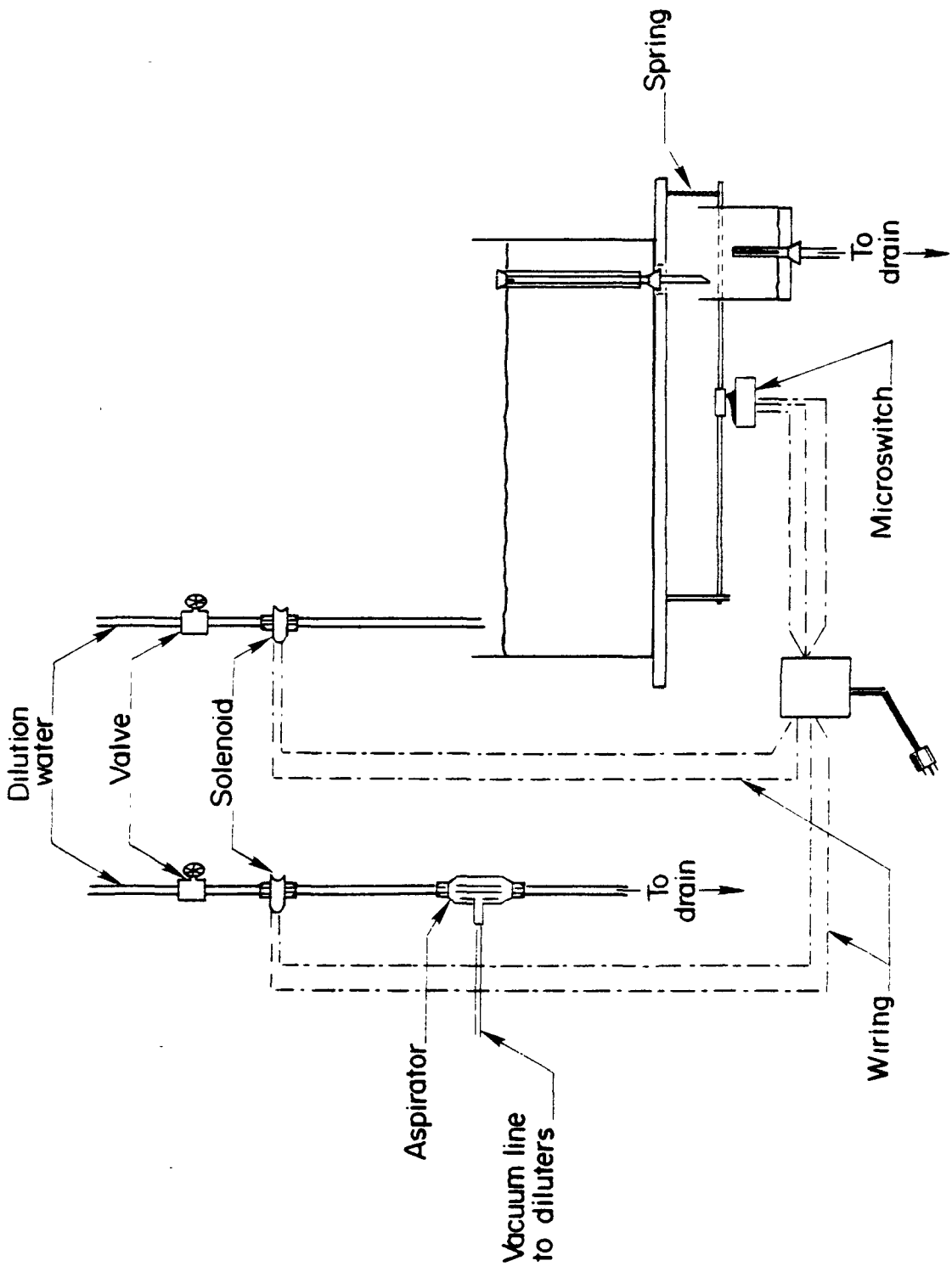


Figure 16. Timing system for multiple diluter system.

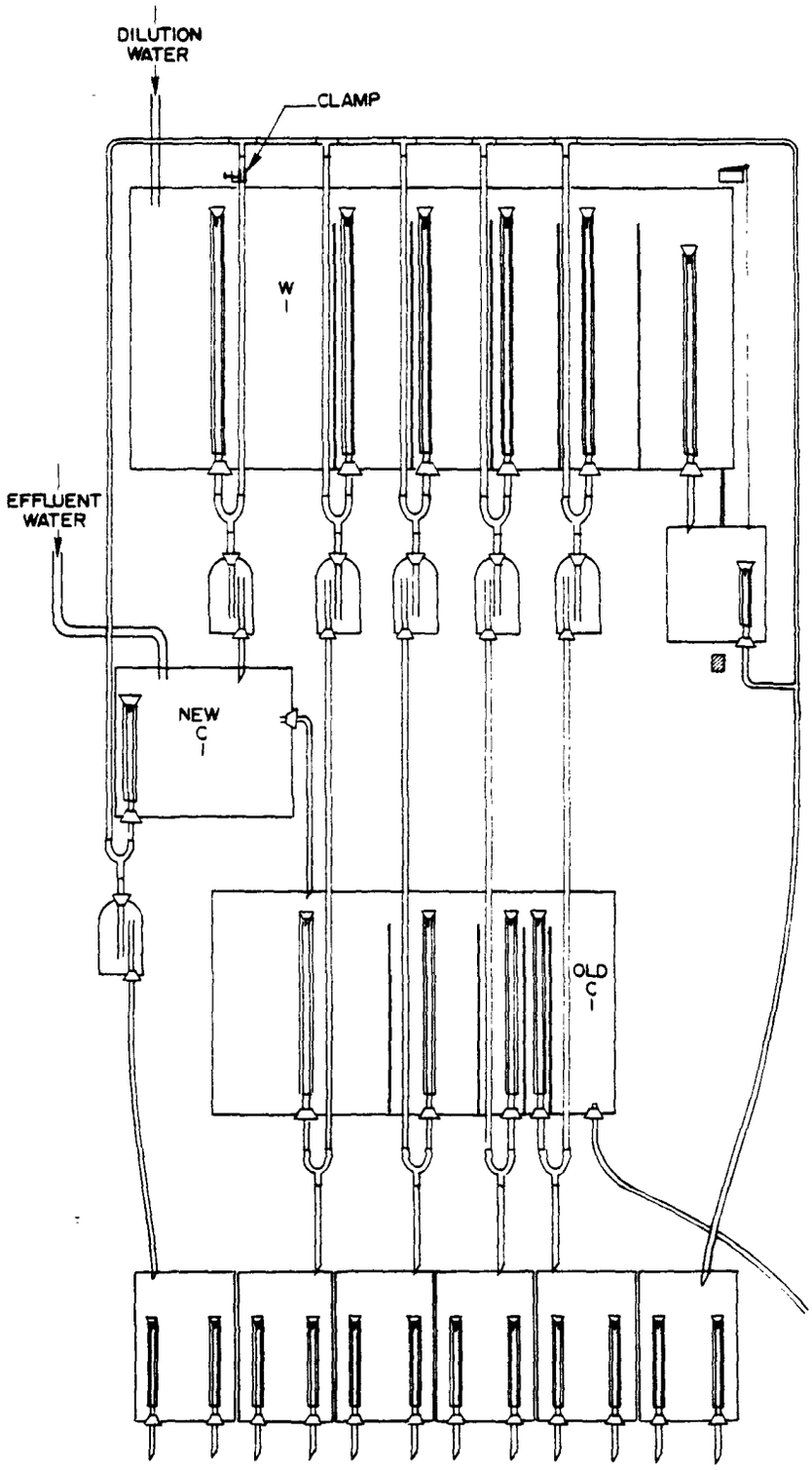


Figure 17. Diluter modifications for effluent dilutions.

3) A new C-1 is constructed and placed on the M cell shelf. It will have a standpipe siphon to deliver 2 liters. A water block is connected to the vacuum line. The vacuum line that had gone to the W-1 cell can be extended to go to the new C-1 cell. The old C-1 cell will empty to waste. The new C-1 cell is built to overflow into the old C-2 cell. The tube from the new cell C-1 water block will go to the same FS cell as did the old cell C-1. The siphon standpipe in the new cell C-1 must be slightly higher than the overflow tube to cell C-2 to prevent a premature siphon.

4) An additional water valve is used to discharge effluent into the new C-1 cell and fill the remaining cells. This valve is also connected to the microswitch under the valve bucket. Consequently, both valves open and close together. The effluent flow rate is set so that cell C-5 overflows into the old cell C-1 and then to waste before the valve is turned off. If the flow rate is too slow, one or more of the last C cells will not be full when the diluter cycles. The dilution-water flow rate may need adjustment since the large W-1 volume is not needed in this mode.

5) All changes can be made in a temporary fashion for easy conversion back to the basic diluter.

If there is a continuing need for an effluent-testing diluter, the above modifications can be incorporated into the original design.

Additions of food, suspended solids, or other similar materials in equal concentrations to all of the test tanks (Brungs and Bailey, 1967) as well as variable toxicant concentrations can be accomplished as follows. A special chamber much like that described by Brungs and Mount (1967) is used. Each diluter receiving the material requires one cell (Figure 18). An extra cell is used for timing, and the water coming from it is sent to waste. Water flowing from those cells used to supply the diluter is used to activate a metering device that meters the desired amount of material into the mixing chamber, which in turn empties into the W-1 cells of the diluters of standard design. Operation of this system is quite similar to regular operation. This modified system differs in two important ways: (1) each of the initial chambers must be calibrated to deliver exactly the required amount of water to feed the diluter including the control (i.e., 12 liters for five concentrations and a control on a 2-liter diluter); and (2) a microswitch or dilution-water valve is not needed on the diluter since the diluter itself does not control the flow rate. If suspended solids are used and settling of these solids is undesirable, the siphon tubes can be set to nearly empty the cells and thus reduce settling.

The injector system of DeFoe (1975) described in Section 6 is an additional useful modification. In the standard diluter, when materials with high toxicity and low solubility are tested, acetone, ethyl alcohol, or similar low-toxicity water-miscible organic solvents are used to disperse the low-solubility material in the water. This dispersant is then diluted in the same proportions as the toxicant. In certain cases it is necessary to maintain a constant dispersant concentration and vary only the toxicant concentration. This system is a series of equal-sized chambers. As these empty, a ratchet-driven syringe is activated for each concentration. The

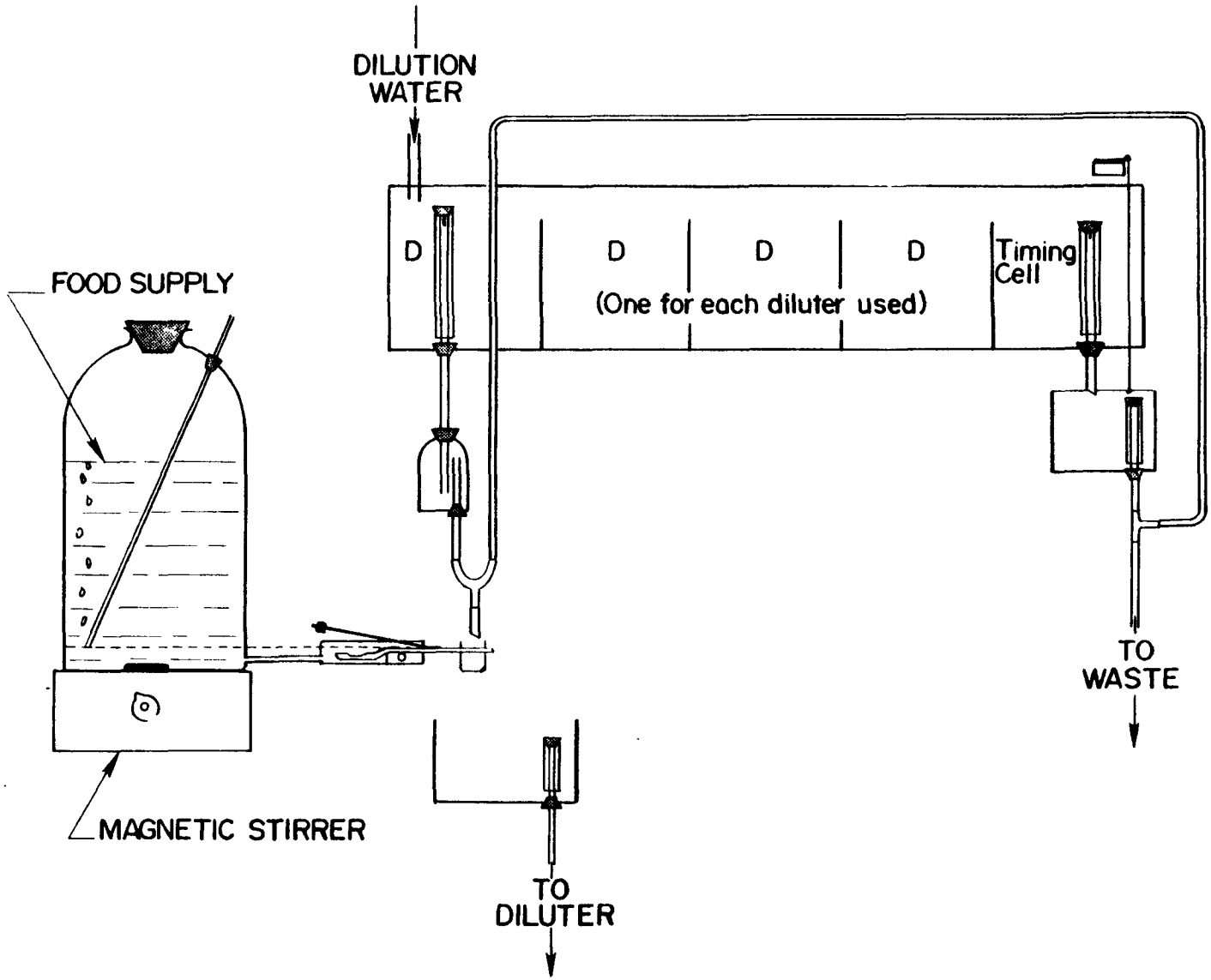


Figure 18. Diluter system modified to include the addition of a food supply (microorganisms). The system may also be used for the addition of sediment or other similar materials

syringes are set to deliver a constant amount of dispersant containing various amounts of the toxicant. It is also possible with this system to test several compounds simultaneously for screening purposes, if desired.

SECTION 8

TROUBLE-SHOOTING

Most diluter malfunctions occur during the first days of operation or after modification or calibration. Listed below are some of the most common problems (A-D) and their causes (1-4).

A) Loss of water flow.

1) Electric power failure or accidental disconnection.

2) Defective microswitch or water valve.

3) Weak valve-bucket spring that will not lift valve bucket.

4) Too much water remaining in valve bucket after emptying. Adjust siphon sleeve.

B) W cell siphons won't start.

1) No water in one or more of the water blocks.

2) Disconnected vacuum line.

3) Dirt or other debris in vacuum tube.

4) Venturi T not functioning properly.

C) Diluter-cell or test-chamber overflow.

1) W-1 cell siphon does not break before the dilution-water valve opens, water will continuously siphon and the M cell will commonly overflow (see Section 9 for safety devices). The timing needs adjustment. The problem can usually be eliminated by slowing the flow through the valve bucket.

2) C-2 or other C cell siphon not functioning properly. If the C-2 or other C cell siphon does not break before the M cell empties, water will continuously siphon until the M cell is empty. The timing needs adjustment. For example, the siphon tube from the W-1 cell can be restricted to slow the filling of the M cell.

D) Premature siphon.

1) Top of W or C siphons too low as compared to the cell dividers.

2) Water tubes not totally emptied by previous siphon action. This difficulty should be eliminated by the 45° angle cuts at the ends of the water tubes. The FS siphons may also function improperly.

SECTION 9

DILUTER SAFETY DEVICES

Several procedures or devices will alleviate problems that occur during diluter operation and increase the probability of conducting successful toxicity tests. If no tests are expected to last more than a few days, then the importance of these devices is reduced.

The use of a calibrated stock-solution container and an electric counter connected to the microswitch (see Section 6) will monitor the chemical-metering apparatus with regard to diluter cycle time and toxicant introduction.

The dipping-bird metering device can occassionally overflow into the M cell if the air-pressure changes significantly in the Mariotte bottle or if air leaks into this bottle. A two-chambered toxicant enclosure (Figure 19) can be used with a central divider over which excess toxicant can flow to a drain tube. This overflow commonly occurs when the Mariotte bottle is refilled and the air pressure inside is stabilized.

A safety device to indicate the continued operation of a proportional diluter is very important. This device can initiate an audible or visual signal, turn on aeration in the test chambers to protect against lowering dissolved oxygen concentrations, or perform any other important function. We have used the W-6 cell most frequently with a small capillary drain at the bottom of this cell (Figure 20). This permits the water level in W-6 to fall below the bottom of the siphon sleeve. Since the flow is slow through the capillary, several minutes or more are required for this level to drop very far. A float level switch (FPC level switch, Fluid Products Co., Hopkins, Minn. 55343) located in the W-6 cell is used to activate alarms and aeration when the water level drops significantly as the result of an elimination of dilution-water flow to the diluter. This capillary drains to waste. The capillary tube should be checked daily and frequently cleaned as clogging can occur.

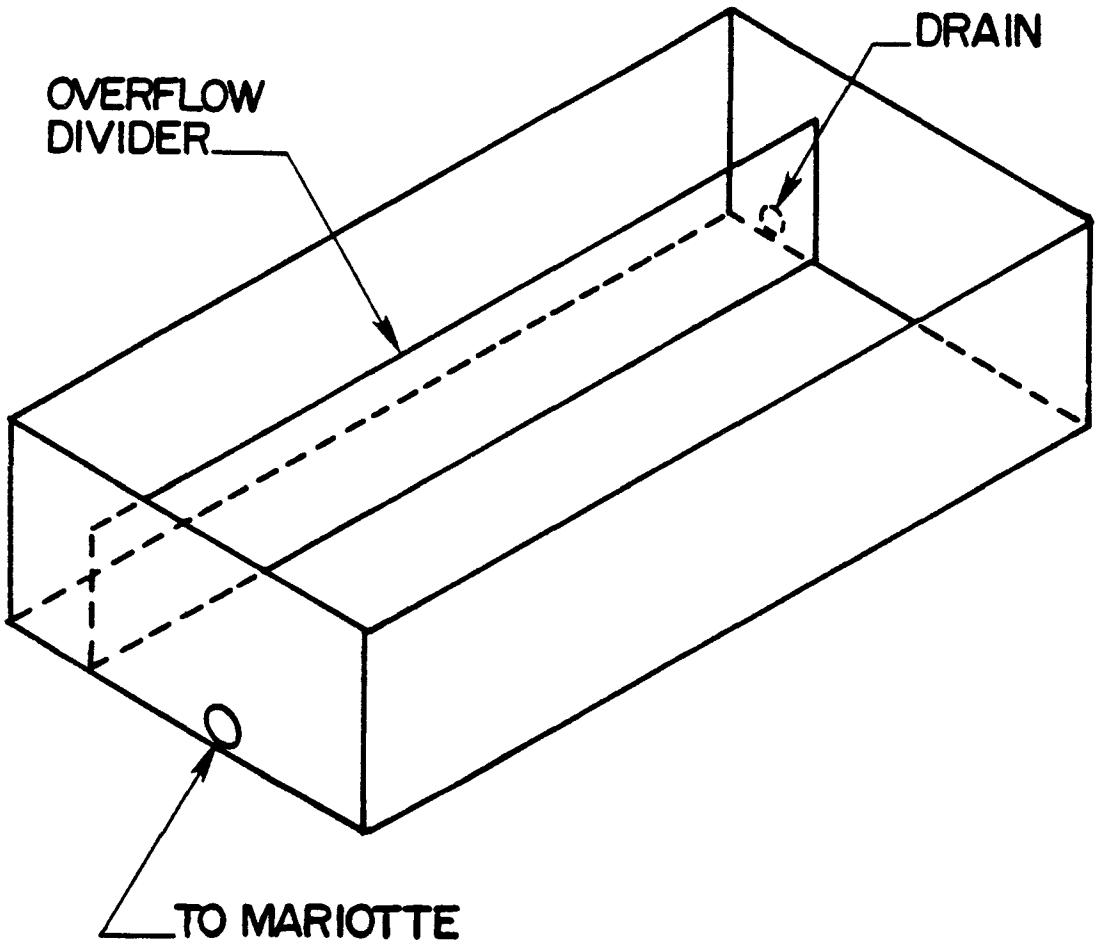


Figure 19. "Dipping-bird" safety device.

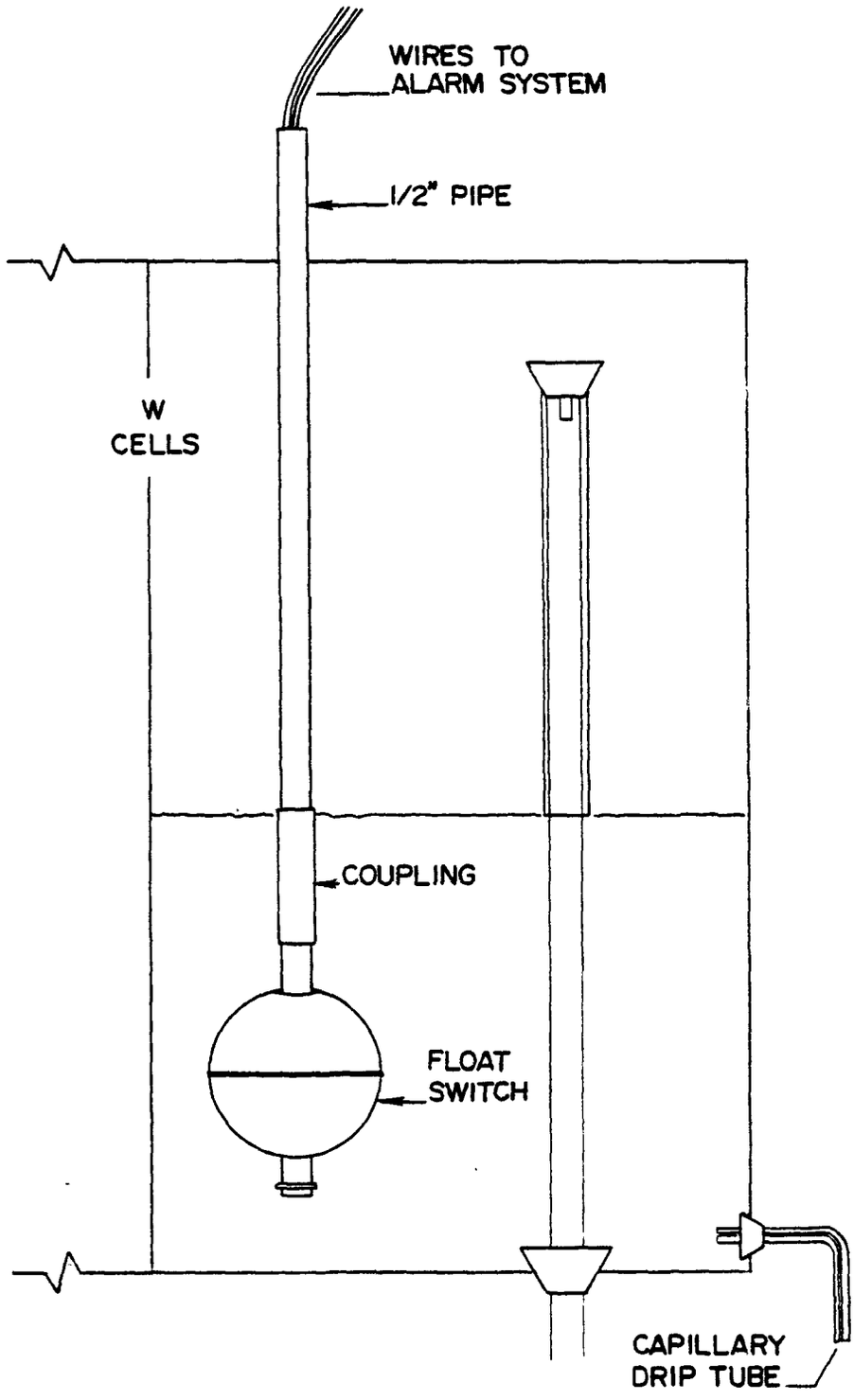


Figure 20. Diluter malfunction safety system.

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APPENDIX A

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A SIMPLIFIED DOSING APPARATUS FOR FISH TOXICOLOGY STUDIES

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Abstract—A simplified diluter for maintaining a series of constant concentrations of a material in flowing water is described. It depends on water flows, metering cells, and venturi tubes to proportion volumes of water and toxicant to give desired concentrations. Construction requires less than 2 days, and only readily available materials are needed. An injector for mixing pesticides in water is also described.

INTRODUCTION

MOUNT and WARNER (1965) have described a serial dilution apparatus suitable for maintenance of constant concentrations of materials in flowing water. They have discussed the need for reliable systems that cannot deliver an excessively high concentration of toxicant in long-term fish toxicity studies. At the Newtown Laboratory of the Cincinnati Water Research Laboratory, Federal Water Pollution Control Administration, Cincinnati, Ohio, we have used this system for several years in fish toxicology studies and have been well satisfied with its performance. Because of a need for more narrow concentration series, such as 1, 0.8, 0.64, 0.51, etc., we have modified the serial diluter in order to make it more suitable for such uses. We have also found that some of those who have constructed serial diluters have had problems before they were able to achieve satisfactory operation. Apparently selection of tubing sizes was troublesome. Some of the components and principles herein described, particularly the water delivery system, can be used advantageously on the serial diluter and are discussed later.

The modified diluter, called a proportional diluter, is not based on serial dilution but rather on simultaneous dilution of one concentration. It has these advantages over the serial diluter: (1) water is delivered to each chamber each half cycle so that the flow rate can be twice as great; (2) timing problems are minimal; (3) operation is much simpler and easier to understand; (4) malfunctions are less frequent than in the serial diluter system; (5) it can deliver a series of concentrations, each concentration as much as 90 per cent of each preceding concentration; and (6) much less vertical space is needed. The main disadvantage is that it is impractical to deliver a series of concentrations with a dilution factor greater than 50 per cent between each concentration; e.g. a concentration series such as 1, 0.1, 0.01, etc.

The proportional diluter shown in FIG. 1 and described in this paper is one that can deliver 5 toxicant concentrations and a control at any desired flow rate per concentration up to 400 ml/min, and with a dilution factor from 50 to 25 per cent between

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successive concentrations. Metering and chemical cells can be exchanged so that the dilution interval between successive concentrations can be decreased down to 10 per cent, that is, a concentration sequence such as 1, 0.9, 0.81, 0.73, etc. Because persons have requested additional details of the serial diluter, more specifications are given in this paper. Throughout the following description, a delivery vol. of 500 ml per concentration is assumed with a maximum flow of 400 ml/min per concentration.

MATERIALS

As before (MOUNT and WARNER, 1965), every effort has been made to utilize materials readily available. Four sheets of 12 × 24-in. single-strength window glass, appropriate glass tubing, glass glue, a hand glass cutter, rubber stoppers, a 1-in. plastic hose "T", plastic bottles, and optionally a mechanical counter, constitute the materials needed. If one wishes, local glass stores will cut the glass to desired sizes, and for a very modest price they will cut the necessary three holes. The availability of an excellent silicone rubber glass glue (Clear Seal produced by General Electric or Glass and Ceramic glue produced by Dow-Corning*) has made the construction of the chambers extremely simple. Clean glass can be glued without etching or scratching, and the pieces can be assembled by simply pressing the pre-glued edges together. TABLE 1 lists the recommended cell sizes for the diluter described in this paper.

TABLE 1. DIMENSIONS AND CAPACITIES OF METERING CELLS

Cell No.	Size (cm) H W L	Maximum capacity (ml)
W-1	12 × 6 × 23	1656
W-2	12 × 6 × 4	288
W-3	12 × 6 × 6	432
W-4	12 × 6 × 7	504
W-5	12 × 6 × 7	504
W-6	12 × 6 × 8	576
M-1	10 × 11 × 16	1760
C-2	12 × 3 × 11	396
C-3	12 × 3 × 9	324
C-4	12 × 3 × 7	252
C-5	12 × 3 × 5	180
C-1	12 × 3 × 6	216

Height does not include 3 cm of freeboard for sides and ends.

PRINCIPLES OF OPERATION

A series of water-metering cells are filled, the water is turned off, the cells are emptied, and the water flow is restored. (The reader is referred to FIG. 2 for a better understanding of the following.) Cell W-1 fills first from IT then overflows into W-2, etc. When cells W-2 to 5 are emptied, appropriate quantities of a higher concentration

* Mention of commercial products does not constitute endorsement by Federal Water Pollution Control Administration.

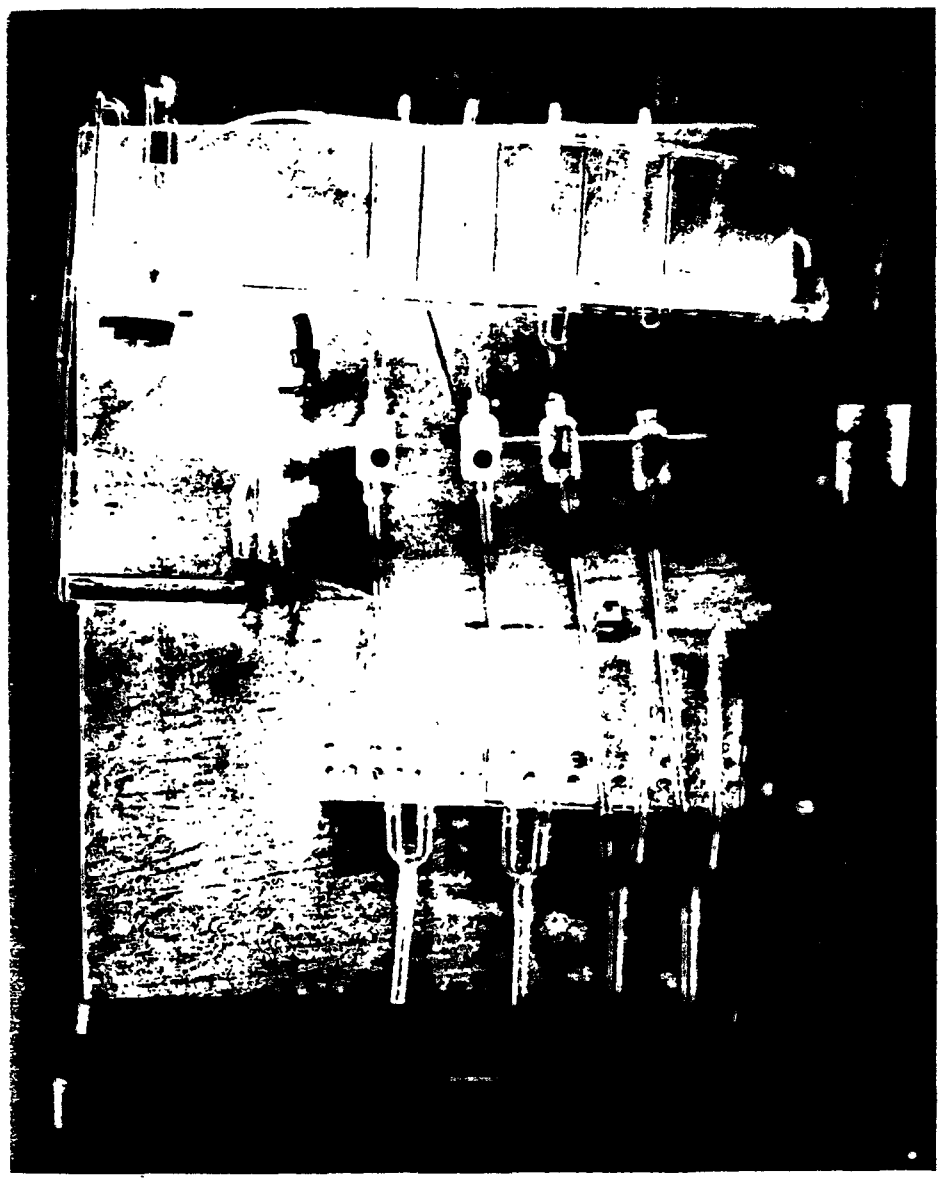
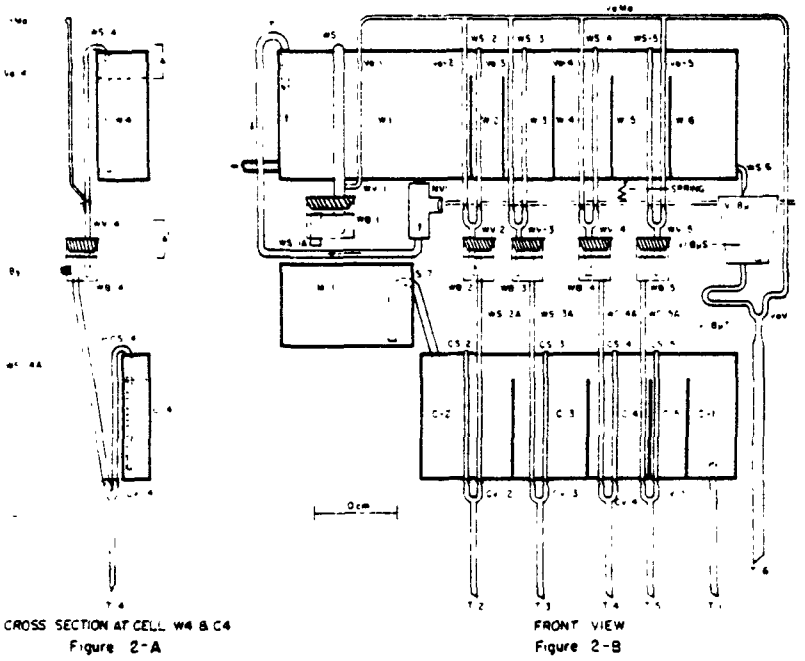


FIG. 1. Photograph of a proportional diluter built as suggested in this paper.

(concentration 1) from cells C-2 to 5 are mixed with the diluent water to give the desired lower concentrations. While the water from cells W-2 to 5 is being emptied through tubes WS-2 to 5 and WS-2A to 5A, the water from W-1 is emptied through WS-1 into mixing chamber M-1 where the toxicant is added, and then the chemical cells C-2 to 5 are filled from cell M-1 through tube S-7. Cell C-2 fills first then overflows into cell C-3, etc. The vol. of W-1 is adjusted so that after cells C-2 through C-5 are filled, 500 ml flows into C-1 and then to the test chamber to furnish test water for the high concentration. Water for a control test chamber is emptied from W-6 and operates the water valve (NV1) to turn off the influent water from tube IT while cells W-1 to 5 empty. It also flows through the vacuum venturi, (VaV) to produce a partial vacuum in the vacuum manifold (VaMa), which is connected to each water venturi (WV-1 to 5) by the tubes Va-1 to 5. The partial vacuum applied by the water venturi causes water from the water cells to rise through the water siphon tubes (WS-1 to 5) and start the siphoning action to empty the water cells. The water blocks (WB-1 to 5) serve to prevent air from entering the system through water siphon tubes WS-1A to 5A. The distance from the water level of each filled water-metering cell to the top of its water siphon tube, distance "A" (FIG. 2A), must be less than the distance from the water level in its respective water block to the bottom of the "U" in its water venturi, distance "A'". Otherwise the water siphons will not start but rather



CROSS SECTION AT CELL W4 & C4
Figure 2-A

FRONT VIEW
Figure 2-B

FIG. 2. Semi-schematic scale drawing of diluter.

Legend: B—block; Bu—bucket; By—by pass spout; C—chemical; I—influent; M—mixing; Ma—manifold; N—needle; S—siphon; T—tube; V—venturi; Va—vacuum; Vl—valve; W—water.

water will enter the vacuum tubes Va-1 to 5. As the water passes the chemical venturi tubes, CV-2 to 5, the chemical siphons, CS-2 to 5, are started and chemical cells C-2 to 5 are emptied.

Only two timing adjustments must be checked: (1) the water flow through the vacuum venturi (VaV) must be fast enough to produce sufficient vacuum to start the water siphons but slow enough so that the water valve remains closed sufficiently long to allow water siphon WS-1 to empty cell W-1 before the influent water again enters W-1 from tube IT; and (2) cells W-2 and C-2 must be emptied and the siphon in tube T-2 broken before water from mixing cell M-1 enters cell C-2 through tube S-7. Obviously, the siphon in T-3, T-4, and T-5 must also be broken before their respective cells fill. This latter problem should not occur if the tube sizes suggested in

TABLE 2. TUBE USED ON DILUTER

Tube No.	o.d. (mm)	Material
WS-1	15	Glass
WS-2 to 5	8	Glass
WV-2 to 5	8	Glass
WS-2A to 5A	8	Glass
WS-1A	15	Glass
WS-6	8	Glass
S-7	10	Glass
CS-2 to 5	10	Glass
CV-2 to 5	10	Glass
T-1 to 5	10	Glass
VaV	5	Glass
T-6	8	Glass
Va-1 to 5	5	Plastic
VaMa	5	Plastic
VlBuT	7	Rubber
IT	10	Glass
NV1	25 (1 in.)	Plastic hose "T"
V1BuS	6	Glass

TABLE 2 are used. If water enters C-2 too soon, the flow rate through tube WS-1A can be slowed by restricting the opening. The siphon WS-6 is adjusted in height so that the total vol. delivered from cell W-6 is 500 ml. The valve bucket (V1Bu) should have a capacity of approximately 500 ml and is best made from a polyethylene bottle. The tube WS-6 must fill the valve bucket at a rate so that the valve closes quickly, giving ample time for the water level in the water cells to drain down to the top of the cell partitions. This drainage must be completed before the water begins flowing through the valve venturi. The time required for drainage is reduced by sloping the cells approximately 1 cm in 10 cm. The chemical cells should be sloped as well. This can be accomplished by sloping the back board of the diluter or by sloping the two shelves on the board as shown in FIG. 1.

CALIBRATION

The vol. delivered from water cell W-1 can best be measured by catching the delivery from tube WS-1A. The delivery vol. from cells W-2 to 5 can be measured by opening

the bypass (By) on the water blocks WB-2 to 5 (shown only on FIG. 1 and 2A) and catching the flow. The bypass must be sufficiently large and positioned so that no water goes down WS-2A to 5A. These vols. should be checked while the diluter is cycling normally in the event that the drain-down is not entirely complete. The delivery vol. of cells C-2 through C-5 are determined as follows. The influent water to the diluter is stopped just as the water cells begin to empty. After the "C" cells have been filled and the overflow into cell C-1 has stopped, 5–10 ml of water should be added to cells W-2 through W-5 to prevent air from entering WS-1 to 5. Suction should then be applied to tube T-2 with a suction bulb and the water delivered caught in a graduated cylinder. This procedure should be repeated for cells C-3 to 5 and then the water flow restored.

The volumes in W-1 through W-5 and C-2 through C-5 cells are adjusted as needed by increasing or decreasing the depth to which the siphon tubes extend into the cell. The WS-1 to 5 tubes and CS-2 to 5 tubes should be glued to the outside of the water and chemical cells so they are rigid, but they should be cut off approximately at the top level of the cell partition and then an adjustable extension added to furnish the desired length. This arrangement allows for maximum adjustment of vol. The cell ends of the WS-1 to 5 and CS-2 to 5 tubes should be exactly parallel to the water surface in the cell so that the siphon breaks abruptly. Placing a funnel-shaped flare on the end of the tube enhances abrupt breaking.

TABLE 3. REQUIRED WORKING VOLUMES OF EACH CELL FOR DILUTION FACTORS OF 50 AND 25 PER CENT BETWEEN CONCENTRATIONS

Cell No.	Vol. (ml) 50 % Factor	25 % Factor
W-1	968	1525
W-2	250	125
W-3	375	219
W-4	438	289
W-5	469	342
W-6	500	500
M-1	968	1525
C-2	250	375
C-3	125	281
C-4	62	211
C-5	31	158
C-1	500	500

TABLE 3 lists the requisite working volumes for 50 per cent and 25 per cent dilution factors between concentrations. These two series of concentration intervals represent the recommended extremes for this particular diluter. One should construct water and chemical cells of different dimensions for better accuracy for greater or smaller dilution factors.

DETAIL FOR SPECIFIC COMPONENTS

(A) *Needle valve*

FIGURE 3, from MOUNT and WARNER (1965), is reproduced here for convenience in constructing the needle valve. For the diluter described in this paper, an inlet and outlet rubber tube of $\frac{1}{8}$-in. i.d. is suggested and a needle made of 13-mm glass tubing. The glass rod should be approximately 5 mm in dia. The taper below the vacuum venturi should be from 5–8 mm in a distance of 1.5–2.5 cm. A string, pulley, and bucket filled with sand makes a fine counterbalance weight to replace the valve spring.

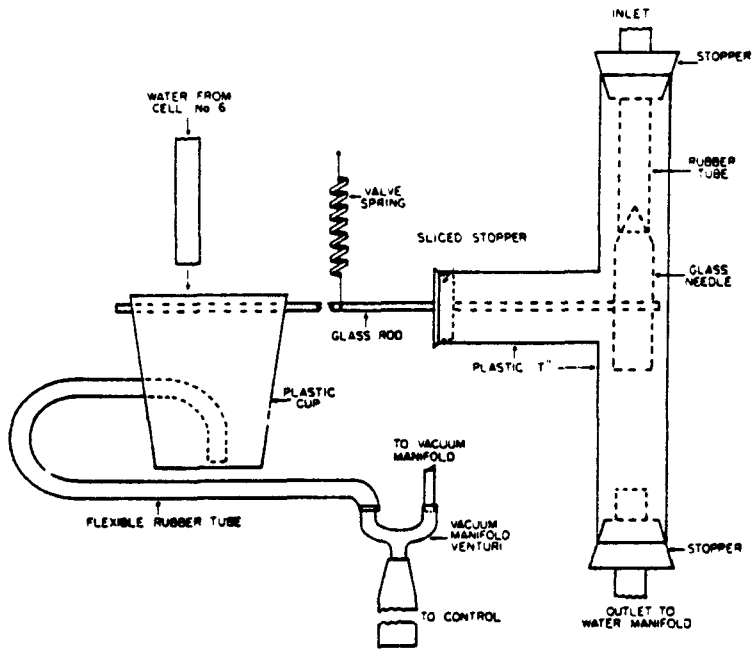


FIG. 3. Needle valve and vacuum venturi detail.

(B) *Vacuum connexion for WS-1 tube*

Since "U" shaped connecting tubes are not easily obtainable in 15 mm o.d., the vacuum line Va-1 is best connected to the WS-1 tube by blowing or grinding a small hole in the side of the tube and gluing over the hole a short piece of 3 mm o.d. glass tubing. Care must be taken to keep the A and A′ distances in the proper relationship as discussed earlier.

(C) *Chemical-metering apparatus*

Many types of metering apparatus can be used to introduce the toxicant; the specific choice depends on the chemical characteristics of the toxicant. Pumps can be used satisfactorily for short-duration tests in which no great damage will occur if the water flow fails or slows drastically. (The pump would continue to introduce to-

and kill the animals.) For longer tests, a safer device is needed. FIGURE 4 (taken from MOUNT and WARNER, 1965) illustrates the method of choice for highly water-soluble materials. By this method, the water solution is kept at a constant level in the funnel by a Mariotte bottle. (The bottle must be insulated against rapid air temperature fluctuations or the funnel may overflow.)

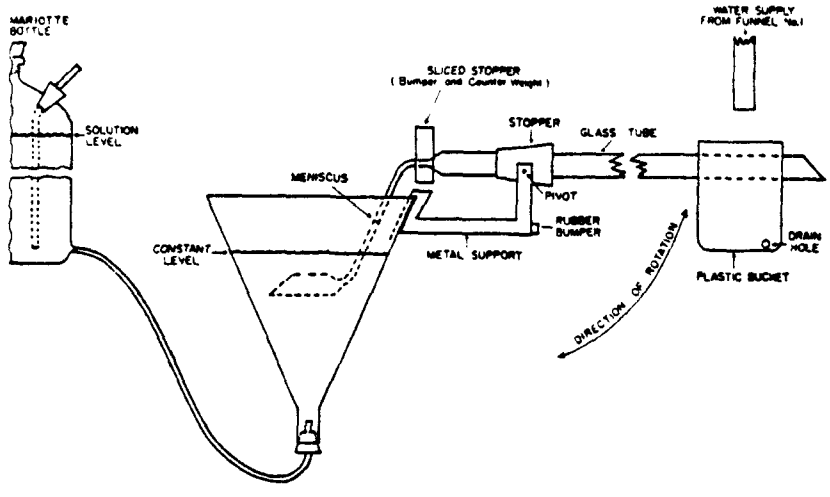


FIG. 4. Detail of chemical-metering apparatus.

When water enters the plastic bucket of the chemical-metering apparatus from cell W-1 (labelled funnel No. 1 on drawing), the tube rotates and the toxicant solution runs through the tube and into the mixing chamber (M-1) beneath. The tube is made by heating and drawing an appropriate sized piece of glass tubing and then bending it to the necessary angle. By experience, we have found that partially closing the funnel end of the tube (by firepolishing) and cutting a hole in the top for filling and releasing air gives slightly better accuracy. (Note: The Mariotte bottle is not drawn to scale.)

For organics that are slightly soluble in water, we have used an injector as sketched in FIG. 5. It is simply a lever arm actuated by the water filling the plastic bucket and causing the arm to rotate. On the end opposite the bucket, a small pawl advances a gear, 1, 2, or 3 teeth; the gear wheel turns the nut a few degrees, advancing the bolt and piston a very short distance and displacing a few μl of solution through the needle into the water from cell W-1. We have used a gear with 42 teeth and a bolt with 40 threads/in. so that by advancing the gear, one tooth at a time, there are 1680 injections/in. of piston travel. With a 1-ml syringe, this gives approximately 0.25 μl/injection; this can be increased up to 30 μl if a 50-ml syringe is used and the gear is advanced three teeth. Thus, one full syringe lasts for 3–10 days, depending on the cycle time of the diluter. Although the injector may seem difficult to construct, if one has a suitable gear and the bolt, the rest can be made from glass tubing, rubber stoppers and burette clamps.

Acetone solutions of organics can be used in the syringe as a stock solution, or if the toxicant is a liquid and is water miscible, it can be used without further dilution.

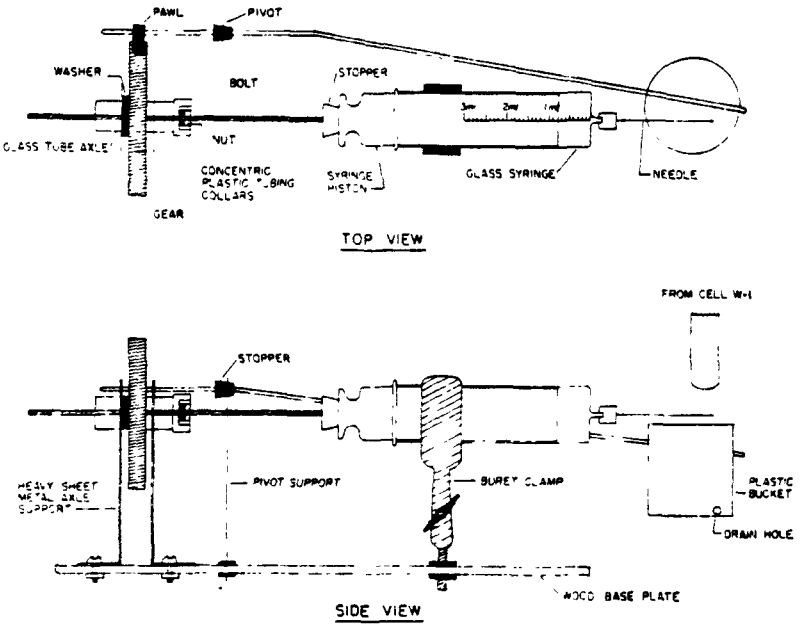


FIG. 5. Injector system for adding μl quantities to the water.

For such toxicants as pesticides we have found the following procedure to be the only way in which we can measure as much pesticide in the water as is introduced.

First the injector is used to inject *air* into a small closed vessel such as a 60 ml stoppered bottle. The air then forces the slurry through a capillary tube from the bottle into the water in chamber M-1. (The syringe and bottle must be insulated against sudden temperature changes.) The bottle is placed on a magnetic stirrer located slightly below the M-1 cell, and the slurry is stirred continuously.

The slurry is made as follows: (1) 25 mg of Triton X-100 is dissolved in 15–25 ml of water; (2) 1–2 ml of acetone containing the requisite amount of pesticide is then added, or if the pesticide is a liquid, it is added directly without being dissolved first in acetone; (3) the mixture is shaken vigorously and then made up to 50 ml for use. Depending on the amount of pesticide present, the slurry is usually white.

We have successfully maintained as much as 10 g of parathion in suspension in 50 ml of slurry in this way without exceeding 10.0 ppb of Triton in the test water, and *no* acetone was present. The decided advantage of this slurry is that it is a micro-suspension that disperses readily in the water and then goes into true solution, whereas when pesticides are dissolved in acetone and introduced directly into water, they usually precipitate and only violent agitation will disperse them in the water. Ludzack (personal communication) stated that in tests he performed there was a marked tendency for aldrin and dieldrin to appear in the surface film or above the water surface on the sides of the container, when they were dissolved in organic solvents before they were introduced into water.

(D) *Modifications of the serial diluter*

The type of water-metering cell described in this paper is superior to that described by MOUNT and WARNER (1965). The main advantage is that the problem of pushing water over the water siphon tubes does not exist because the system is an open one and no pressure can develop. In addition, volume adjustments can be made more readily, either by moving the tubes or using volume displacers.

Only one minor change need be made to adapt the open cell system to the serial diluter. The water siphon tubes WS-1 to 5 must be set so that the siphons start in proper order. This is achieved by setting WS-1 and WS-5 as close to the cell edge as possible (as shown in FIG. 2B) and then raising WS-4 approximately 5 mm, WS-3, 10 mm, and WS-2, 15 mm above the cell edge. Funnels may be used for water blocks as previously described or plastic bottles may be used as shown in FIG. 1. It is necessary, as for the proportional diluter, to slope the cell unit so that when the influent water is shut off by the valve, the water will drain down quickly to the level of the partition tops.

SUMMARY

The diluter herein described has been found by testing to be as dependable as or more dependable than the serial diluter described by MOUNT and WARNER (1965). It operates simply and is much easier to understand and construct. The diluter shown in FIG. 1 was built in approximately 13 hr. For very wide concentration ranges with very large dilution factors between each concentration, the serial diluter (MOUNT and WARNER, 1965) is best, but for dilution factors, 50 per cent and smaller, the one described here is superior.

REFERENCE

MOUNT D. I. and WARNER R. E. (1965) *A Serial-dilution Apparatus for Continuous Delivery of Variou Concentrations of Materials in Water*. U.S. Public Health Service Publ. No. 999-WP-23, 16 p.

APPENDIX B

Multichannel Toxicant Injection System for Flow-Through Bioassays

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DEFOE, D. L. 1975. Multichannel toxicant injection system for flow-through bioassays. J. Fish. Res. Board Can. 32: 544–546

A mechanical injection system needing little maintenance is described which offers a dependable and flexible method for maintaining multiple toxicant concentrations in flow-through bioassays. Falling water from a series of water-metering cells similar to those of a proportional diluter, activates a series of mechanical syringe injectors. The system dispenses equal amounts of toxicant dispersants (solvents, wetting agents, or additives) to all toxicant concentrations and to a dispersant control and allows changing unlimited toxicant concentrations independently of each other.

DEFOE, D. L. 1975. Multichannel toxicant injection system for flow-through bioassays. J. Fish. Res. Board Can. 32: 544–546

L'auteur décrit un appareil d'injection mécanique ne requierant que peu d'entretien et fournissant une méthode fiable et flexible pour maintenir plusieurs concentrations de toxique lors d'analyses biologiques dans un courant continu. L'eau qui tombe d'une série de cellules compteuses d'eau semblables à celles d'un appareil à dilution proportionnelle active un groupe de seringues d'injection mécaniques. L'appareil distribue d'égales quantités d'agents dispersifs du toxique (solvants, agents mouillants ou additifs) à toutes concentrations de toxique, ainsi qu'à un agent dispersif témoin, et permet de varier à l'infini les concentrations de toxique indépendamment les unes des autres.

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THIS report describes modifications made to the *proportional diluter* and *injector* of Mount and Brungs (1967) to dispense equal amounts of toxicant dispersant or additive to all toxicant concentrations and to a dispersant control.

In addition to dispensing equal amounts of toxicant dispersant, the system has the following advantages and qualities:

1) An unlimited number of toxicant concentra-

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tions can be changed independently of each other and to any proportion with only minor adjustments.

2) The device can be used in bioassays where measurement of toxicant in solution, such as a polyelectrolyte, is impractical or difficult. The toxicant concentrations of the test water can be precisely calculated from the amount of toxicant injected and the volume of water used.

3) The system operates mechanically by using the force of falling water; the operation of each injector unit ceases to operate if the water flow stops. This reduces the possibility of toxicant overdosing or flushing of individual concentrations in the event of a support-system failure.

4) The commercial availability of the injection units minimizes the amount of construction done by the researcher, and the durability and simplicity reduce the maintenance demand.

During the 30-mo evaluation of the injector system, the coefficient of variation (C.V.) of dispensing water soluable toxicants into bioassay systems ranged from 8 to 12%. The injected volume of polyelectrolytes measured over a month period showed a C.V. of 8%. The C.V. for a 4-mo cadmium zinc mixture study ranged from 10 to 12%. Concentrations ranged from 4.8 to 8.0 and 25–140 μg/liter, respectively, for the two metals. These C.V.s represent injector error and analytical error.

For insoluble compounds, such as PCBs and methoxychlor, the C.V. increased. A 14-mo PCB exposure with concentrations ranging from 0.1 to 10 μg/liter, resulted in C.V. of 24–42%. Methoxychlor measurements after a month period showed a C.V of 20%. These C.V.s include dissolution, sorption, and analytical error in addition to injector error.

Operation — The delivery system is diagrammed in Fig. 1A. Incoming water controlled by a solenoid enters water-metering cell 0. Consecutively, each cell fills and overflows, and fills the subsequent cell. The last cell, 7, has a self-starting standpipe siphon described by Benoit and Puglisi (1973). This siphon delivers water from cell 7 to a bucket suspended over a microswitch which shuts off the incoming water. From this bucket the water siphons off and flows through a U-tube creating the venturi vacuum, which starts the flow through the remaining metering cell siphons (1–6). Equal volumes of water are discharged to each of the mixing cells below.

Each mixing cell is mounted on the operating lever of the injector (Fig. 1B) and triggers an injection each time water enters it. The operating lever turns a cogged wheel that drives a threaded rod against the syringe plunger. The injected liquid is transported by a teflon tube from the syringe to the mixing cell, where the turbulence of the incoming water ensures adequate mixing. Proper calibration of the standpipe siphon in the mixing cell ensures that nearly all of the toxicant and water is present and mixed before being discharged into the flow-splitting cell. This cell is also equipped with a standpipe siphon for more accurate flow splitting and easier cleaning.

Control water can come from any of the water-metering cells; in this system cell 2 was used (Fig. 1A). An individual metering cell ensures that the control will always receive an amount of water (1 liter) equal to that of other test tanks. The proportional diluter uses the water remaining after all metering cells are filled to supply control water. Therefore, if flow rates change for any reason, the control water volume could vary as much as 50% until adjustments are made.

The dispersant control has one injection unit to dispense the dispersant only, while the control water empties directly into the flow-splitting cell.

Total cycling time for this system depends on the flow rate of incoming water; in practice the fastest was approximately one cycle per minute

The toxicant concentrations can be varied by one or all of the following adjustments:

1) The toxicant stock solution for the syringes can be changed. This adjustment will vary each concentration independently of the others.

2) The quantity of toxicant solution injected can be controlled by syringe size (10–50 ml) and by adjusting the set screws on the operating lever (Fig. 1B). These screws control the amount of pivot of the operating lever, which can turn from 1 to 10 cogs of the wheel per cycle. These adjustments allow an injection volume range of 2.3–85 μl.

3) The amount of water receiving the injection can be altered by recalibrating the siphon in the water-metering cell.

Materials — Most of the materials needed are similar to those described by Mount and Brungs (1967) for the proportional diluter. The following materials are needed for the injection units — one mechanical injector for each toxicant concentration and for the dispersant control. The injectors are available commercially; however, there is no patent on the injector so it can be copied or constructed by any individual. For each injector one gas-tight gas-chromatograph syringe is required. Sizes 10–50 ml have been used successfully This type syringe was used because of the resistance of the teflon plunger tip to toxicants and their dispersants. Teflon tubing

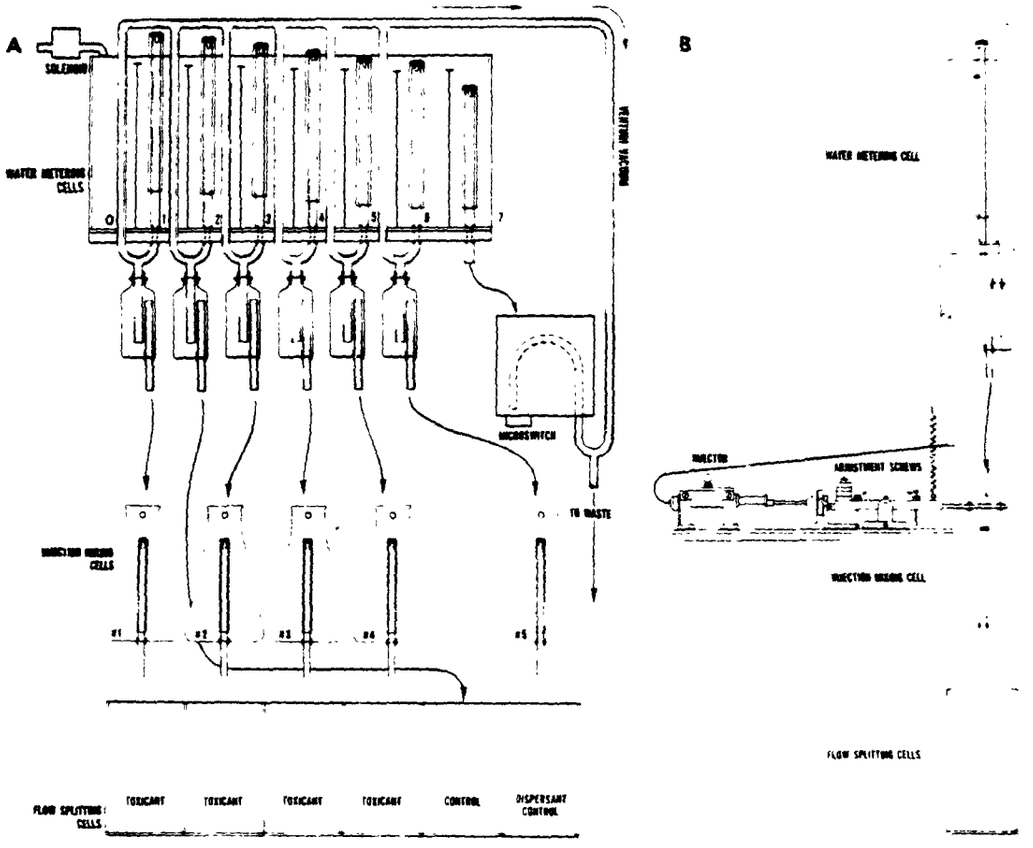

FIG. 1. Front (A) and lateral (B) views of injector system.

was also used to transport the injected material. The injection mixing cells are Erlenmeyer flasks; one flask is mounted on each injector operating lever. A spring is needed to return each mixing cell and operating lever to original position. Counterbalances can be used instead of springs, if smaller mixing cells are necessary.

The approximate cost for one complete injection unit is from $100 to $130, this includes the injector cost of $85 and syringe cost which varies with size. The cost of this system containing five units is approximately $600–700.

BENOIT, D. A., AND F. A. PUGLISI. 1973. A simplified flow-splitting chamber and siphon for proportional diluters. Water Res. 7: 1915–1916.

MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21–29.

APPENDIX C

Water Research Pergamon Press 1973. Vol. 7, pp. 1915–1916. Printed in Great Britain

NOTE

A SIMPLIFIED FLOW-SPLITTING CHAMBER AND SIPHON FOR PROPORTIONAL DILUTERS

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(*Received* 9 *May* 1973)

Abstract—Simplified flow-splitting chambers and siphons were designed and tested for use with proportional diluters in bioassay systems. The apparatus allows each concentration from the diluter to be thoroughly mixed and divided four ways for delivery to duplicate fry and adult exposure tanks. Test water delivered to each exposure tank varied by only 5–10 per cent of the calculated volumes.

THE PROPORTIONAL diluter developed by MOUNT and BRUNGS (1967) provided a dosing apparatus which can maintain a series of constant concentrations of toxicant in flowing water for bioassay systems. A typical bioassay system usually requires that each concentration be divided four ways for delivery to duplicate fry and adult exposure tanks. Flow-splitting chambers and siphons described in this report were designed to be used with a proportional diluter which delivers 2 l. per concentration, but the system can be modified to fit a diluter of any size. The simplified siphon has several advantages over the conventional U-tube siphon: difficult tube bending is eliminated, water volumes siphoned through each flow-splitting tube are easily adjusted, and tubes can be disassembled for cleaning simply by removing each sleeve from the standpipe.

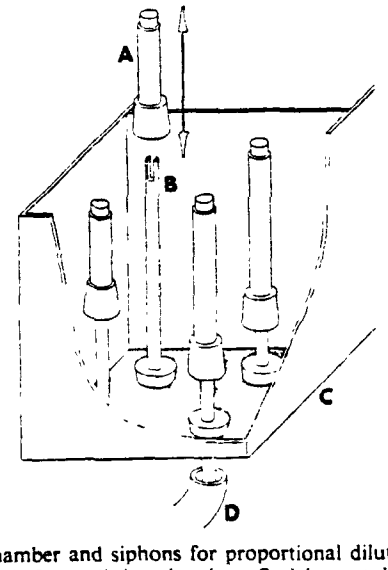

FIG. 1. Flow-splitting chamber and siphons for proportional diluters: *A*, siphon sleeve; *B*, siphon standpipe; *C*, flow-splitting chamber; *D*, delivery tube to exposure tank.

The apparatus consists of six identical chambers with four flow-splitting siphons per chamber (FIG. 1). Glass flow-splitting chambers measured 10 × 15 × 18 cm high. Four 2·5 cm holes were drilled in the bottom of each chamber. Glass-tube standpipes, with two notches cut in one end (0·6 cm wide by 1·6 cm deep) and a neoprene stopper around the other end, were inserted in each of the four holes so the bottom of the notches measured 15 cm above the bottom of the chamber. Glass-tube sleeves, with a neoprene stopper around the outside of one end and the core from the hole bored in the stopper inserted in the other end, were placed over each standpipe. The flow-splitting chambers with siphons were then positioned beneath the diluter so the toxicant-bearing water and dilutant water fell directly into each chamber. This arrangement allows additional mixing before delivery to each exposure tank. As the test water rises slightly above the top of the sleeves in each chamber, water is forced through the notches and down the standpipes. This action creates a siphon which empties the chamber and delivers test water to each exposure tank.

The diameter of each standpipe and sleeve was determined by the flow rate of each concentration delivered from the 2-l. diluter into each chamber. If the diameters of the standpipes are too large, some siphons will not start; and if they are too small, some siphons will start ahead of the others. Siphon tubes for each duplicate fry and adult exposure tank delivered 150 and 850 ml per cycle, respectively, and were calibrated by moving the stoppers, in or out, on either end of the siphon sleeve. Siphon sleeves for the adult tanks must be kept as long as possible so each mixing chamber empties after every cycle. Test water delivered to each exposure tank varied by only 5–10 per cent of the calculated volumes.

Delivery tubes from the flow-splitting chamber to each exposure tank should be large enough to fit loosely over the lower end of each standpipe. The air break between standpipe and delivery tube eliminates back pressure which can cause the siphon to malfunction. If delivery tubes must be attached directly to the standpipes, they must slope downward toward the exposure tanks so each delivery tube empties after every cycle.

REFERENCE

MOUNT D. I. and BRUNGS W. A. (1967) A simplified dosing apparatus for fish toxicology studies. *Water Research* **1**, 21–29.

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16 ABSTRACT

This paper presents a discussion of the testing procedures using proportional diluters. The construction, calibration, and operation of the equipment is explained, and trouble shooting techniques necessary for successful use of such equipment are given.

A bibliography includes many related published materials that are not discussed in the text but which should be useful to the reader. Included are numerous citations on physical toxicity testing methods, but papers on statistics or biological test procedures are not included.

17 KEY WORDS AND DOCUMENT ANALYSIS

a DESCRIPTORS	b IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field Group
Dilution Construction Toxicity	Testing procedures Dilution equipment Diluters proportional Operation calibration	14-B

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