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BIOASSAY DILUTER CONSTRUCTION, TRAINING MANUAL

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ENVIRONMENTAL PROTECTION AGENCY
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June, 1973

AUTHOR'S PREFACE

This manual has been prepared to assist individuals in the construction of constant flow bioassay and biomonitoring equipment. While it is written from the point of view that such persons will come to Cincinnati in order to take advantage of the construction tools available here, and also the personal counseling in regard to needs and design; it can also be a useful supplement to the literature when used alone in one's own home laboratory. Even in Cincinnati, the individual is expected to work largely by himself after the initial interviews and demonstrations are completed, although an instructor is always available for questions and assistance.

It must be recognized that the design and details of equipment in this field are still under development, and that what is "standard" today may readily be supplanted by a new concept or device tomorrow. Consequently if use is made of this manual more than a year or so after its date of issue, the worker should either contact the author, or the National Water Quality Laboratories at 6201 Congdon Boulevard, Duluth, Minnesota 55804 to determine if significant changes should be incorporated.

H. W. Jackson, Ph. D.
Cincinnati, Ohio
May 1973

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The Construction of Proportional Dilutors for Bioassay and Biomonitoring

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PLATES

NOTE: A mixture of metric and English units is employed due to the custom of employing metric units for scientific volumetric expressions, while the equipment is constructed of "double strength window glass" which is 1/8 inch thick. See Table 2.

APPENDICES

THE CONSTRUCTION OF PROPORTIONAL DILUTERS FOR BIOASSAY AND BIOMONITORING

I INTRODUCTION

A The following step-by-step instructions are intended for use in the National Training Center at Cincinnati, Ohio, to supplement official publications. They are designed to be carried out under the general supervision of an instructor. The trainee will be expected to work largely by himself after an introductory discussion with one or more consultants. Occasional personal demonstrations are given by the instructor of "tricks" and procedures difficult to describe succinctly in words.

B The equipment described is based on the following publications which are attached as Appendices 1 to 4.

1 Mount, D. I. and W. A. Brungs. A Simplified Dosing Apparatus for Fish Toxicology Studies. Water Research, 1:21-29, 1967.

2 Brungs, W. A. and D. I. Mount. A Water Delivery System for Small Fish-holding Tanks. Trans. Am. Fish. Soc., 99(4):799-802. October 1970.

3 Jackson, H. W. and W. A. Brungs. Biomonitoring Industrial Effluents. Industrial Water Engr., 14-18. July 1966.

4 Esvelt, Jarry A. and Jerrold D. Connors. Continuous-Flow Fish Bioassay Apparatus for Municipal and Industrial Effluents.

These papers are part of these instructions and should be read before coming to Cincinnati, although complete comprehension of the operating mechanisms will probably not be clear until working models are available for study.

Two additional references are cited.

C The reference to a paper by Mount and Warner cited at the end of Mount and Brungs '67 may be useful for "trouble shooting." It contains the original description of this type of equipment.

D For further information, current refinements, and special methods, contact the author or the Director, National Water Quality Laboratory, 6201 Congdon Boulevard, Duluth, Minnesota 55804.

II PROGRAM IN CINCINNATI

A Discuss needs and objectives with instructor on arrival, including decision as to whether or not to incorporate flow splitter tanks.

B Observe flow-through equipment in operation.

C Review the Mount and Brungs 1967 paper again, hereafter referred to as "M&B '67."

D Study M&B '67, Figure 2 and the section "Principles of Operation" beginning on page 22. Also study Plate 1 of this supplement until the operation of the apparatus is understood.

E Remarks

1 For long term or larger scale operations, it is desirable to scale up equipment to deliver larger working volumes. For example, the working volume can be readily increased from 0.5 to 1 liter by doubling the thickness of the W and C tanks and increasing the capacity of the M-1 tank in proportion. This is the procedure currently in use by the National Water Quality Laboratories in Newtown, Ohio, and Duluth, Minnesota.

2 Plans and procedures which follow are for the original 0.5 liter delivery size as described in M&B '67. Note that total flow per 24 hours can be increased or decreased by appropriate adjustments. (Discuss adjustment of the diameter of the WS-6 siphon in particular [M&B '67, Fig. 2-B and Table 2] with your instructor.)

3 Mixing and Flow-splitting Tanks.

a Experience has shown that an additional set of tanks is advisable between the C tanks and the experimental or test aquaria. Their main function is to completely mix the discharge of each C chamber with that of the corresponding W chamber before passing the entire volume on to the test tanks. They are incorporated in the designs used in Appendix 4. Details of construction are described below.

b In addition to mixing, they can also be used to distribute and adjust the flow between two or more replicate tanks, as shown in Plates 8 and 9.

4 Improvement in statistical validity can be achieved not only by operating two or more replicate sets of test tanks as suggested above, but also by arranging these tanks on the laboratory bench in a random manner, instead of in sequence of concentrations. A short table of random numbers is included as Table 6 for your convenience in this regard. Test tanks shown in Plate 1 are so arranged.

III CONSTRUCTION

It is important to begin the construction of equipment as soon as possible in order to provide time for overnight drying of the cement if testing and/or assembly before departure is anticipated.

A Obtain instruction in the use of the sheet glass cutter and the carborundum wheel from the instructor. (This is important as both equipment and operator may be seriously injured through improper use.) This will immediately be followed by a demonstration of techniques for the assembly of glass cells by edge cementing. Items not described below are covered in M&B '67. Note Section X below for suggestions as to how to proceed when equipment used in this course is not available in the home laboratory.

B Lay out, cut and label major blocks of glass for the W, M, C and flow splitter tanks (see Plate 2).

C Assembling the M-1 Tank

1 Subdivide the M sheet as shown in Plate 3, and bend the support tube for the discharge siphon. Cut a notch in one end plate to receive same. (Sections of glass Xed out are spare, and may be used to replace pieces broken, miscut, etc.)

2 Assemble the pieces on the bench top and determine exactly how they will be put together. Cf: Plate 3. Always check pieces cut for each tank assembly against Table 2 (or 4) to assure a complete set before beginning assembly.

a It may be assumed that edges, which have just been cut, are chemically clean. The glass surfaces to which the edges will be cemented, however, are seldom clean enough to permit perfect adhesion by the cement, and may contaminate the experiment. Clean all surfaces that will be "in" thoroughly with a good laboratory solvent such as acetone.

b Spread a sheet of protective paper (such as newspaper) over the bench top to protect it from the cement.

3 Cement the M-1 Chamber together.

a Fill a disposable plastic syringe with cement, if not already done. Clear "silicone rubber" cement, available in tubes over the counter

at hardware stores has been found best for this work. Disposable plastic syringes in 10 or 20 cc sizes are available from scientific supply houses.

b Consult your plans and be sure that the notch for the siphon tube will be properly placed.

c Apply cement to the bottom edge of the "front" piece and lay it flat on the paper, top edge toward you. Application of cement to the edges of the glass plates is one of the most critical procedures in the entire operation, and should be practiced until it can be executed with speed, precision, and thoroughness. Neither too much nor too little, and no gaps!

Joints should be filled so that internal corners can be completely cleaned (no pockets), but exposure of cement inside chambers should be kept to an absolute minimum.

d Set the bottom piece lightly against the coated edge and prop it roughly in position with some object.

e If siphon support is to be placed in corner as shown on Plate 3 and time is critical, apply a bed of cement and place it in position. Prop upper end 1/4 inch or more away from front piece, and proceed as in f. If time is not critical, wait until cement on rest of M-1 has dried for 1/2 to 1 hour before setting regardless of position.

f Coat the appropriate three edges of the notched end piece (including the inside of the notch) and set it in position on the end of the side piece. Bring the bottom piece up until enough contact is made to hold it in position. Carefully smooth cement around siphon base, using minimum necessary for strength.

g Now coat the edges of the other end piece, and set it in position.

h Set the remaining side piece in position after applying cement to the bottom edge, and press the entire assembly gently but firmly together. Be careful not to slide cemented surfaces. After 5 or 10 minutes, turn the cell upright to rest on its bottom for final alignment. Do not attempt hard pressure to force contact where there is insufficient cement, or an improperly cut piece of glass. If such a problem appears, fill in after partial drying.

NOTE: In fabricating the deeper tanks (see below) the siphon base (or straight drain piece) may be set before or after assembly as seems most expedient. If set before allow 20 to 30 minutes for cement to stiffen before assembling remaining parts.

i After cement has set (20 to 30 minutes) have the instructor check and comment. He will show you how to detect probable pin-hole leaks visually, and how to correct them.

D Slack time can be used to lay out and cut flat pieces for remaining tanks and to prepare siphon pieces, valve bucket, water blocks, valve, etc. The instructor will be available for assistance as needed.

E The Larger Tank Assemblies

1 The W and C chambers of the standard diluter were designed by M&B '67 with sufficient capacity to deliver any of the dilution ratios cited in Table 1 (Cf: M&B '67 Table 3).

The concept of the flow-splitter tanks has developed more recently, but it will be assumed from hereon that they will be incorporated.

2 Unless special needs were determined in the initial conference, cut the pieces of the W, C, and flow-splitter tanks as diagrammed in Plates 4, 5, and 6, and check them against the list (Table 2).

F Assembling the Larger Tanks

1 Assemble the various pieces and determine exactly how they will fit together. Make sure that the notch for the W-6 siphon support or the C-1 drain is properly cut and positioned.

2 Mark the positions of the various divider pieces as shown in Plate 7 on the outside of both sides and the bottom. Lay these pieces out on the bench top so that the markings show through, but will be on the outside in the finished assembly. Place the bottom edge of the front side on the bench, away from you.

3 Apply cement to proper edges as before (III-C-3-h above). Apply cement to all appropriate edges of a given piece at one time. Cement dries relatively slowly, so that a few minutes delay before laying on matching piece can be tolerated. Extreme delay (10-15 minutes) will, however, result in poor adhesion.

a Install in the following sequence: bottom against "front" piece, dividers over marks, "back" side "on top" and against bottom, ends on. Note that cement must be applied to the ends of front, back and bottom pieces (in contrast to sequence in M-1 cell) in order for the end piece to be properly sealed.

b The W and C tanks should be permitted to set up for at least an hour if possible before cementing the siphon tupes (WS-1 to 5 and CS-2 to 5, M&B '67) in place. Tops of all bends should be equidistant above edge of tank. The completed units must be permitted to dry overnight before further assembly or testing. As noted in the M-1 section (III-C-3-e), siphon bases or drain tubes in W-6 or C-1 may be installed in advance, or later. Not at this time. You might prefer to mount siphons as described in Appendix 4.

c After overnight curing smooth all exposed sharp edges with a carborundum stone or flat file. Provisional internal extensions of siphons, and also the W-6 overflow siphons may now be installed, using a thin film of stopcock grease to prevent permanent "locking" of plastic tubing sleeves to glass.

1) Preliminary determination of the depth of internal siphon extensions may be made at this time (or if preferred before siphon tubes are cemented in place). Refer to Table 1 (or your own special design figures) and note the working volume to be delivered by each cell. Prop the end of each tank in turn up approximately one inch on the lab bench (W-1 or C-2), so that the tank slopes down to the discharge end as in Plate 13. Seal the discharge tubes with rubber tubing and a pinch clamp.

2) Fill the tanks with a rubber tube led into the first chamber, allowing the water to overflow naturally into succeeding chambers.

3) Siphon off the desired working volume from each chamber into a graduated cylinder.

4) Mark the water level remaining in the chamber on the outside of the glass.

5) Extend the internal ends of the siphons down to this mark (or to the surface of the water) as noted above.

6) In W-6 or any terminal chamber to be emptied by an overflow siphon draw the water level down 1/2 to 1 inch below the lip of the last divider and mark this level. Now draw off the working volume, and again make a mark. The top of the overflow siphon should be placed at the

upper mark, the intake at the lower mark.

7) All siphon extensions will empty more cleanly if flared as described in Appendix 4. Ask your instructor for a demonstration, or consult Reference 1.

d For final "fine tuning" calibration, see M&B '67.

G Toxicant Delivery Systems

1 If a dipping bird mechanism is desired for chemical metering, suggested plans for a cup and associated devices are presented in Plates 10, 11, and 12. These differ slightly from those illustrated in M&B '67, but serve the same purpose. Note: a safety factor is introduced if the glass (back) mounting piece is cemented to a piece of wood or metal, or even wire loops, for attachment to the diluter panel (Plate 13).

2 The dipper itself can easily be fabricated from a discarded volumetric pipette as will be demonstrated by the instructor (see also Plate 12, A).

3 Injector mechanisms for micro quantities of toxicants are introduced at the end of M&B '67. Further developments are noted in Esvelt et al, 1971 (Appendix 4).

4 A simple toxicant metering device which has no moving parts has recently been developed at the Fish-Pesticide Laboratory at Columbia, Missouri (see reference: McAllister et al, 1972). Structural features are shown in Plate 18. For further information contact the authors at the above address, or consult the publication cited.

H Electrical Control Systems

1 Solenoid valves activated by micro switches and floats are more flexible and efficient for larger systems, and can be readily wired to ensure "fail-safe" operation.

2 An excellent description of one such plan is given following page 163 of SERL Report No. 71-7. See Appendix 4.

I Mixing and Flow-splitting Devices

1 The general principle of flow-splitter tanks is shown in Plate 8A. The plan view (center of plate) illustrates various possible arrangements of nozzles or siphons.

Since many holes are required for this plan, and since the test solutions only dwell in these tanks for a relatively few seconds per cycle, some laboratories resort to plexiglass for their construction. Boring holes in glass (especially large ones) is not as difficult as it might seem, however, (see below) and glass is still the recommended material. Use smallest possible holes.

2 If distribution only is desired (and mixing is no problem), simple small bore glass nozzles may be employed as shown in Plate 8B. Care must be exercised, however, that the supply tube from the C and W tanks does not direct more liquid into one tube than the other. This should be checked by catching and measuring the discharge per cycle from each nozzle separately.

3 If complete mixing prior to splitting is important (as is usually the case), some device such as the Duluth Flow Distributor (see Plate 9) is recommended. This retains the discharge from the C and W tanks until they are thoroughly mixed. As the cycle is nearly finished, both (or all) distributor siphon tubes tip over simultaneously, and the completely mixed liquid is distributed to the various tanks.

4 Since the lengths of the inner siphon tubes are identical, the rates of discharge will likewise (supposedly)

be identical. However, if, on catching and measuring the discharge from each siphon tube as described above (Paragraph 2), it is found that one tube is delivering more than another, the offending collar tube can be slipped upward a few millimeters on the stopper seal. This will break the siphon sooner and thus the flow to that particular tank, and leave more for the other(s). The principle is clearly illustrated in Plate 9.

5 If glass-blowing equipment is available, a much less fragile construction for the Duluth Flow Distributor is as follows:

a Close the top of the central siphon tube, leaving the tip with a short spike (approximately 1/8 to 1/4 inch long).

b Blow a hole (or two) in the side of the siphon tube, just below the tip, the total cross sectional area of which is approximately equal to the cross sectional area of the tube itself. (If two holes are blown, they should be at exactly the same level.)

c Fashion a central cavity in the underside of the sliding rubber seal of the outer collar tube to receive and "center" the spike of the central siphon tube.

d Install and adjust the central siphon tube to the desired height in the mixing chamber.

e Adjust the position of the sliding rubber seal in the outer collar tube so that the bottom of the tube is at the desired height when the outer tube is set over the inner one. It is not necessary that a rigid connection be made.

The above system greatly simplifies the adjustment of relative volumes dispensed to the various replicate tanks.

J Siphon Breakers

1 The distributor devices for the flow splitter tanks above all depend in one way or another on an equal (or proportional) delivery rate from each. In order to accomplish this, there must be no additional suction from the supply lines which catch the various discharges and deliver them to the proper test chambers. This is easily prevented by the use of suction or "siphon breakers," which are simply devices for freely admitting air into the delivery lines immediately below the flow splitter. Probably the simplest system is that illustrated in Plate 8, B2. The plan suggested in Plate 8, B1 permits the use of a smaller size of tubing for distributing the discharge to the test tanks.

IV TESTING AND MOUNTING

A Before assembling equipment on the 2-1/2' x 4' panel, each chamber should be tested for leaks.

1 Place the tank on sheets of brown paper toweling on the laboratory bench. Close the outlets of W-6 or C-1 with short pieces of rubber tubing and pinch clamps.

2 Lead water from tap into first chamber and fill within half an inch of the divider top. Stop and look for leaks. If any are noted, mark the point of their first appearance.

3 Now overflow the first compartment and allow the next one to nearly fill. Mark leaks as before. Continue until all chambers are tested.

B Stopping Leaks, Big and Little

1 "Pinhole" leaks between chambers inside may be ignored. The chambers empty so quickly when the siphons start that these tiny leaks will be inconsequential and will eventually seal themselves with detritus. Large leaks should be stopped.

2 External leaks of any size must be plugged.

a Dump water out and wipe exterior dry.

b Insert a 6-inch piece of glass tubing in the end of a long piece of rubber tubing, and attach to the compressed air cock. Turn air on hard, and blow water out of the chambers at the points of serious leakage.

c Force cement into crack from outside with finger or disposable syringe. Avoid adding to area of exposed cement on inside of tanks, if possible.

C Glass tubing should be well covered with stopcock grease before slipping on plastic tubing. This will prevent it from "freezing" and will greatly facilitate testing and adjustment.

D Plate 13 suggests a basic layout for a 2-1/21 x 4' panel. One half inch exterior (or marine) plywood, painted on both sides, is good for a permanent assembly. If the equipment is to be disassembled for shipment, 2 x 4 ft. pegboard panels are provided for test assemblies in the laboratory.

V BIOMONITORING

A If the need developed in the preliminary conference is simply for surveillance or monitoring to detect change (usually deleterious) of an effluent, rather than to assay or measure an exacting parameter, the physical equipment may be somewhat simplified. This is particularly true of the toxicant administration, as the degree of toxicity involved is usually much, much less. Note paper by Jackson and Brungs '66 (Reference 3).

B Before constructing such a system, however, consideration should be given to using the M&B '67 plan appropriately modified. For example, waste could be delivered directly to the C tanks, by-passing M-1 and eliminating the W-1 siphon (simply clamp off the vacuum line to W-1). A separate (probably solenoid) valve, would need to be installed to cut off the waste flow during cycling. Proportional dilutions could then be maintained, including full strength waste (from C-1 chamber) and pure dilution water (stream or lake) from W-6, as described in Jackson and Brungs '66.

C A simplified version of this equipment which could meet biomonitoring requirements is shown in Plates 14 and 15.

1 Although a full range of dilutions from full strength waste to pure dilution water is shown here, special circumstances might indicate that pure dilution water, pure waste water, and perhaps one dilution only would meet the needs of the moment. In this case, the remaining chambers could either be deleted from the original structure, or not used.

2 A suggestion is offered in Plate 15 for a system to permit continuous flow of both effluent and dilution water. This can be controlled "hydraulically" as shown, or electrically. Such a flow-shifting device is particularly helpful where suspended solids are involved.

3 Plate 14 shows an extra overflow or "waste" chamber on the C tank in order to permit exact metering of the 100% waste. If this is not important, the C tank structure may be shortened to nine inches or less (7 inches, for example), and an ample supply of pure waste run through the C-1 chambers on a simple excess flow-through basis. This eliminates the necessity of a water block and siphon setup for the C-1 chamber. One should realize, however, in exercising this option, that should C-1 metering ever become desirable, a new tank would be needed.

4 The water block for C-1 must, of course, be below the level of the C tank, as the others are below the level of the W tank. As a matter of fact, the system generally works

best if the W-2 water block is also placed low, just above the entrance of the C-2 siphon.

5 This system will work faster if 10 and 12 mm tubing is used for the W-3 and W-4 siphons, and 14 mm tubing for W-2 and C-2.

VI SPECIAL DESIGN PROBLEMS

A In case special needs arise which cannot be achieved by the standard (0.5 liter) diluter, note the design factors in Table 3. Factors for 1 liter diluters are also cited. Proportional diluters up to four liters per concentration per cycle are now in use in some laboratories.

B Each flow-splitter chamber must hold the combined flow of a W cell and the corresponding C cell, leaving one or more inches of freeboard for safety.

C The discharge from the W-6 and C-1 cells may be used as "volumetric cushions," since solutions passing through them are not necessarily proportioned (mixed with) to any other flow. However, the intent of the original M&B '67 design was that the siphon depth in W-6 and the working volume discharged from W-1 would be so adjusted that the W-6 and C-1 working volumes (discharges) would each be 0.5 liters, and thus equal to the other flows.

D If additional dilutions are desired or if it is desired to meter pure control and pure M-1 water from special chambers, it is a relatively simple matter to add on one or more chambers to both tank series. For example, W-6 and C-1 could be fitted with siphons and vacuum lines, and calibrated for desired working discharge volumes. Both then might discharge directly to test tanks (via flow-splitters if desired), without being mixed with any other solution. Overflow from W-6 could operate valve bucket through a "W-7" chamber, and overflow from C-1 could be wasted. Variations are infinite.

VII EXPERIMENTAL TANKS

A Tanks or aquaria as such are not strictly speaking a part of the "proportional diluter." However, they are so fundamental to its proper use, that a brief description is offered here of a type of in-house construction found to be cheap and effective. Construction details are shown in Plate 16.

B Nearly any size may be employed, but the "workhorse" of the National Water Quality Laboratories for fish bioassays has been the two cubic foot size illustrated.

C The exact locations of overflows, screens, use or nonuse of dividers, etc. are all optional. Glass is ordered in bulk in a ratio of three pieces of 12" x 24" to two of 12" x 12". Half of the 12" x 12" pieces have holes drilled as shown. All water is delivered via glass tubing over the top.

VIII WATER SUPPLY

A A simple "head box" is not hard to devise, but if a float chamber and valve is involved, examine the fittings to assure that no metal other than some acceptable form of stainless steel comes in contact with the water. A constant overflow system may be safer.

B This is fine if an acceptable water supply is available which requires no treatment before use. However, some individuals are faced with the alternatives of either treating tap water, or not running experiments. There is also the situation where the temperature of the water supply must be controlled.

C In either case, it is desirable to maintain a constant head without losing any of the treated water (whether chemical or thermal treatment). One approach is to use a realtively small "box" composed of two chambers, connected by a small but adequate aperture or tube. The raw water supply (valve or overflow) operates in and out of one chamber, while the experimental water supply is taken from the other. No treated water is thus wasted.

D Plate 17 illustrates an arrangement whereby not only the above results are achieved, but the water is also mixed, aged, and aerated (this would probably not be desirable for thermal control). The chemical treatment indicated might be, for example, thiosulfate, to counteract chlorine or an activated charcoal filter might be inserted. In any event, a chemist should calculate the amount and type of treatment required in order to obtain the desired quality.

E For information on acceptable water quality for experimental use as such, consult "Standard Methods" or other references.

IX CONSTRUCTION HINTS

A If a sheet glass cutter is not available in the laboratory, order pre-cut sizes of glass sheets (from Table 2) from local hardware store. Specify approximately 1/32nd inch tolerances.

B Sheet glass may be cut by hand (especially smaller pieces) using a hand glass cutter and a meter stick, and extra thick yard-stick, or other straight-edge. Be sure edge is truly straight. Clamp, or have another person hold straight edge in position. Practice first on scrap pieces.

C Wire hacksaw baldes with silicon-carbide grit advertised in hobby shops for cutting glass bottles etc. are excellent for cutting notches if a carborundum cutting wheel is not available. Use long, slow strokes. WEAR FULLY ENCLOSED SAFETY GLASSES.

D Butane gas torches with wide wing tips are excellent for bending glass tubing.

E Boring Holes

1 Best: Order holes to be bored as specified, by glass supplier.

2 If you wish to bore your own, procure a six-inch length of brass tubing slightly smaller in diameter than you want the hole to be. Cut the end of the tubing off square, and file several notches around the cutting edge. Chuck the tubing in a drill press. If drill chuck will not accept large enough tubing, fit a short squat section of the tubing desired with a one-hole rubber stopper. Cut the head from a 1/4" or 5/16" bolt, run a nut up the thread end not farther than the length of the rubber stopper, and slip on a washer as large or larger than the diameter of the cutting tube to be used. Force the thread end of this assembly into the stopper hole, and this in turn into the cutter tube. Now chuck the bolt shaft in the drill press as above. This will not be as easy to line up and get started as a straight piece of tubing but once seated, grinding can proceed as described below.

In order to reduce chipping when the drill breaks through, support the glass to be cut on a flat board (a scrap of "Formica" shelf topping is excellent). Even better: put a layer of plaster of paris between the glass and the board and let it set, or apply a small patch of masking tape to the underside of the glass.

Build a little coffer-dam around the area where the hole is to be cut, using putty. Pour in about one fourth of a teaspoonful of No. 220 silicon carbide grit or equivalent, and add a few drops of water to make a thin slurry. If cutting action slows, add more grit and water. Never let center get stiff. With the drill press running about 300 revolutions per minute (faster for small holes, slower for large holes), lower the end of the tubing gently into contact with the glass. You will hear a grinding sound as the action starts. Raise and lower the tubing about once every five seconds. You should be through a piece of 1/8-ince glass in a few minutes.

Take it easy as the drill comes through the glass--to avoid splintering chips off the edges of the hole on the bottom as noted above. Drilling through rounded objects is more difficult, but can be accomplished with proper care.

3 Special steel drill bits for boring holes in glass may be obtained from professional glass working equipment supply houses. (Reference 1, page 189)

F Repairing Breaks

1 Glass cracked previous to assembly (such as a "bottom" in which several holes have already been notched or drilled) may be "assembled" as any other two pieces: apply cement to broken edges and press them into position (do not attempt if fragmented into several pieces).

2 Glass cracked after assembly may be waterproofed by a narrow bead of cement applied to both sides. Be sure preliminary surface cleaning is thorough.

ACKNOWLEDGMENT

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This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, Direct Technical Training Branch, Manpower Development Staff, WPO, Environmental Protection Agency, Cincinnati, OH 45268.

Table 1

WORKING VOLUMES IN STANDARD 0.5 LITER DILUTER

Cell No.	0.5 Factor (1)	Log Series (2)	.25 Factor (3)	1:2:1 (4)	Vol. Std. (5) Diluter Chambers
W1	968	1080	1525	1250	1580
W2	250	220	125	125	375
W3	375	340	219	250	470
W4	438	410	289	375	750
W5	469	450	342	×	750
W6	500	500	500	500	930
M1	968	1080	1525	1250	1650
C2	250	280	375	375	460
C3	125	160	250	160	350
C4	62	90	211	125	280
C5	31	50	158	×	200
C1	500	500	500	500	230 (6)

(1) Each successive dilution is half as strong as the one before.

(2) The ratio of the strength of each successive dilution to the one before it is approximately 1:1.8. This is the logarithmic series recommended in "Standard Methods" for static bioassays. Starting with C-1 as = 100%, the strength of C-2 + W-2 would thus be 56%, C-3 + W-3 = 32%, C-4 + W-4 = 18%, and C-5 + W-5 = 10%.

(3) Each successive dilution is 3/4 as strong as the preceding one.

(4) Starting with 100% pure test solution from C-1, C-2 + W-2 = 75%, C-3 + W-3 = 50%, and C-4 + W-4 = 25%. C-5 + W-5 would not be used, and W-6 would deliver pure dilution water.

(5) It is evident by inspection that the Standard Diluter is so designed that any of the ratios cited may be obtained without structure change.

Table 2

PIECES REQUIRED FOR DILUTER TANKS (*)

Standard 0.5 Liter Diluter

Tank	No. of pcs.	Part Name	Dimensions in inches	Notes
M	2	sides	4 × 7	
	2	ends	3-3/4 × 4	one with notch
	1	bottom	4 × 7	
W	2	sides	6 × 24	
	2	ends	2-1/2 × 6-1/8	one with notch
	5	dividers	2-1/4 × approx. 5	
	1	bottom	2-1/2 × 24	
C	2	sides	6 × 15	
	2	ends	1-1/2 × 6-1/8	one with notch
	4	dividers	1-1/4 × 5	

*Vacuum and siphon systems for 0.5 liter diluter are well described in Table 2, page 24, M&B '67 and are not included here.

One Liter Diluter (selected items)

Tank	No. of pcs.	Part Name	Dimensions in inches	Notes
M	2	sides	6 × 7	
	2	ends	6 × 5-3/4	one with notch
	1	bottom	6 × 7	
W	2	sides	6 × 24	
	2	ends	4-3/4 × 6-1/8	one with notch
	5	dividers	4-1/2 × 5	
	1	bottom	4-3/4 × 24	
C	2	sides	6 × 15	
	2	ends	2-3/4 × 6-1/8	one with notch
	4	dividers	2-1/2 × 5	
	1	bottom	2-3/4 × 15	

Siphon Systems			Dimensions o. d. in mm
W	4	WS-2 to 5	10
	1	WS-1	16
C	4	CS-2 to 5	12
	1	S-7	12
	5	T-1 to 5	12
All U's			12 (or 1/2")

Table 3

DESIGN FACTORS: VOLUMETRIC EQUIVALENTS AND CALCULATIONS

1 inch = 25.4 mm = 2.5 cm

1/4 inch = 6.35 mm

1 square inch = 642 mm^2 = 6.5 cm^2

1 cubic inch = 16.4 cm^3 (ml)

1 liter contains 61 $in.^3$

Factors for 0.5 Liter Diluter

For partitions 1-1/4 inches wide, count 8 cm^2 per inch of height, or 40.3 cm^2 for 5 inches.

cc per running inch of C tank: 102.5

For partitions 2-1/4 inches wide, count 14.5 cm^2 per inch of height, or 72.6 cm^2 for 5 inches.

cc per running inch of W tank: 186

Ends of M chamber (per Table 2) contain 94 cm^2

cc per running inch of M tank: 235

Table 4

PIECES REQUIRED FOR BIOMONITOR SETUP (cf: Plates 14, 15)

	No. Pcs.	Part Name	Dimensions (in.)	Notes
W	2	sides	6 × 9	
	2	ends	2-1/2 × 6-1/8	one with notch
	3	dividers	2-1/4 × 5	
	1	bottom	2-1/2 × 9	
C	2	sides	6 × 10-1/2	
	2	ends	2-1/2 × 6-1/8	one with notch
	4	dividers	2-1/4 × 5	
	1	bottom	2-1/2 × 10-1/2	
Control Box	1	mounting plate (back)	2-3/8 × 7	
	1	front	2-3/8 × 5-5/8	
	1	bottom	1-5/16 × 5-5/8	
	3	ends and tall partitions	1-1/16 × 2	
	2	short partitions	1-1/16 × 1-1/2	
	1	slide plate*	1 × 8	2 notches or holes to receive tubes
	2	slide caps	1/2 × 1-5/16	
	1	bottom	1-5/16 × 5-5/8	
	1(or 2)	wire U (or 1/8" hole)		To receive control rod(s)
Miscellaneous	1	float chamber and float		See Plate 14
	1	quadrant wheel and bearings		See Plate 14
	2	control rods		See Plate 14

*Could be of stainless steel.

Table 5

MATERIALS FOR ONE 0.5 LITER STANDARD DILUTER

1 Sheet double strength window glass, 36 × 42

Glass Tubing (not less than:)

OD	4 ft. lengths
8 mm	3
10 mm	3
14 mm	1/2
Capillary tubing or solid rod (approx. 8 mm OD)	1/2
	"Us"
1/4" (8 mm)	4
3/8" (10 mm)	4
	"Ts"
3/16" OD, glass	4
1/4" (8 mm) OD, glass	1
3/8" ID, PVC or glass*	1
3/4" (or 1") ID, PVC	1

Plastic Tubing

6' - 1/8"	aquarium air tubing, thick walled
	assorted short lengths of 1/4, 3/8 and 1/2 inch Tygon tubing (or equivalent) for connections
5	30 ml polyethylene bottles, vials, or equivalent (for water blocks)
3	assorted larger polyethylene bottles
1	or more volumetric pipettes of selected sizes e.g., 5 ml (for dipping bird)
1	Outdoor, or marine, plywood pc. 1/2" × 30" × 48" (optional at Cincinnati)
	assorted rubber stoppers

* "1/2 inch" PVC "street L" and "T" may be substituted as demonstrated.

Table 6

SHORT TABLE OF RANDOM NUMBERS

46	96	85	77	27	92	86	26	45	21	89	91	71	42	64	64	58	22	75	81	74	91	48	46	18
44	19	15	32	63	55	87	77	33	29	45	00	31	34	84	05	72	90	44	27	78	22	07	62	17
34	39	80	62	24	33	81	67	28	11	34	79	26	35	34	23	09	94	00	80	55	31	63	27	91
74	97	80	30	65	07	71	30	01	84	47	45	89	70	74	13	04	90	51	27	61	34	63	87	44
22	14	61	60	86	38	33	71	13	33	72	08	16	13	50	56	48	51	29	48	30	93	45	66	29
40	03	96	40	03	47	24	60	09	21	21	18	00	05	86	52	85	40	73	73	57	68	36	33	91
52	33	76	44	56	15	47	75	78	73	78	19	87	06	98	47	48	02	62	03	42	05	32	55	02
37	59	20	40	93	17	82	24	19	90	80	87	32	74	59	84	24	49	70	17	23	75	83	42	00
11	02	55	57	48	84	74	36	22	67	19	20	15	92	53	37	13	75	54	89	56	73	23	39	07
10	33	79	26	34	54	71	33	89	74	68	48	23	17	49	18	81	05	52	85	70	05	73	11	17
67	59	28	25	47	89	11	65	65	20	42	23	96	41	64	20	30	89	87	64	37	93	36	96	35
93	50	75	20	09	18	54	34	68	02	54	87	23	05	43	36	98	29	97	93	87	08	30	92	98
24	43	23	72	80	64	34	27	23	46	15	36	10	63	21	59	69	76	02	62	31	62	47	60	34
39	91	63	18	38	27	10	78	88	84	42	32	00	97	92	00	04	94	50	05	75	82	70	80	35
74	62	19	67	54	18	28	92	33	69	98	96	74	35	72	11	68	25	08	95	31	79	11	79	54
91	03	35	60	81	16	61	97	25	14	78	21	22	05	25	47	26	37	80	39	19	06	41	02	00
42	57	66	76	72	91	03	63	48	46	44	01	33	53	62	28	80	59	55	05	02	16	13	17	54
06	36	63	06	15	03	72	38	01	58	25	37	66	48	56	19	56	41	29	28	76	49	74	39	50
92	70	96	70	89	80	87	14	25	49	25	94	62	78	26	15	41	39	48	75	64	69	61	06	38
91	08	88	53	52	13	04	82	23	00	26	36	47	44	04	08	84	80	07	44	76	51	52	41	59
68	85	97	74	47	53	90	05	90	84	87	48	25	01	11	05	45	11	43	15	60	40	31	84	59
59	54	13	09	13	80	42	29	63	03	24	64	12	43	28	10	01	65	62	07	79	83	05	59	61
39	18	32	69	33	46	58	19	34	03	59	28	97	31	02	65	47	47	70	39	74	17	30	22	65
67	43	31	09	12	60	19	57	63	78	11	80	10	97	15	70	04	89	81	78	54	84	87	83	42
61	75	37	19	56	90	75	39	03	56	49	92	72	95	27	52	87	47	12	52	54	62	43	23	13
78	10	91	11	00	63	19	63	74	58	69	03	51	38	60	36	53	56	77	06	69	03	89	91	24
93	23	71	58	09	78	08	03	07	71	79	32	25	19	61	04	40	33	12	06	78	91	97	88	95
37	55	48	82	63	89	92	59	14	72	19	17	22	51	90	20	03	64	96	60	48	01	95	44	84
62	13	11	71	17	23	29	25	13	85	33	35	07	69	25	68	57	92	57	11	84	44	01	33	66
29	89	97	47	03	13	20	86	22	45	59	98	64	53	89	64	94	81	55	87	73	81	58	46	42
16	94	85	82	89	07	17	30	29	89	89	80	98	36	25	36	53	02	49	14	34	03	52	09	20
04	93	10	59	75	12	98	84	60	93	68	16	87	60	11	50	46	56	58	45	88	72	50	46	11
95	71	43	68	97	18	85	17	13	08	00	50	77	50	46	92	45	26	97	21	48	22	23	08	32
86	05	39	14	35	48	68	18	36	57	09	62	40	28	87	08	74	79	91	08	27	12	43	32	03
59	30	60	10	41	31	00	69	63	77	01	89	94	60	19	02	70	88	72	33	38	88	20	60	86
05	45	35	40	54	03	98	96	76	27	77	84	80	08	64	60	44	34	54	24	85	20	85	77	32
71	85	17	74	66	27	85	19	55	56	51	36	48	92	32	44	40	47	10	38	22	52	42	29	96
80	20	32	80	98	00	40	92	57	51	52	83	14	55	31	99	73	23	40	07	64	54	44	99	21
13	50	78	02	73	39	66	82	01	28	67	51	75	66	33	97	47	58	42	44	88	09	28	58	06
67	92	65	41	45	36	77	96	46	21	14	39	56	36	70	15	74	43	62	69	82	30	77	28	77
72	56	73	44	26	04	62	81	15	35	79	26	99	57	28	22	25	94	80	62	95	48	98	23	86
28	86	85	64	94	11	58	78	45	36	34	45	91	38	51	10	68	36	87	81	16	77	30	19	36
69	57	40	80	44	94	60	82	94	93	98	01	48	50	57	69	60	77	69	60	74	22	05	77	17
71	20	03	30	79	25	74	17	78	34	54	45	04	77	42	59	75	78	64	99	37	03	18	03	36
89	98	55	98	22	45	12	49	82	71	57	33	28	69	50	59	15	09	25	79	39	42	84	18	70
58	74	82	81	14	02	01	05	77	94	65	57	70	39	42	48	56	84	31	59	18	70	41	74	60
50	54	73	81	91	07	81	26	25	45	49	61	22	88	41	20	00	15	59	93	51	60	65	65	63
49	33	72	90	10	20	65	28	44	63	95	86	75	78	69	24	41	65	86	10	34	10	32	00	93
11	85	01	43	65	02	85	69	56	88	34	29	64	35	48	15	70	11	77	83	01	34	82	91	04
34	22	46	41	84	74	27	02	57	77	47	93	72	02	95	63	75	74	69	69	61	34	31	92	13

Table 7

POTENTIAL RATE OF PROGRESS FOR A SKILLED LABORATORY TECHNICIAN

At end of:	Could expect to have:
Day 1	Discussed objectives. Visited working bioassay laboratory and observed flow through equipment in action.
	Studied working model in laboratory and learned its operation.
	Received instruction in the various laboratory procedures.
	Cut out and assembled an M-1 tank.
Day 2	Cut out and assemble W and C tanks and installed siphon bases.
Day 3	Test tanks for water tightness and repair flaws.
	Cut and assemble dipping bird and tank.
	Cut and assemble assorted tubing and small fittings.
	Mount equipment on temporary pegboard (or pack for shipment). Or:
Day 4	Test and calibrate setup in A. M.
	Disassemble and pack for shipment in P. M.

NOTE: Individuals not routinely engaged in constructing and operating laboratory equipment of this type should plan on five days to accomplish the above objectives. Flow-splitter tanks might not be completed in the above timetable.

PLATES

PLATE 1. FLOW PLAN FOR STANDARD DILUTER

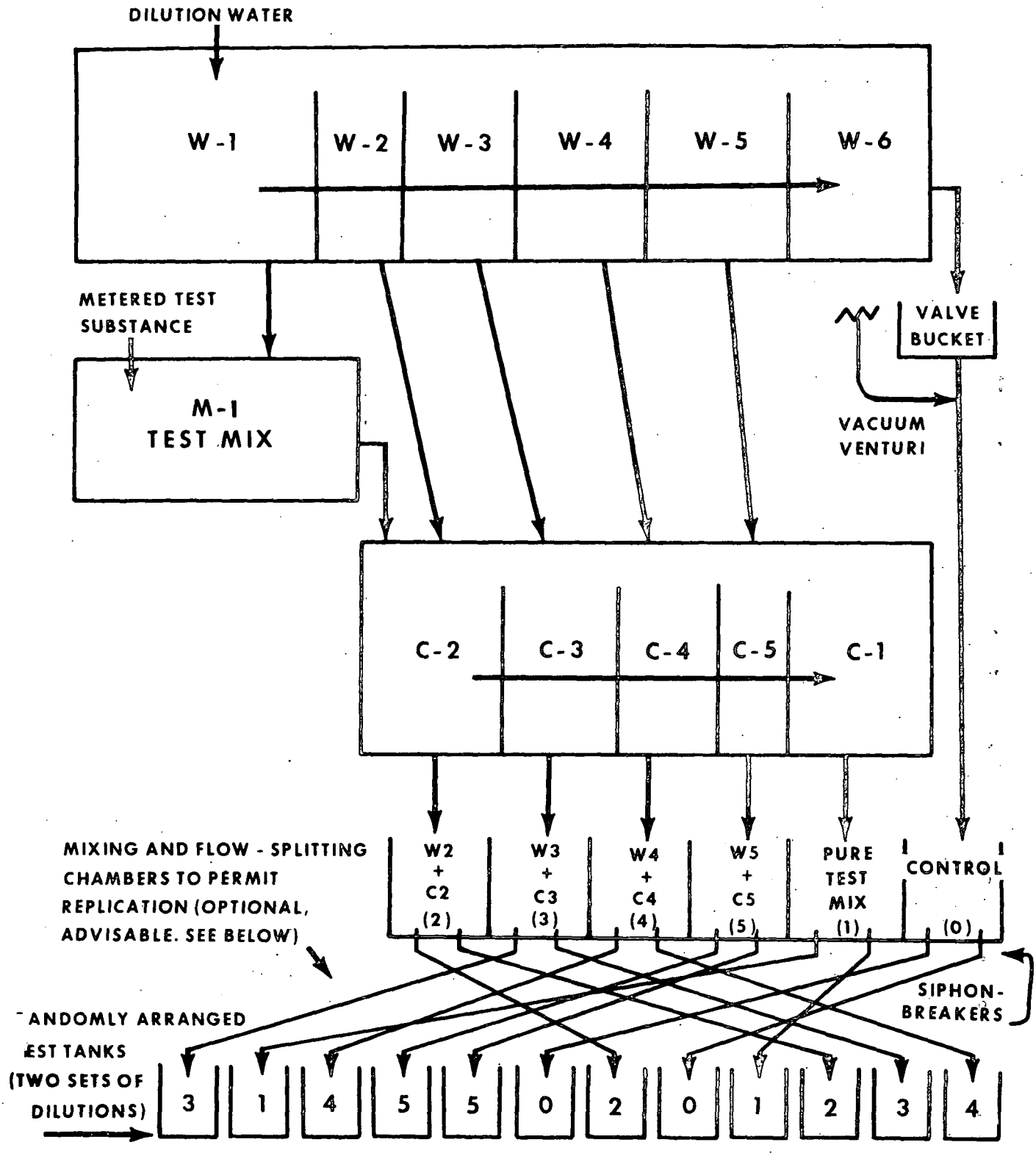
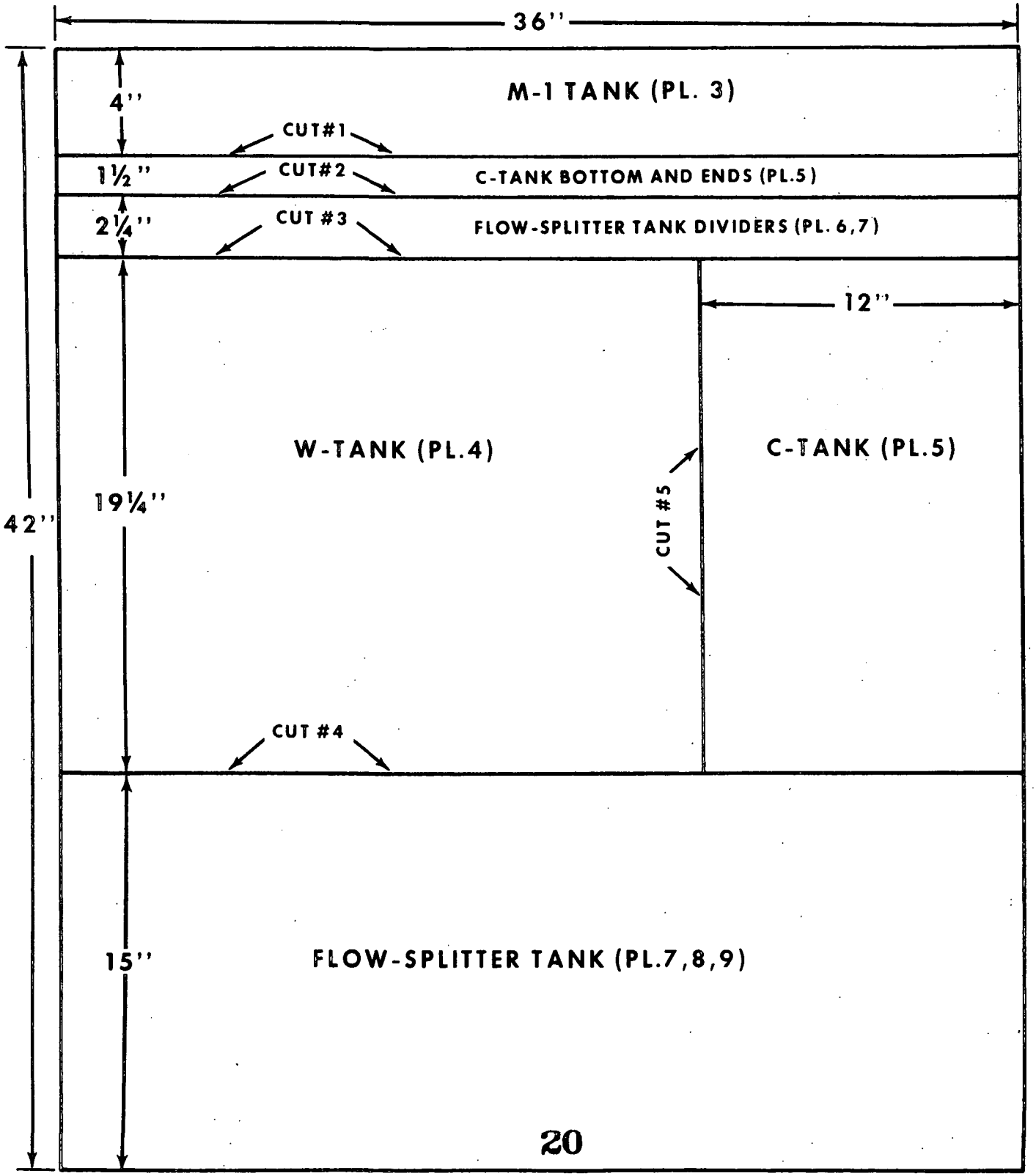


PLATE 2. STOCK SHEETS

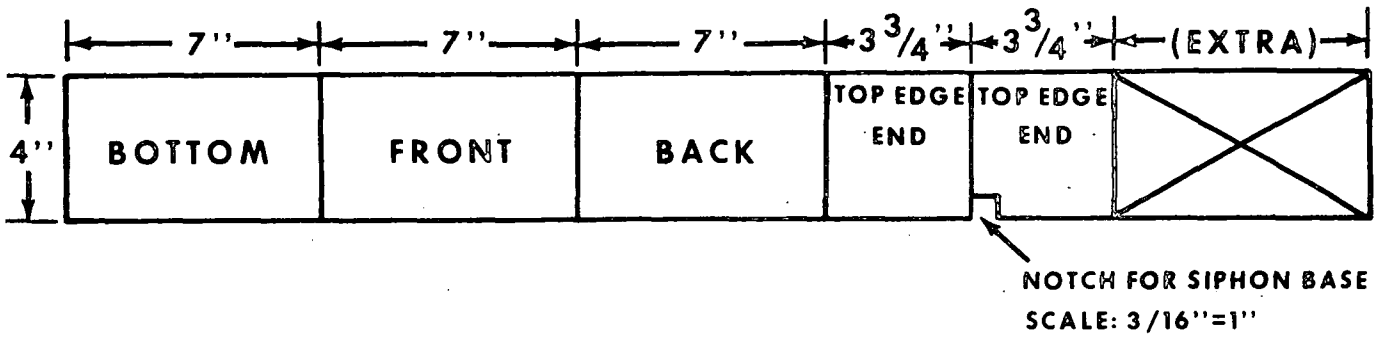
(BASED ON "DOUBLE STRENGTH " WINDOW GLASS, 1/8"×36"×42")

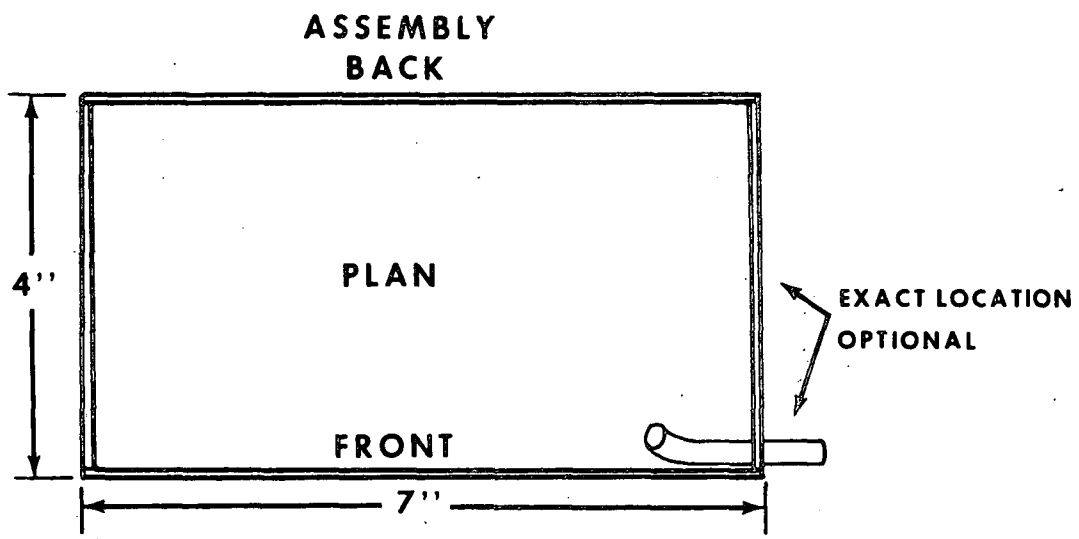
NOTE SEQUENCE OF CUTS. LABEL EACH PIECE WHEN CUT.



SCALE: 3/16"=1"

PLATE 3. CUTTING AND ASSEMBLY: M-1





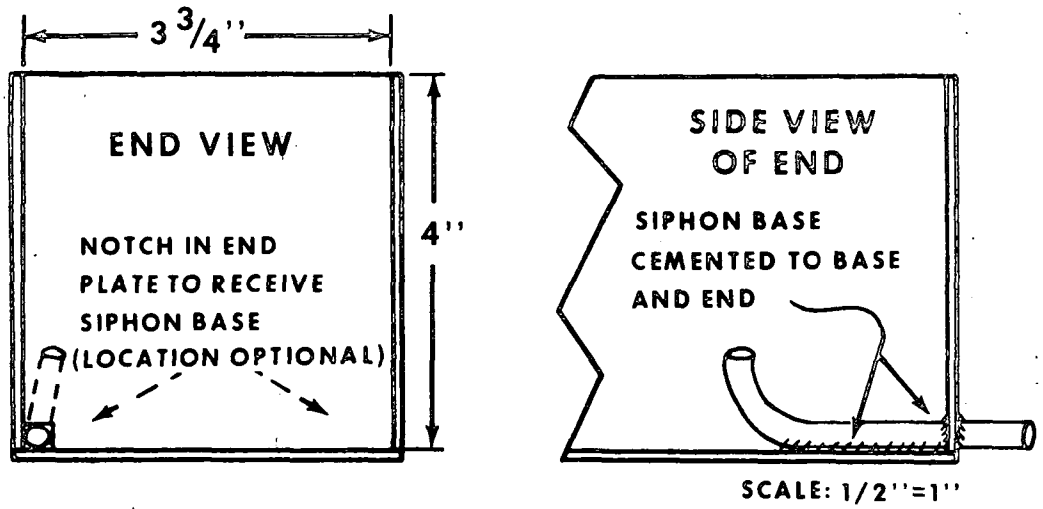
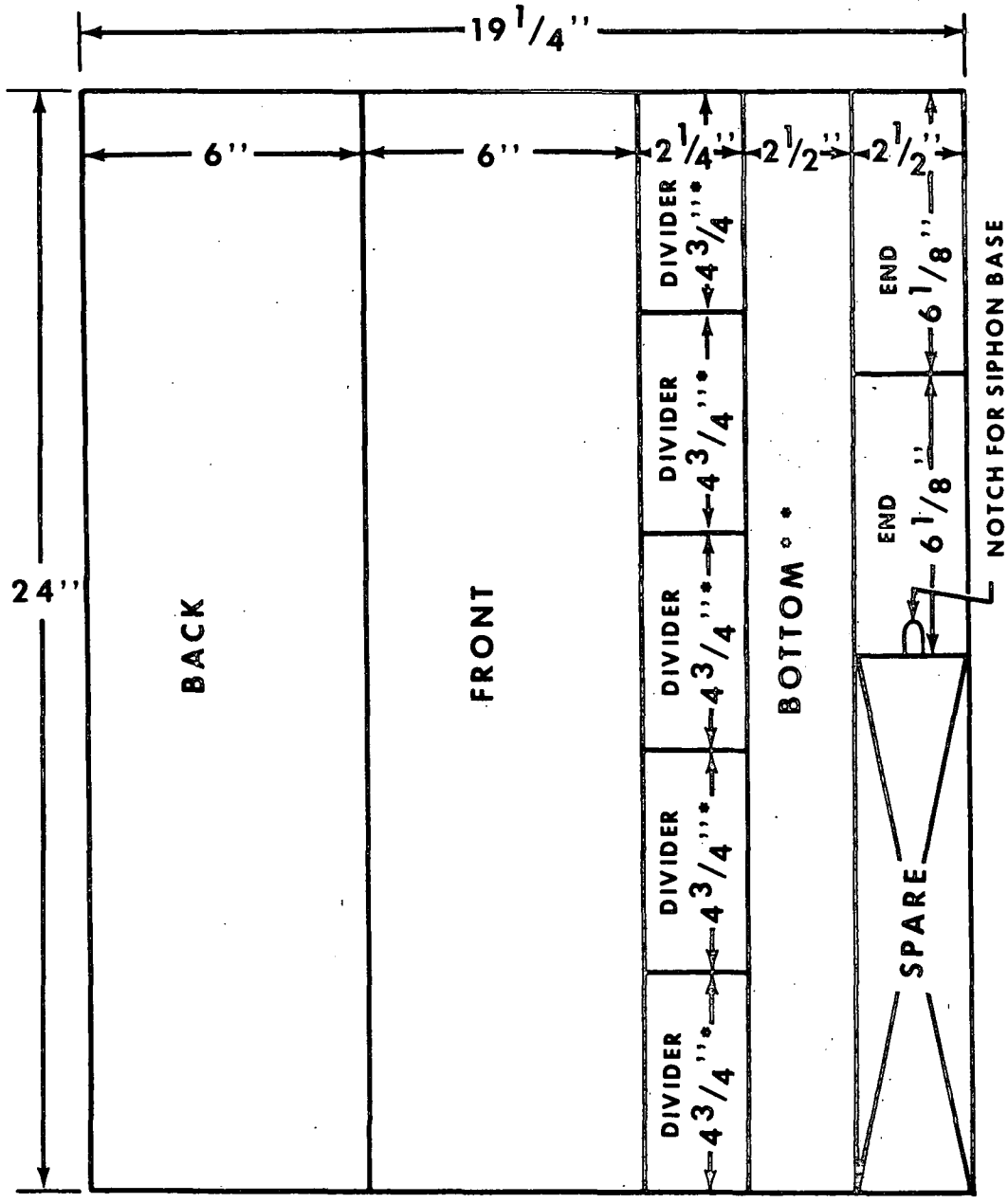


PLATE 4. CUTTING PLAN FOR W-TANK

(Cf: TABLE 3)

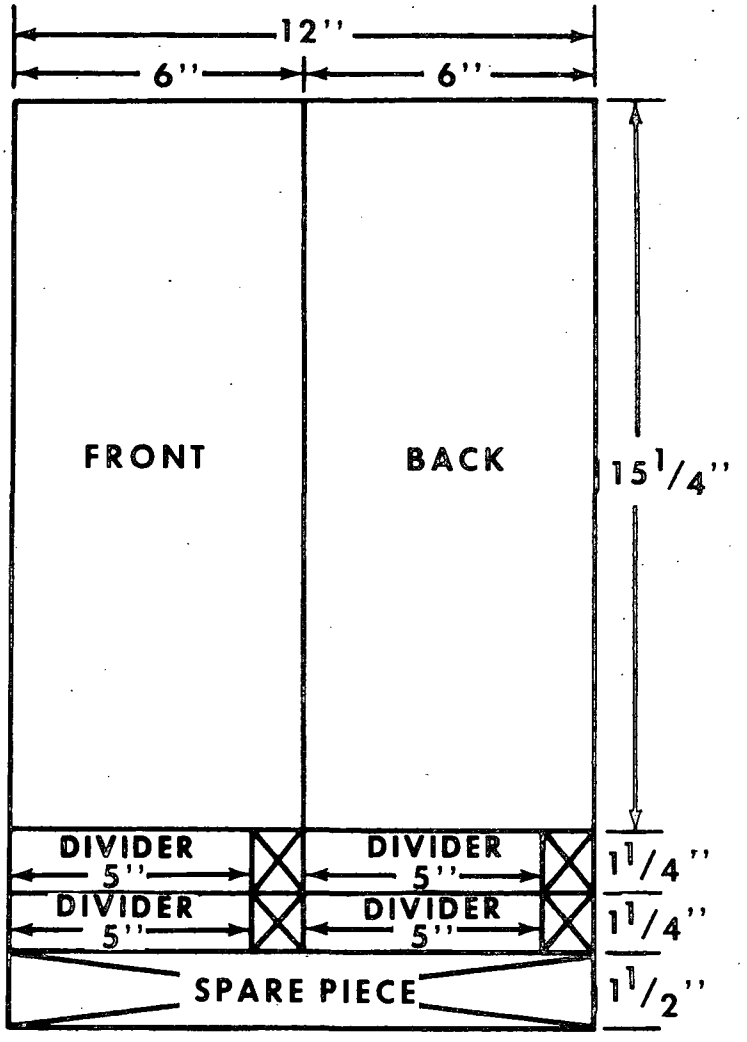


* 5 DIVIDERS @ SLIGHTLY OVER 4 3/4" (4.8") = 24"

**BE SURE THIS PIECE IS FULL 2 1/2" WIDE, OR MORE.

SCALE 1/4"=1"

PLATE 5. CUTTING PLAN FOR C-TANK

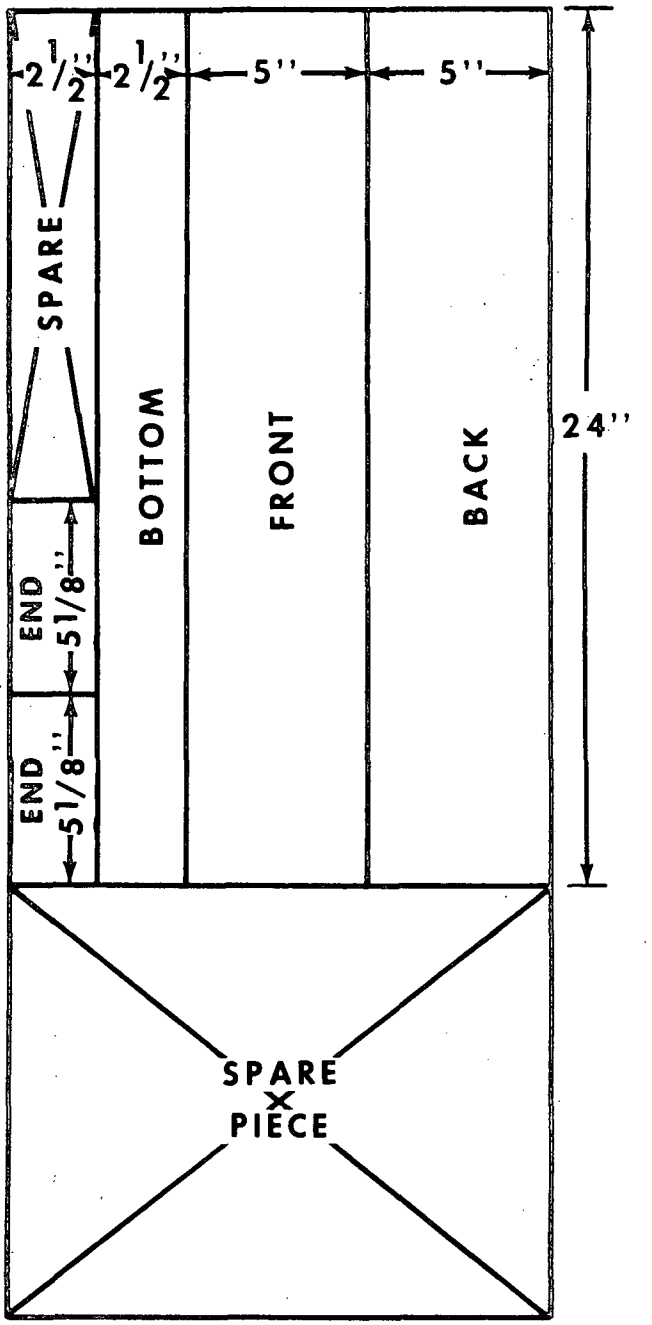


NOTES: A. FROM LONG 1 1/2'' WIDE STRIP DESIGNATED ON PLATE 2,
CUT: 1 PIECE 15 1/4'' LONG FOR BOTTOM
2 PIECES 6 1/8'' LONG FOR ENDS. ONE WITH NOTCH FOR DRAIN (FOR C-1 CHAMBER)
B. DIVIDERS MUST BE EXACTLY RIGHT IN ORDER FOR TANK TO FIT AND SEAL FREE OF LEAKS.

SCALE: 3''=1'

PLATE 6. CUTTING PLAN FOR FLOW SPLITTER TANKS

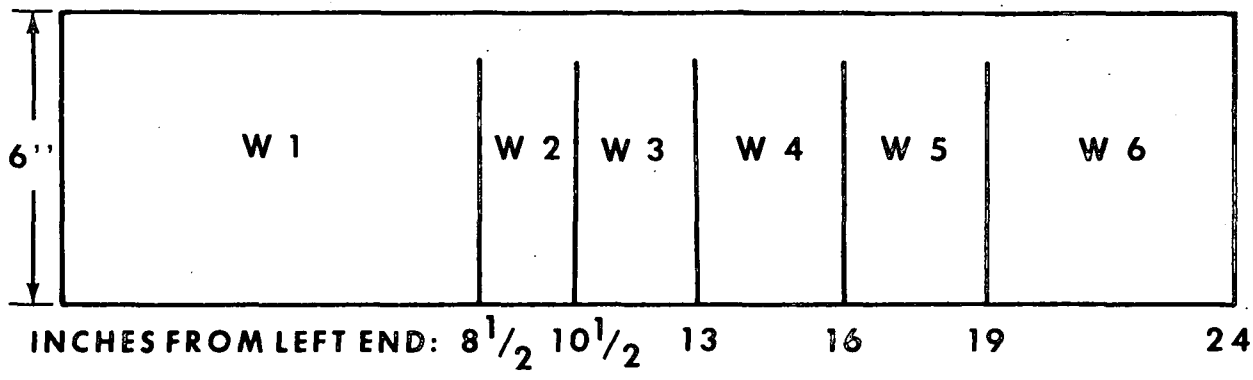
NOTE: FOR DIVIDERS, SEE PLATE 2.



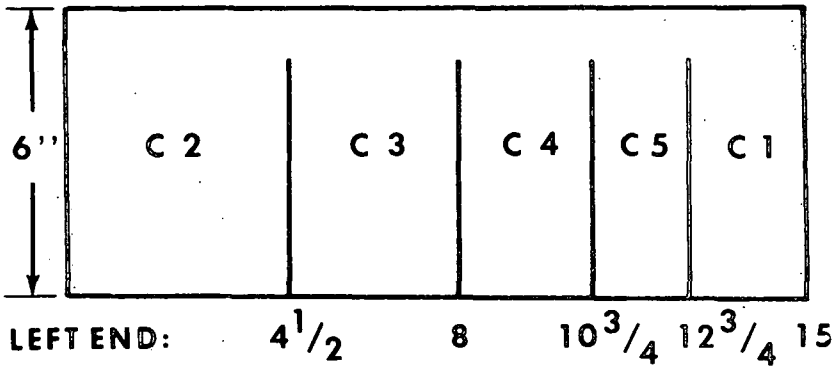
SCALE: 3/16=1"

PLATE 7. DIVIDER SPACING

W TANKS



C TANKS



FLOW-SPLITTER TANKS

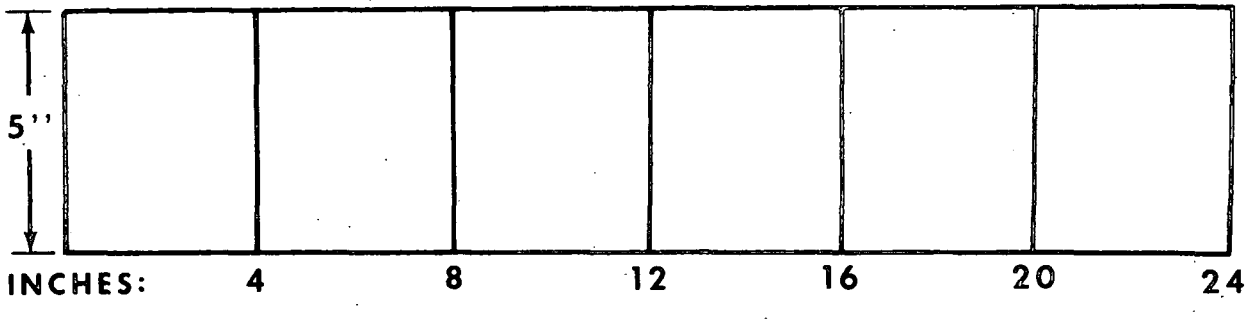


PLATE 8. FLOW-SPLITTERS AND SIPHON BREAKERS

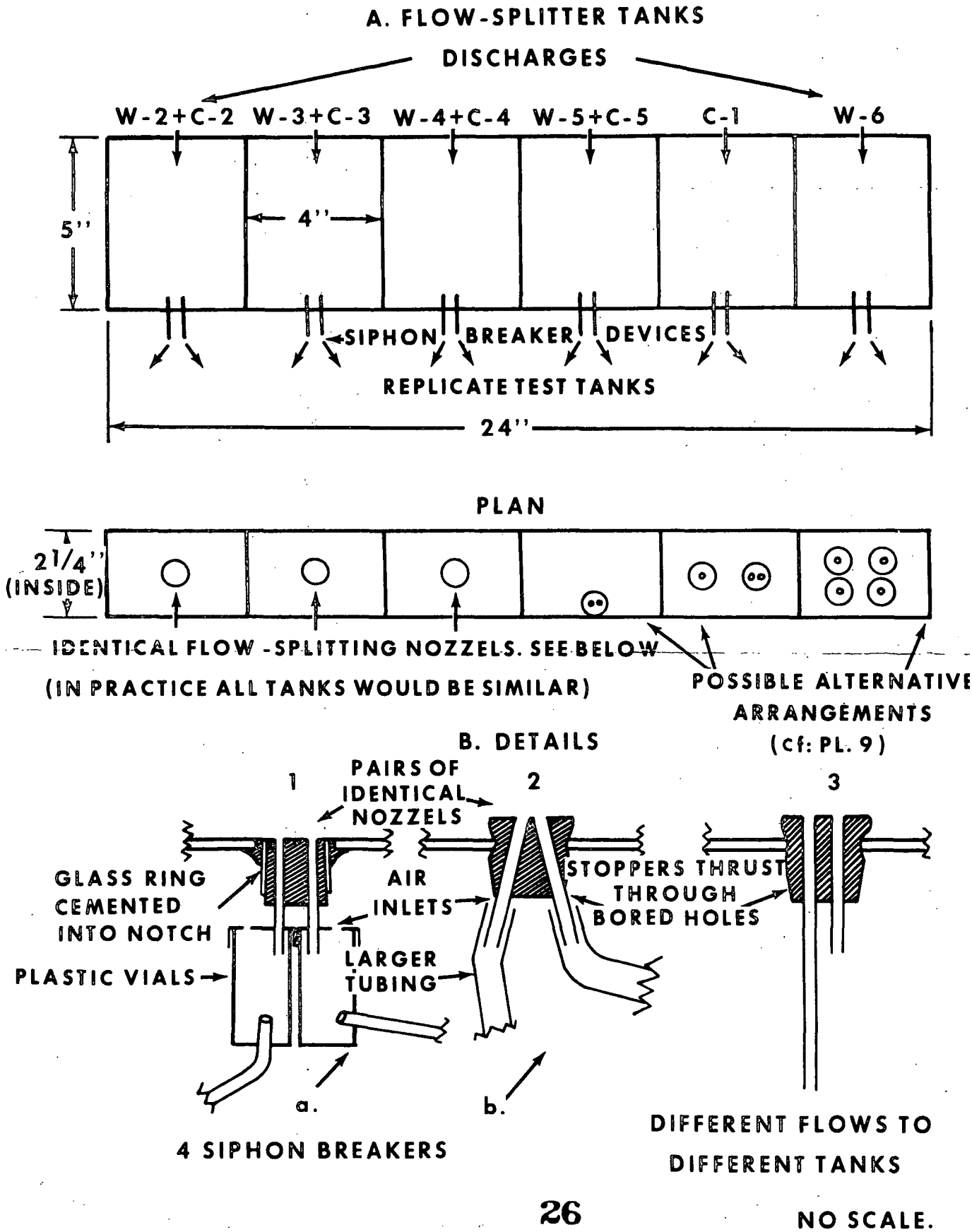


PLATE 9. DULUTH FLOW DISTRIBUTORS

THESE DEVICES WHEN INSTALLED IN FLOW-SPLITTER TANKS CAN BE ADJUSTED TO DISTRIBUTE THE FLOW EVENLY OR DIFFERENTIALLY BETWEEN TWO OR MORE TEST TANKS WITH GREAT PRECISION.

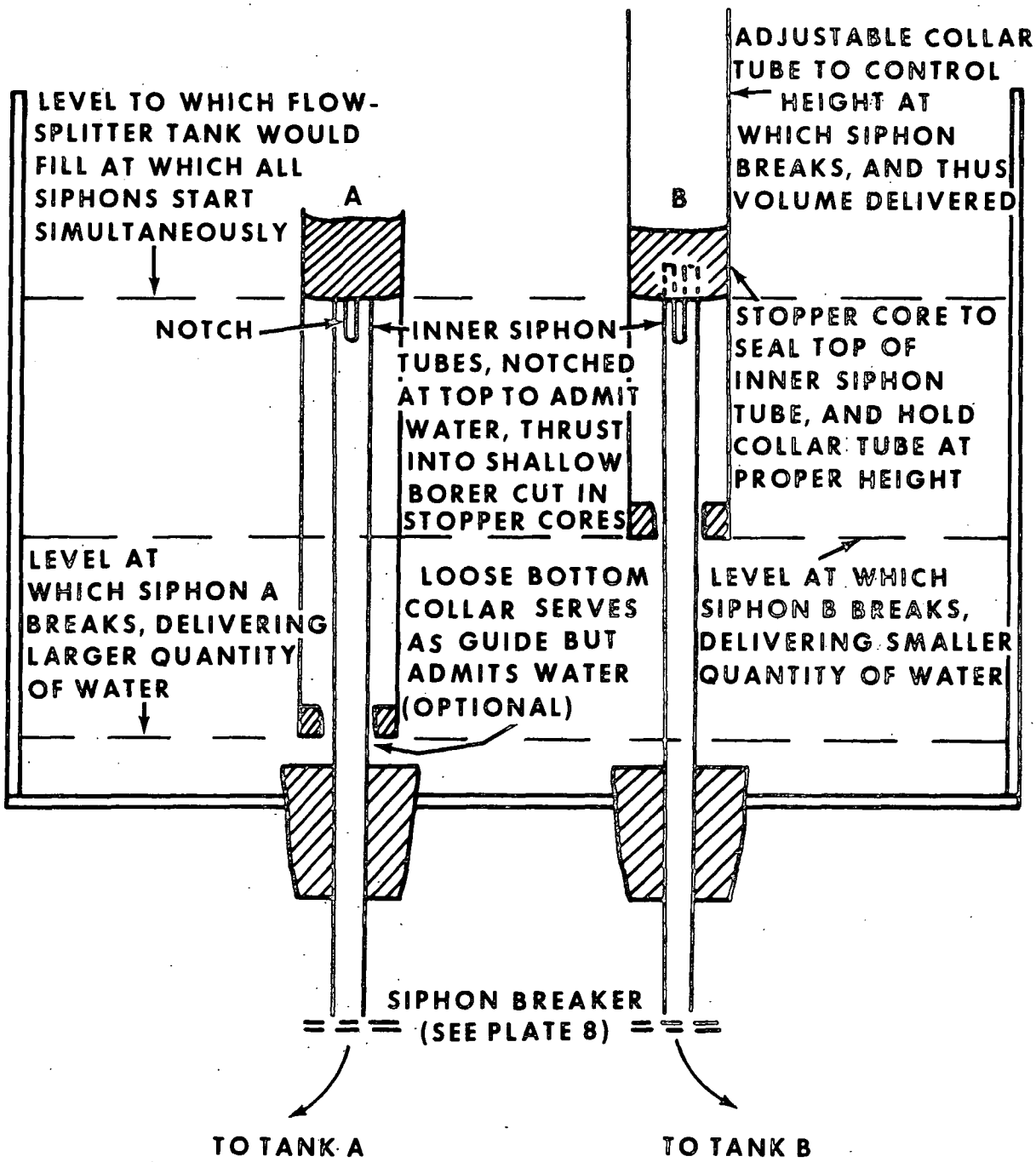
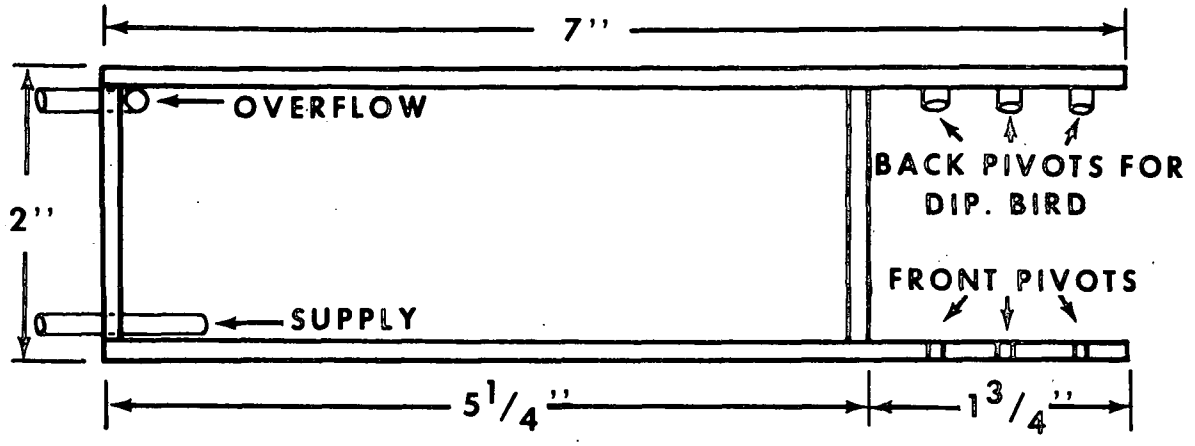


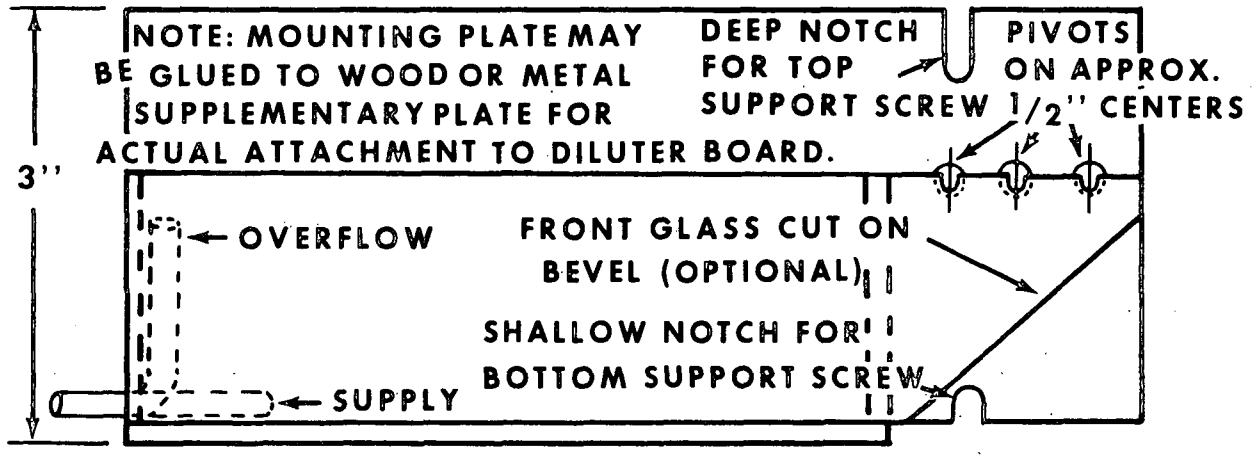
PLATE 10. DIPPING BIRD CHAMBER (OPTIONAL)

NOTE: DIMENSIONS SHOWN WILL ACCOMODATE A 40 ml SCOOP, WHICH IS PROBABLY MAXIMUM THAT SHOULD BE USED WITH STANDARD DILUTER. SMALLER CHAMBER FOR SMALLER SCOOP SHOWN ON PLATE 11.

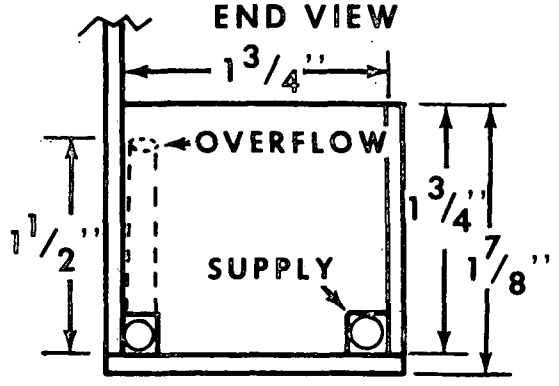
PLAN



FRONT VIEW



END VIEW



1 PC 3×7	MOUNTING PLATE
2 PCS $1\frac{3}{4}\times1\frac{3}{4}$	ENDS, ONE WITH CORNERS CUT OUT
1 PC $2\times5\frac{1}{4}$	BOTTOM
1 PC $1\frac{3}{4}\times7$	FRONT

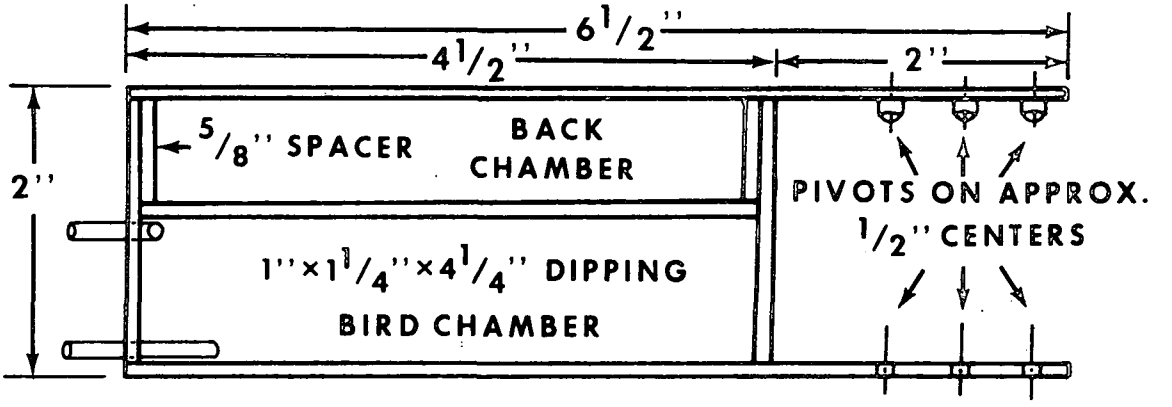
LOWER RT. CORNER BEVELED

SCALE: 3/4''=1''

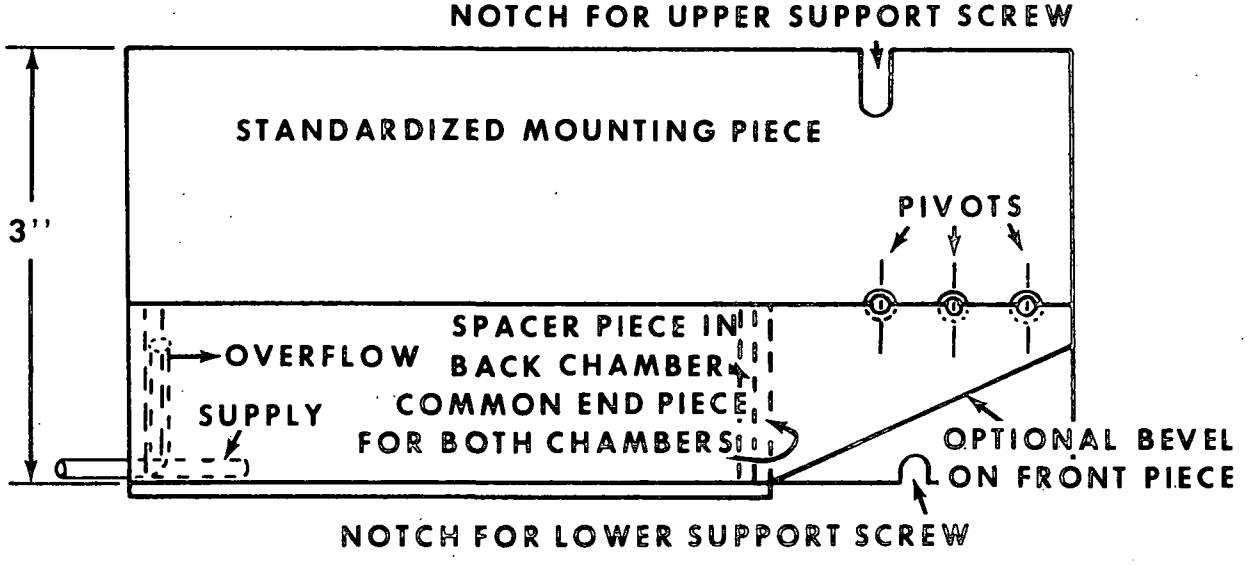
PLATE 11. SMALL DIPPING BIRD CHAMBER

(FOR MORE TOXIC MATERIALS)

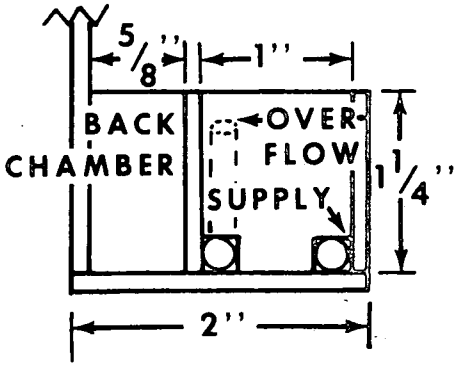
PLAN



FRONT VIEW



LEFT END VIEW

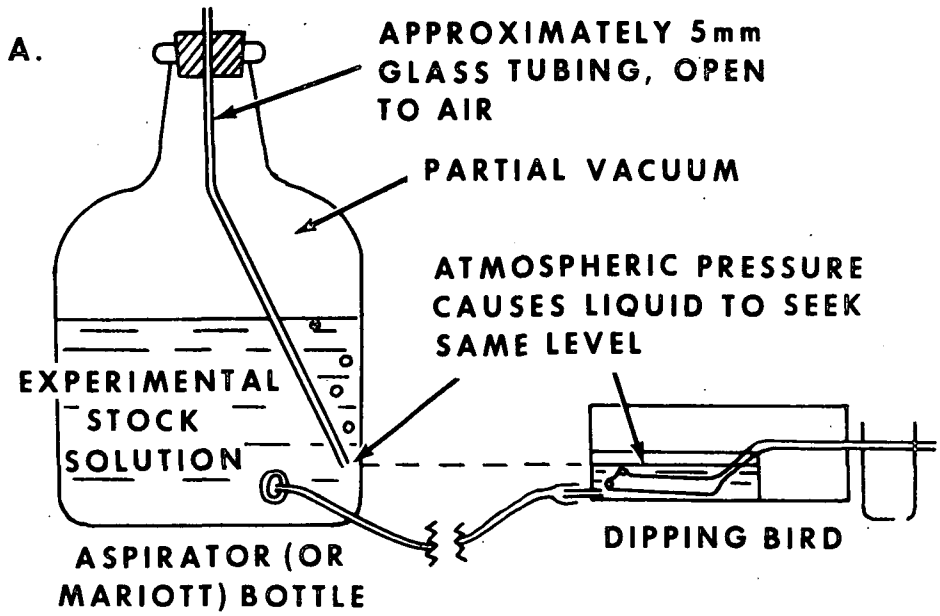


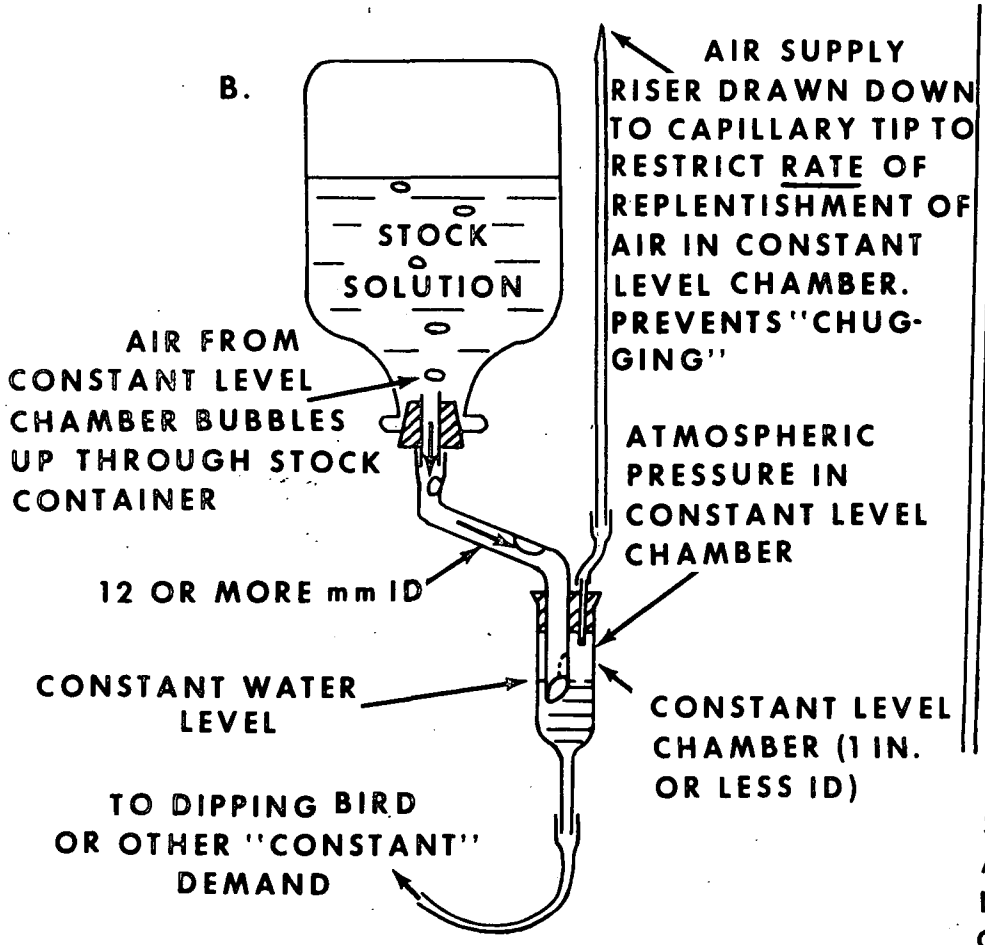
PIECES REQUIRED

1 PC 3×6 1/2	MOUNTING PIECE
2 PCS 1 1/4×1 3/4	ENDS
2PCS 1 1/4×5/8	SPACERS
1 PC 1 1/4 ×4 1/4	CENTER WALL
1 PC 1 1/4×6 1/2	FRONT
(BEVELED CORNER OPTIONAL)	
1 PC 2×4 1/2	BOTTOM

SCALE: 3/4''=1''

PLATE 12. SOME CONSTANT LEVEL DEVICES





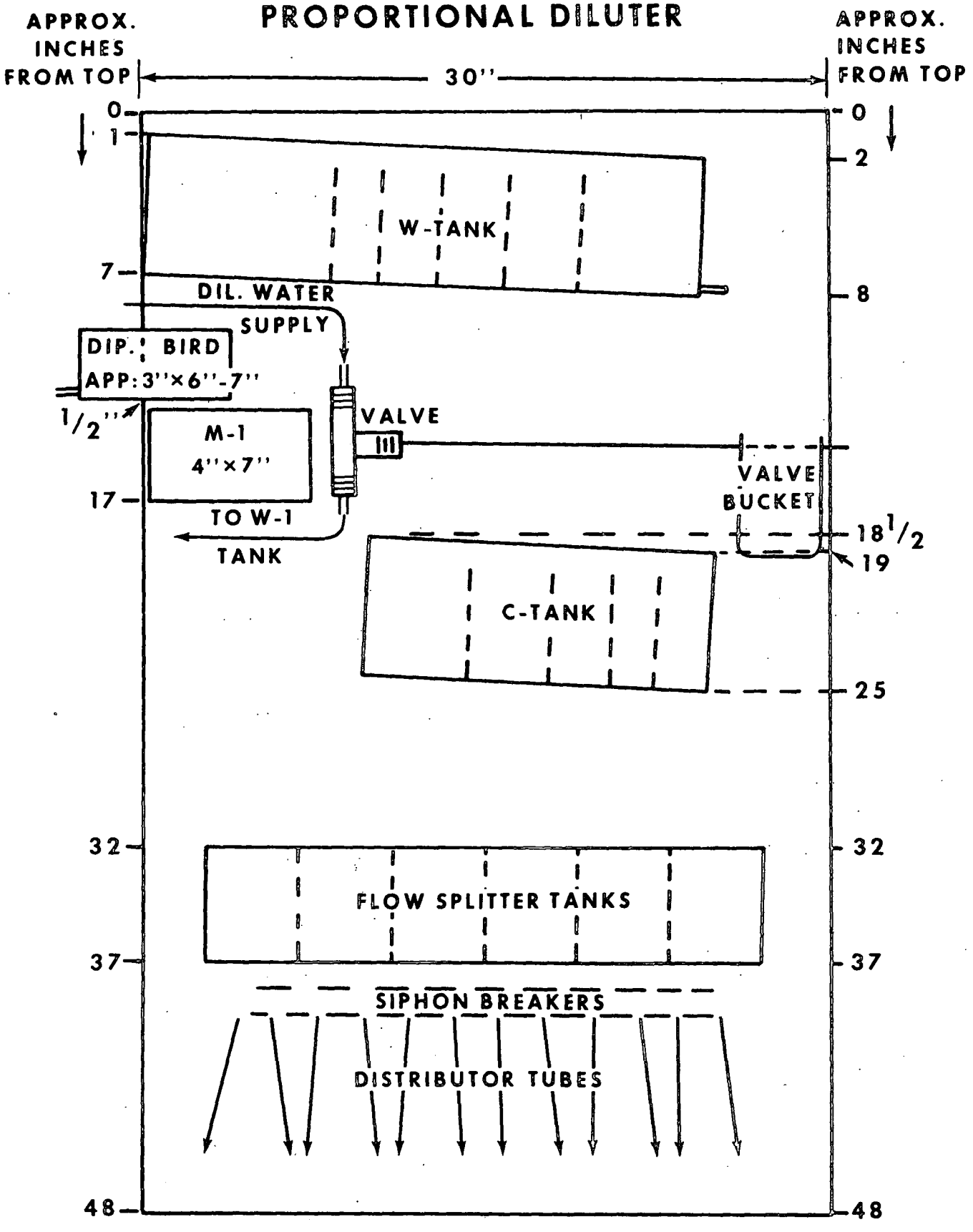
(NO SCALE)

REMARKS:

"A" IS THE SIMPLEST KNOWN CONSTANT LEVEL DEVICE FOR THE TYPE OF USE ILLUSTRATED. THE ACTUAL LEVEL IS ADJUSTED BY SLIDING THE AIR SUPPLY TUBE UP OR DOWN.

"B" IS A DEVICE WHICH MAY BE USED IF AN ASPIRATOR BOTTLE IS NOT AVAILABLE, IF IT CANNOT BE MOUNTED AT THE SAME LEVEL AS THE DIPPING BIRD, OR FOR SOME OTHER USES IF SETTLABLE SOLIDS ARE PRESENT WHICH NEED SLIGHTLY MORE VIGOROUS AGITATION. IF FASTER DELIVERY IS REQUIRED, CUT CAPILLARY RISER TIP OFF AT POINT OF SLIGHTLY LARGER DIAMETER.

PLATE 13. SUGGESTED LAYOUT FOR PROPORTIONAL DILUTER



SCALE: 2''=1'

PLATE 14. A FLOW PLAN FOR BIOMONITORING

(SEE ALSO PLATE 15, AND TEXT SECTION V)

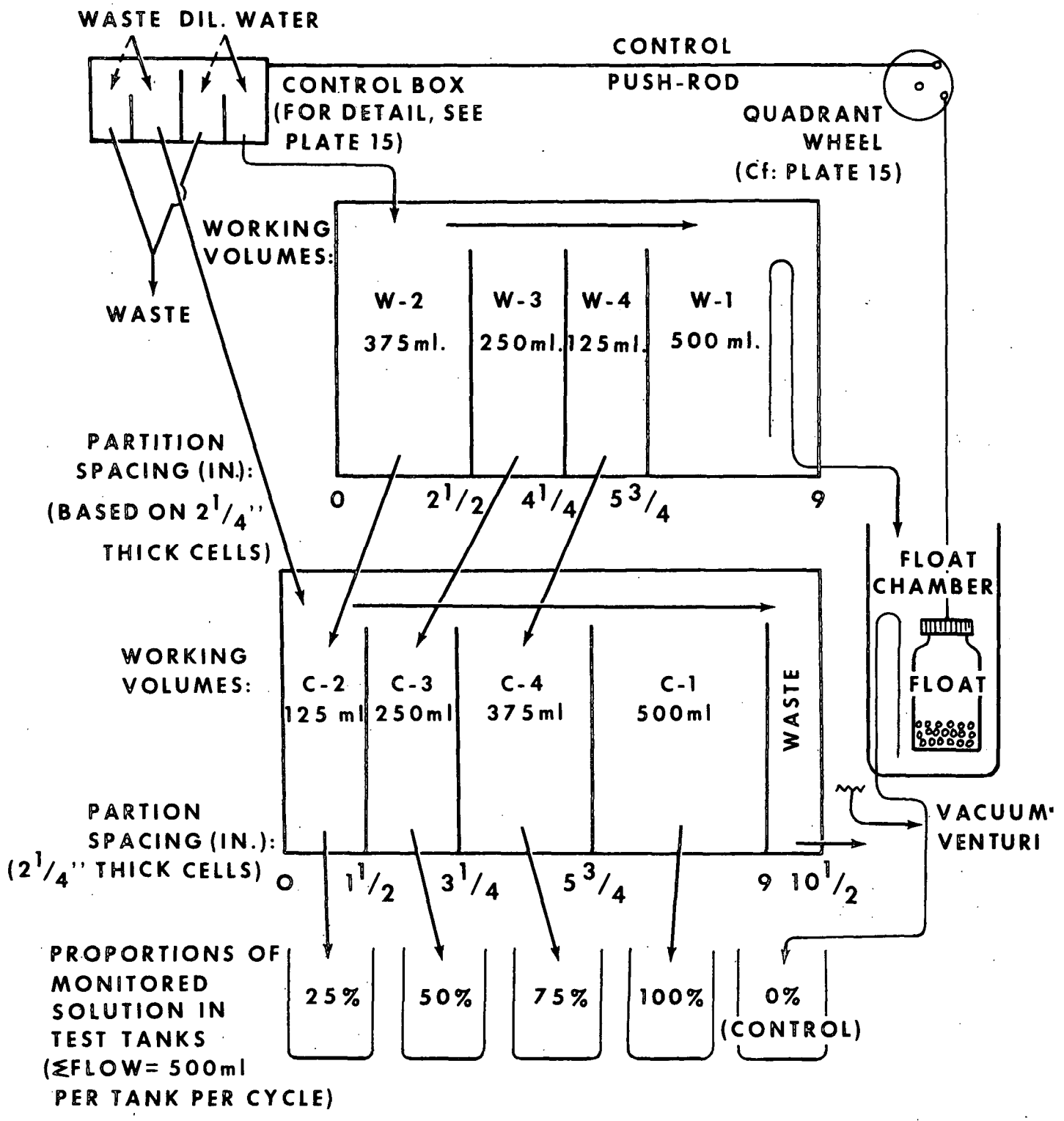
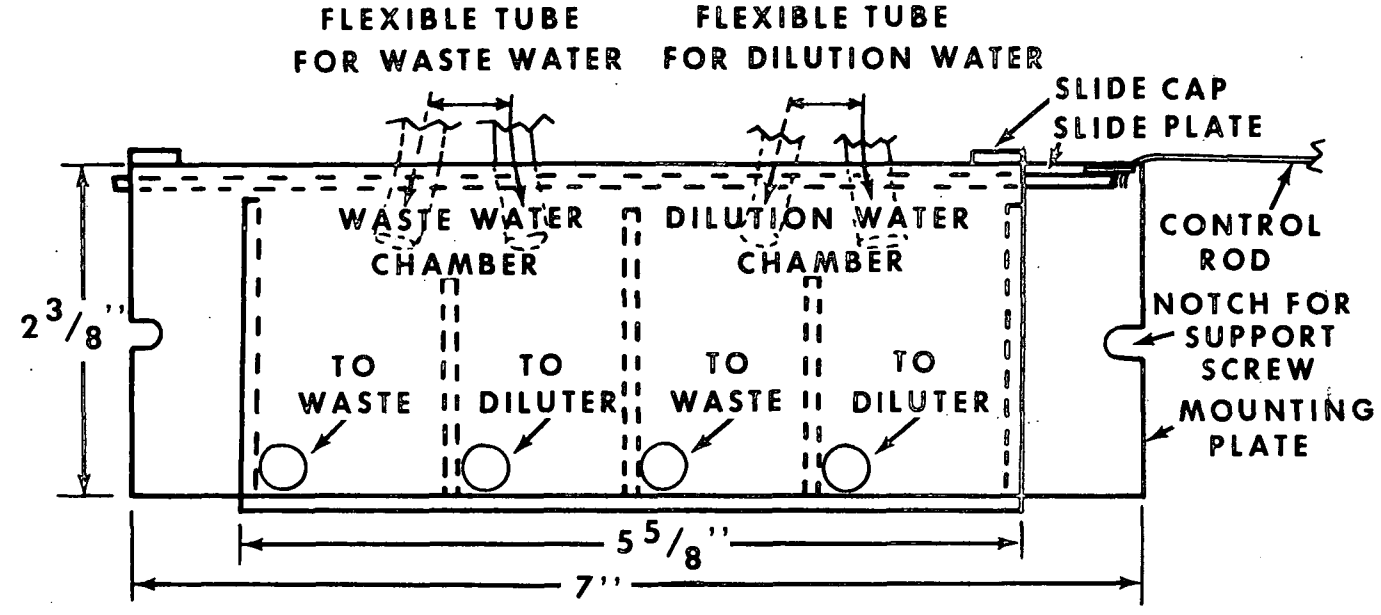
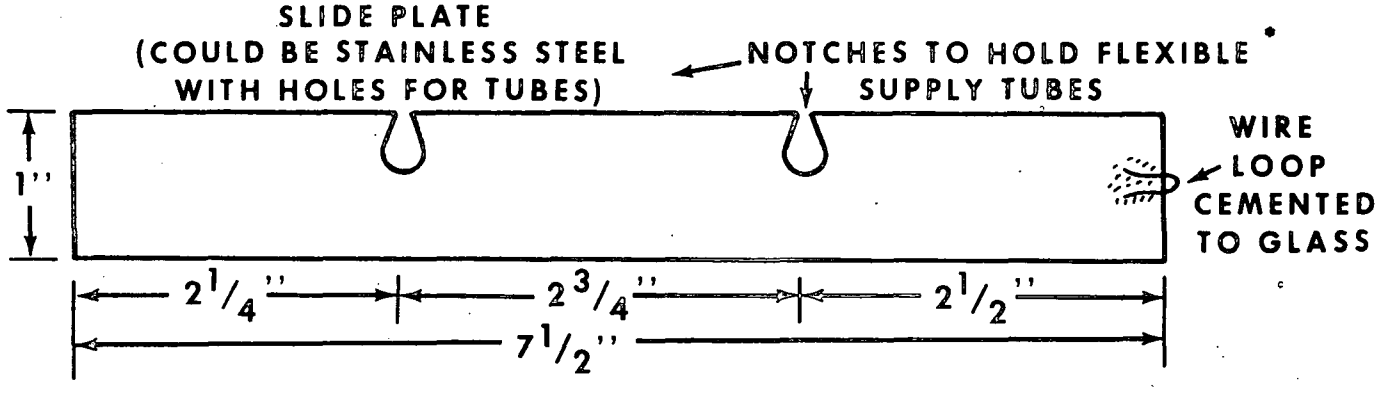
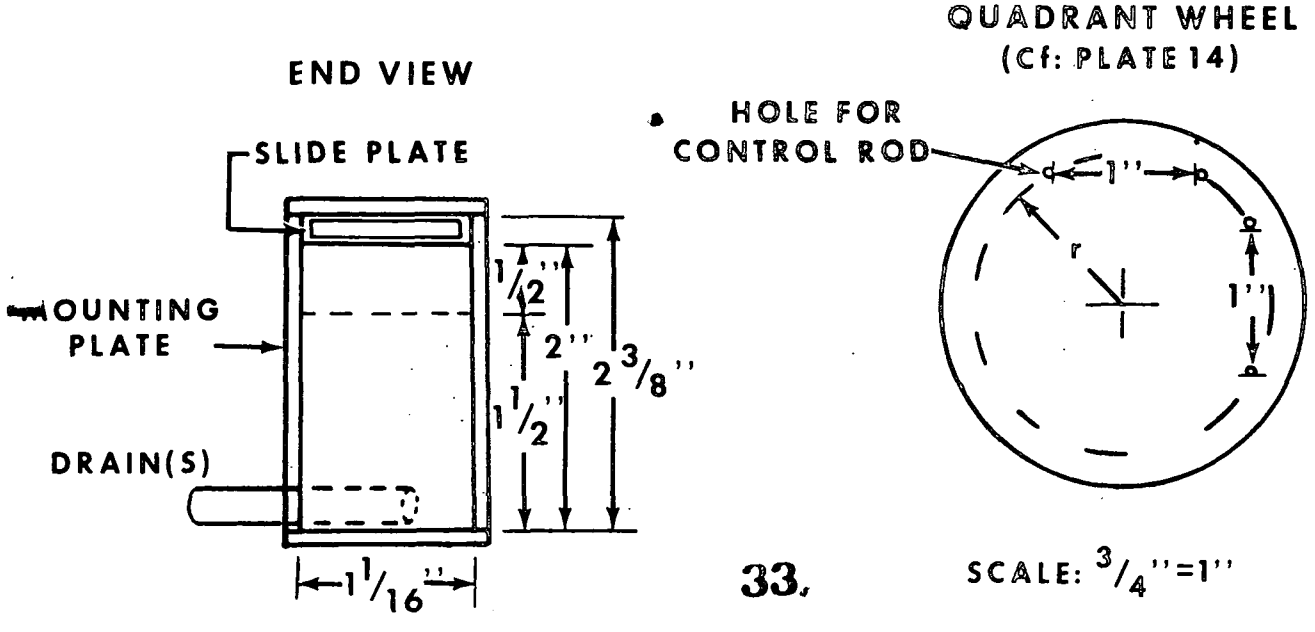


PLATE 15. BIOMONITOR CONTROL BOX







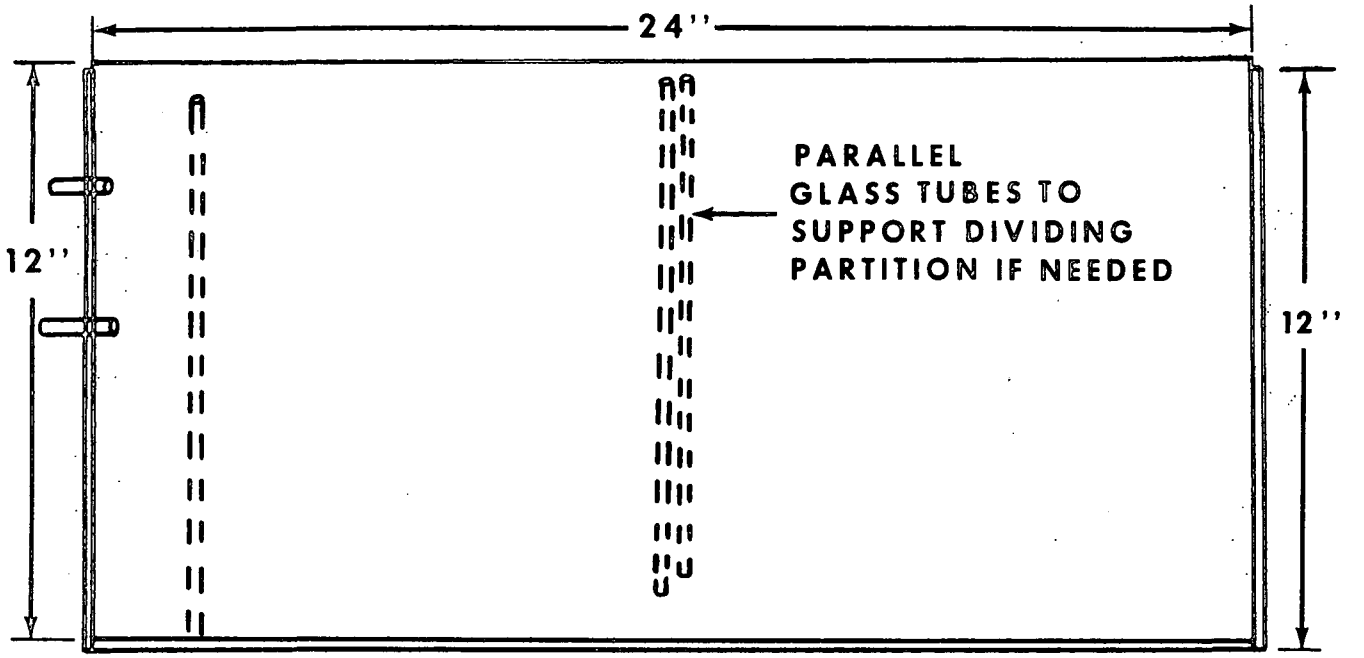
SCALE: 3/4''=1''

° SLIDE PLATE WORKS BEST IF SUSPENDED BY SUPPLY TUBES

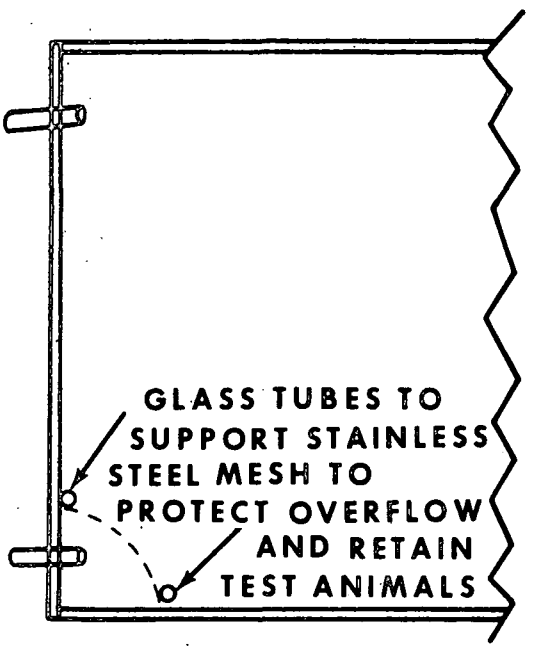
PLATE 16. TEST TANK

(3PCS: 12''×24'', 2 PCS 12''×12'')

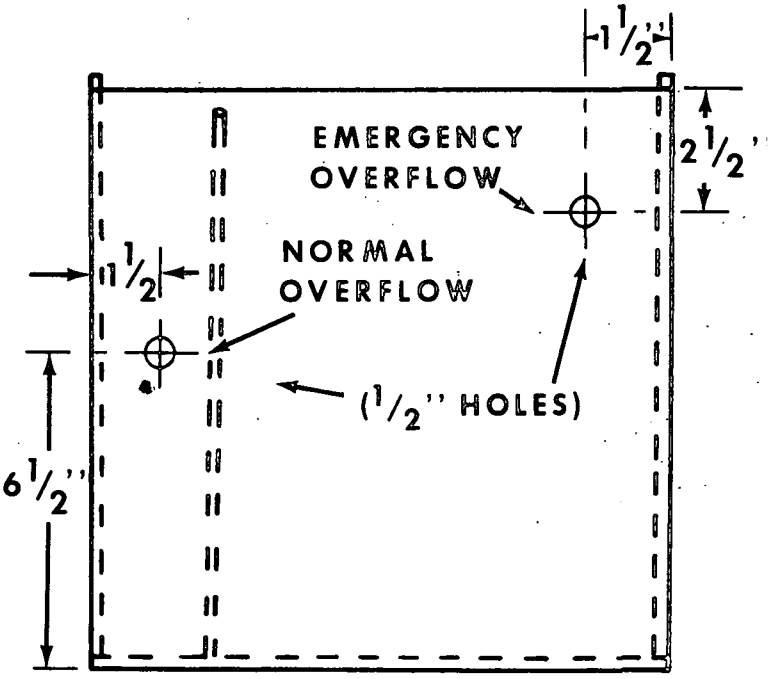
SIDE VIEW



PLAN OF DISCHARGE END



END VIEW



SCALE: 1/4''=1''

PLATE 17. A WATER CONDITIONING SYSTEM

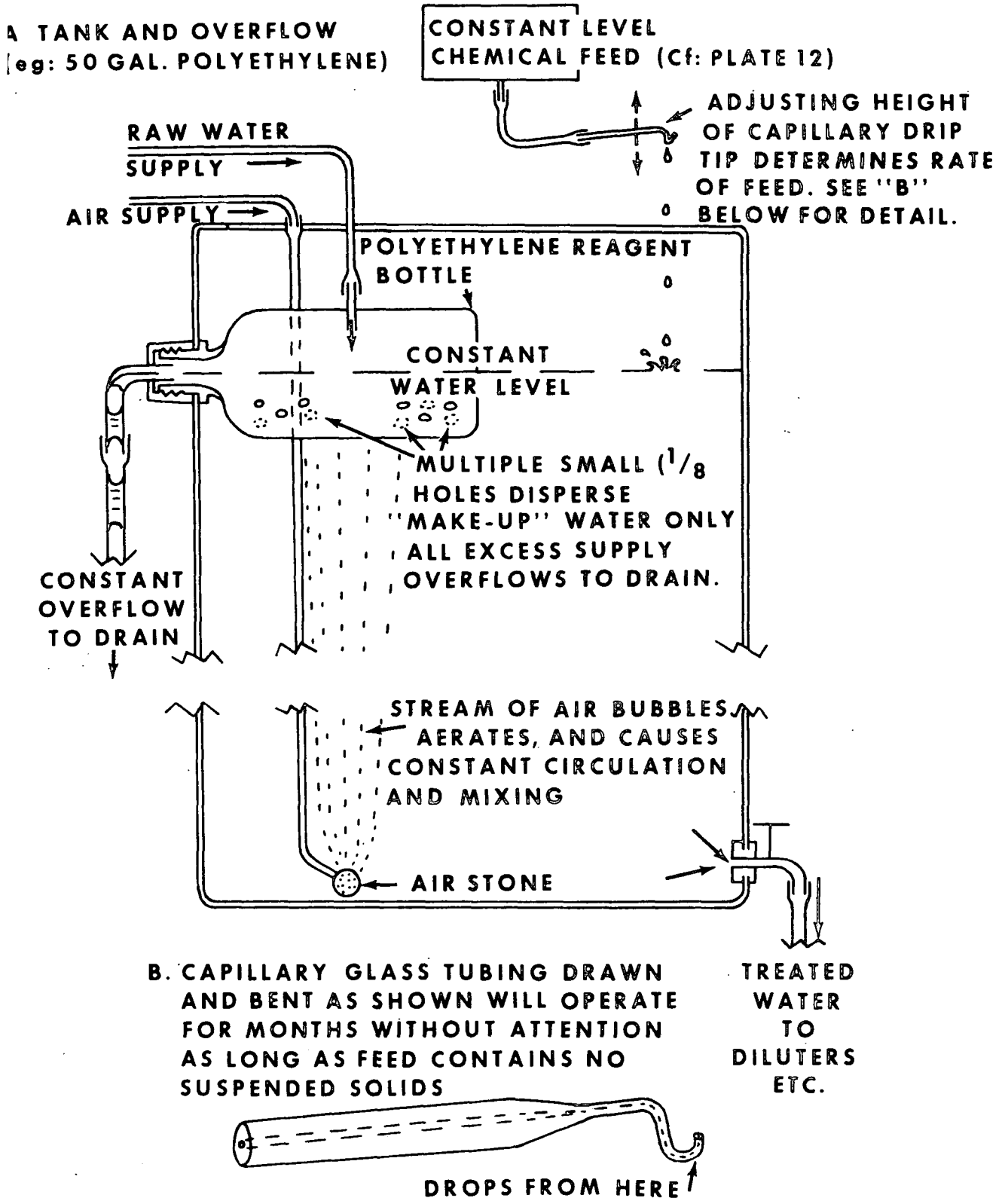
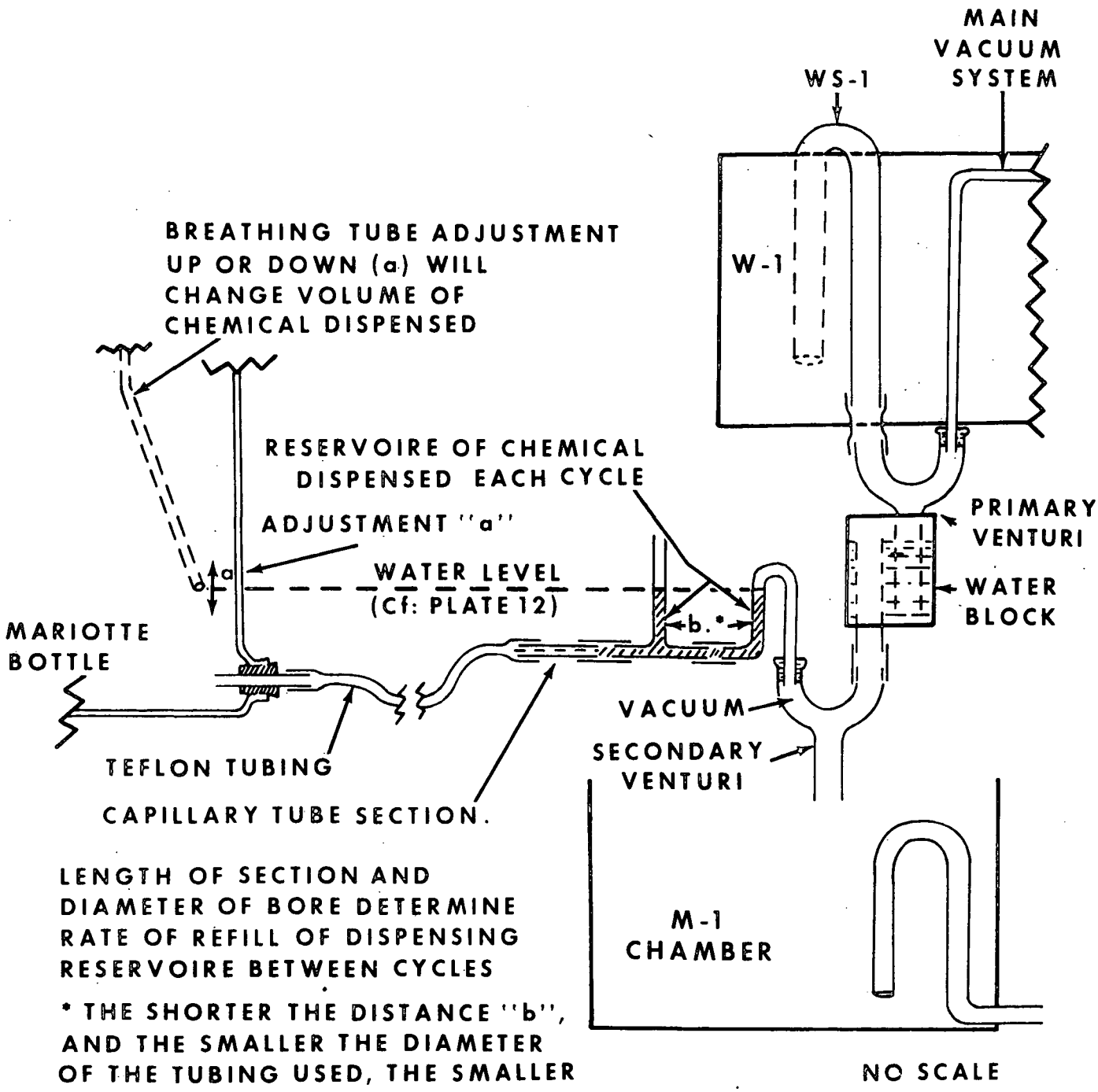


PLATE 18. A SIMPLE TOXICANT METER



LENGTH OF SECTION AND DIAMETER OF BORE DETERMINE RATE OF REFILL OF DISPENSING RESERVOIRE BETWEEN CYCLES

* THE SHORTER THE DISTANCE "b", AND THE SMALLER THE DIAMETER OF THE TUBING USED, THE SMALLER WILL BE THE WORKING VOLUME DELIVERED.

AFTER McALLISTER et al.'72

APPENDICES

APPENDIX 1

Water Research, Pergamon Press 1967. Vol. 1, pp. 21–29. Printed in Great Britain. **

A SIMPLIFIED DOSING APPARATUS FOR FISH TOXICOLOGY STUDIES

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(*Received* 12 *September* 1966)

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

* Respectively Fisheries Research Biologist and Aquatic Biologist.

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.

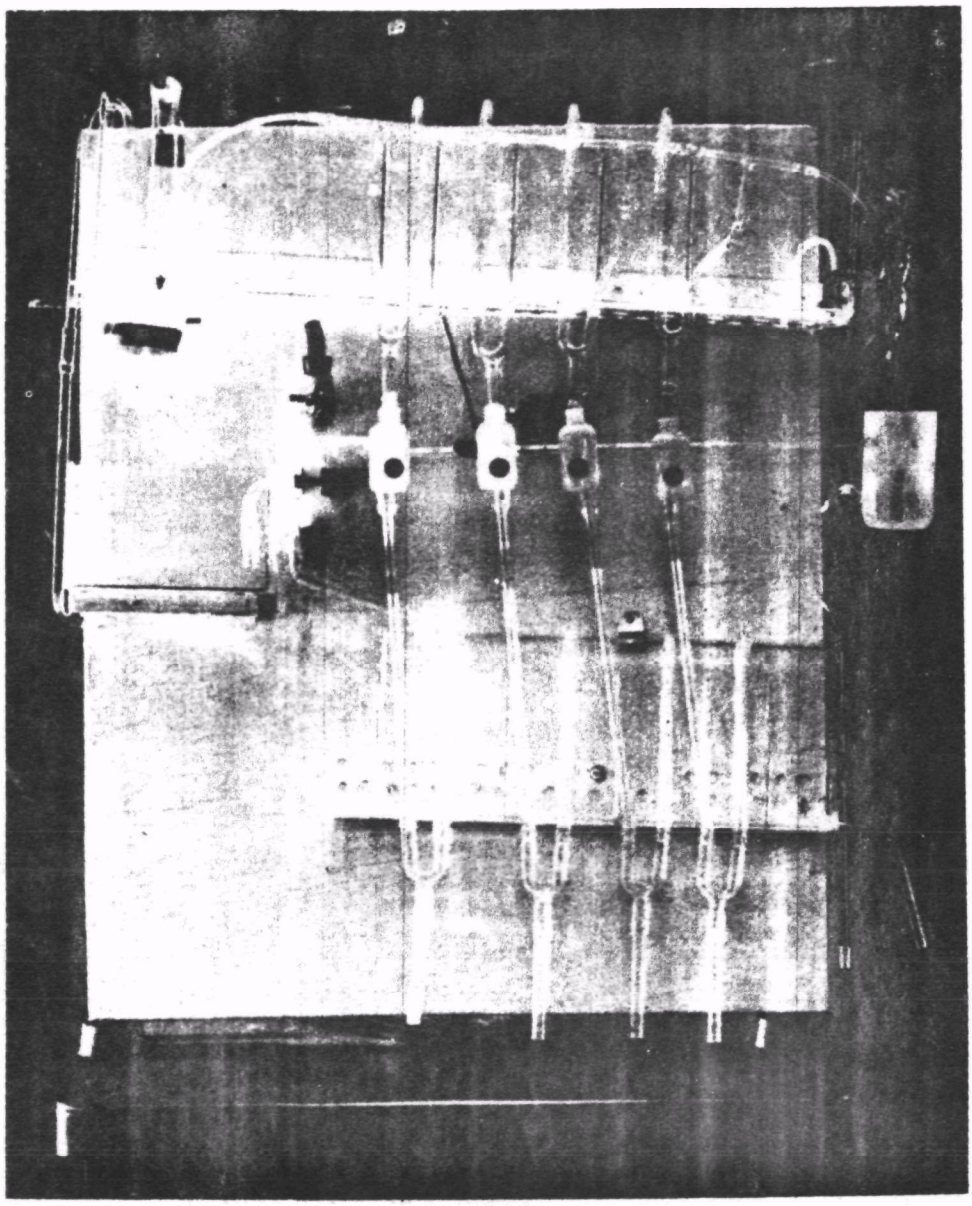

FIG. 1. Photograph of a proportional diluter built as suggested in this paper.

(*Facing p.* 22)

(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

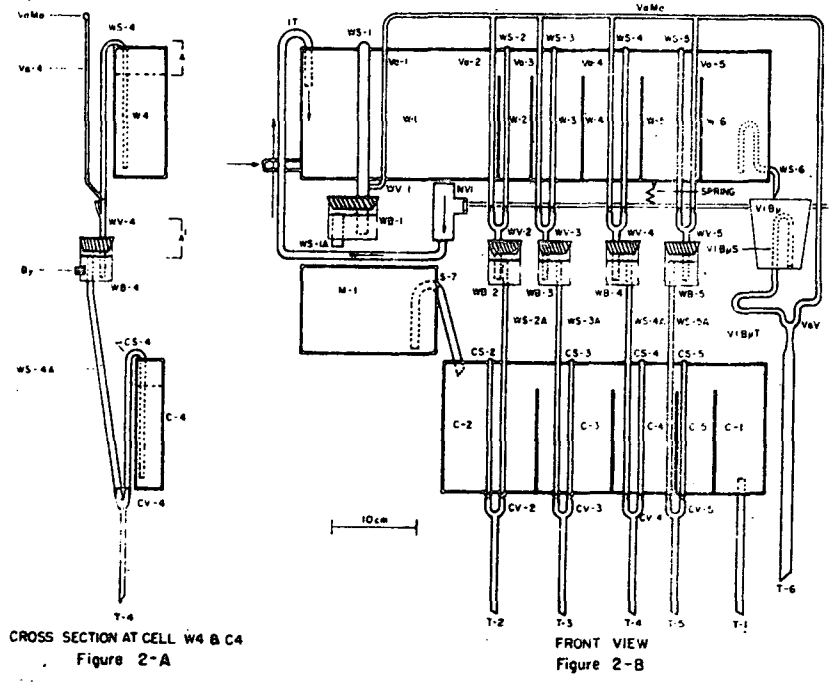

FIG. 2. Semi-schematic scale drawing of diluter.

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"
VlBuS	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 (VlBu) 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 $\frac{3}{8}$-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.

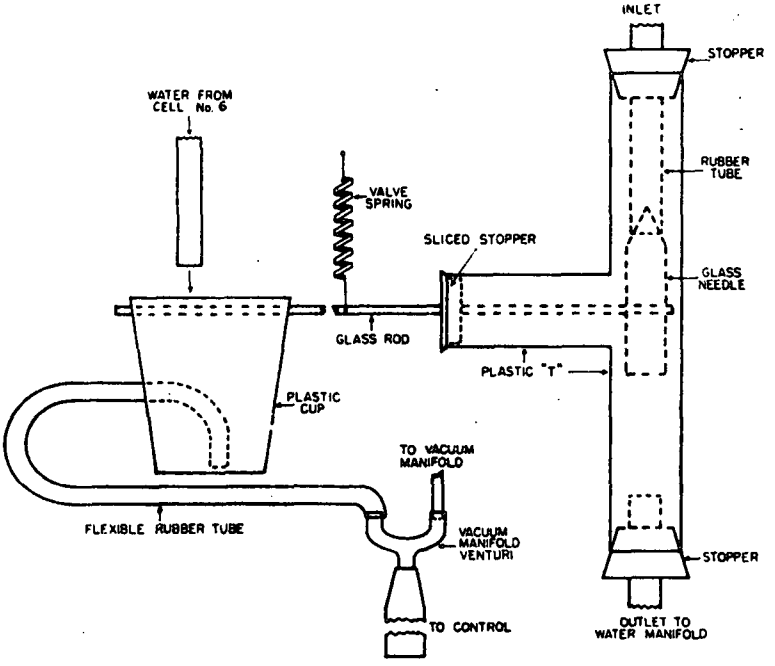

FIG. 3. Needle valve and vacuum venturi detail.

(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 toxicant

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.)

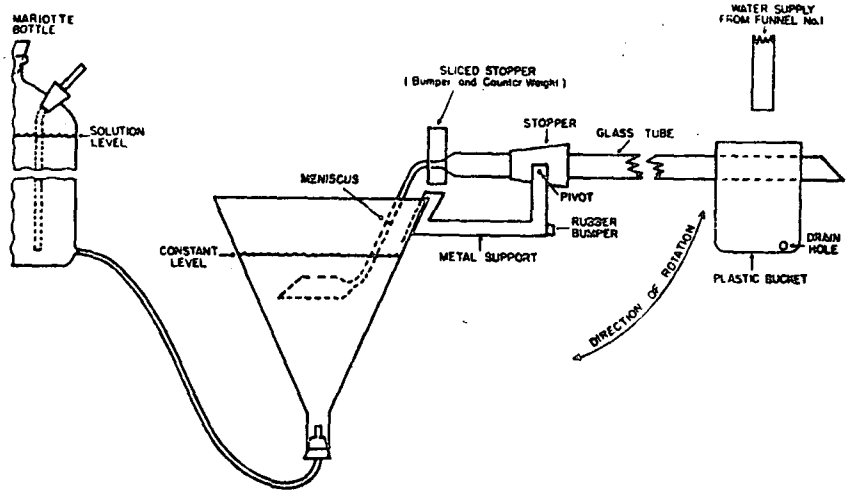

FIG. 4. Detail of chemical-metering apparatus.

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.

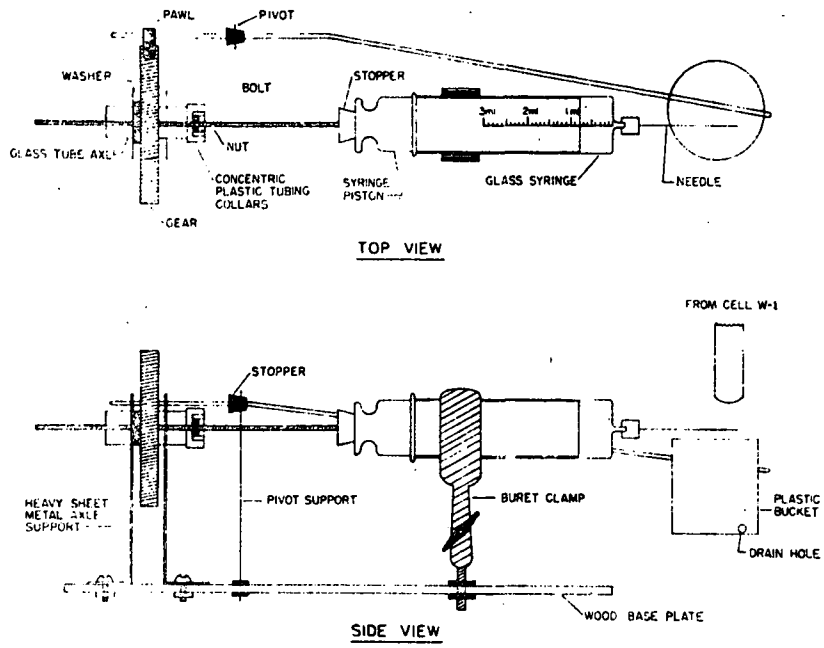

FIG. 5. Injector system for adding μl quantities to the water.

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 Various Concentrations of Materials in Water.* U.S. Public Health Service Publ. No. 999-WP-23, 16 pp.

APPENDIX 2

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Reprinted from TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY
Vol. 99, No. 4, October 1970
pp. 799–802 *

A Water Delivery System for Small Fish-Holding Tanks

INTRODUCTION

The necessity for maintaining small populations of fish and other aquatic life in holding tanks for observation, acclimatization, and so forth is steadily increasing as more investigators become involved in physiological, toxicological, and other investigations of aquatic life. The principal initial problems in utilizing aquatic life in laboratory investigations are of a facilities nature; for example, the design of holding facilities. A more specific problem is that of water flow control. Many of us have regulated water flow to holding tanks by an assortment of techniques such as valves, screw-clamps, and small-bore tubing. Anyone who has worked with such techniques has probably had difficulty in maintaining uniform flow rates because of clogging of the water lines at the point of restriction. This can be especially troublesome if a "natural" water is used. Valuable lots of aquatic organisms can be lost if the water flow stops or becomes insufficient.

The water delivery system described herein is a modification of the proportional diluter described in detail by Mount and Brungs (1967). This system is almost free from clogging caused by suspended solids, cladocerans, snails, and so forth, since it avoids the problem of restricted openings as a means of providing controlled water flows to each holding tank. The particular design discussed here (Figure 1) can be used to deliver 500 milliliters (ml) to each of six holding tanks as often as every two minutes. Comparable systems have been used in the Newtown Fish Toxicology Laboratory for longer than one year with rare malfunctions and little maintenance other than occasional cleaning.

MATERIALS

All materials used for construction of the water delivery system are readily available. Single- or double-strength window glass, appropriate glass and vinyl tubing, glass glue, a hand glass cutter, a 1-inch plastic hose "T", plastic bottles, rubber stoppers and, optionally, a mechanical counter, constitute the necessary materials. If one wishes, local glass stores will cut the glass to desired sizes and cut the necessary hole. An excellent silicone rubber glass glue (Silicone Seal produced by General Electric or Glass and Ceramic glue produced by Dow-Corning)* now on the market has made construction of the water delivery system extremely simple. Clean glass can be glued without etching and the pieces can be assembled by simply pressing the edges together with glue. Disposable plastic syringes of 10-ml capacity filled with the glue are ideal for depositing a fine bead of glue on the edges to be glued. The water delivery system for approximately a 500-ml delivery from each of six cells would measure 24″ wide × 6″ high × 2″ deep, each cell being 4″ wide. Individual cell dividers would be 5″ high. Minor variations in dimensions are unimportant as long as the pieces can be easily assembled with appropriate overlap. The water delivery system is usually placed on a 2-inch deep shelf attached to a piece of ½-inch plywood.

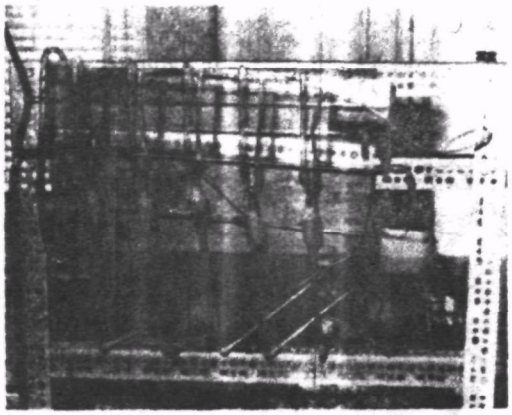

FIGURE 1.—Photograph of operational water delivery system.

* Mention of product and company name does not constitute endorsement by the Federal Water Quality Administration or the U. S. Department of the Interior.

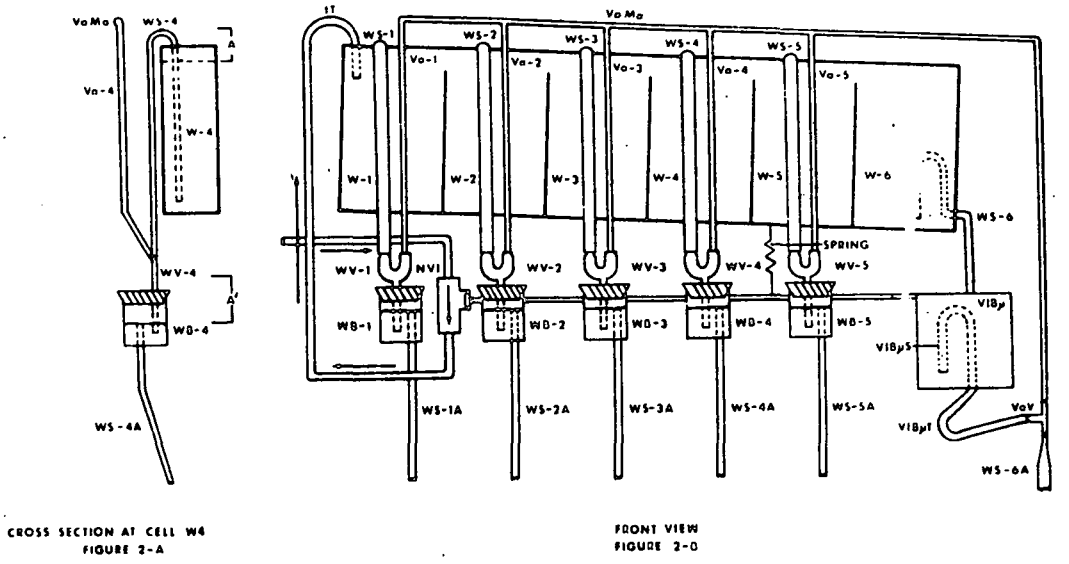

FIGURE 2.—Semi-schematic scale drawing of water delivery system. (Legend explained in text.)

PRINCIPLES OF OPERATION

The details of operation are thoroughly discussed for the proportional diluter (Mount and Brungs, 1967) but will be included here for convenience. The series of water-metering cells is filled, the water is turned off by the valve, the cells are emptied, and the water flow restored. Cell W-1 (Figure 2B) fills first from IT, W-1 then overflows into W-2, and so forth. As cell W-6 is filled it overflows through siphon WS-6 into the valve bucket (VlBu), which should also have a capacity of about 500 ml. A 1-pint plastic refrigerator dish is quite satisfactory. As VlBu fills, the weight of the water causes the water valve (NVl) to turn off the influent water from tube IT while the water metering cells empty. The tube WS-6 must fill the valve bucket at a rate such 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 dividers. This drainage, which is quickened by having the lefthand end of the system about one inch higher than the righthand end, should be completed before the water begins flowing through the valve bucket water line (VlBuT) to the vacuum venturi (VaV). This insures delivery of uniform volumes from each water cell every time the system cycles. As the water originating from W-6 passes through the vacuum venturi, a partial vacuum is produced 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 vacuum venturi causes water to be pushed up the water siphon tubes (WS-1 to 5) by the greater atmospheric pressure. This results in siphons being started in the water siphon tubes. The water blocks (WB-1 to 5) serve to prevent air from entering the system through water siphon tubes WS-1A to 5A. These latter siphons are connected to drain lines to each holding tank. The lines leading to the holding tanks should slope so that they are completely drained after each cycle. For easiest operation it is advisable to have the flow from WS-6A drop directly into the nearest holding tank. This will avoid possible complications in the operation of the vacuum venturi (VaV). It is absolutely necessary that the distance from the water level of each filled water cell to the top of its water siphon tube, distance "A" (Figure 2A), 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 siphons will not start but rather water will enter the vacuum tubes Va-1 to 5.

There is only one timing adjustment to be

TABLE 1.—*Tube sizes for water delivery system*

Number	Outside diameter (mm)	Material
WS-1 to 5	10	Glass
WS-6	8	Glass
WV-1 to 5	10	Glass "U" connector
WS-1A to 5A	11	Glass
WS-6A	8	Glass
VlBuS	8	Glass
VaV	8	Glass
Va-1 to 5	5	Plastic
NVl	25 (1-inch)	Plastic hose "T"
VlBuT	6	Rubber
IT	12	Glass

checked: the water flow through the vacuum venturi (VaV) must be fast enough to produce sufficient vacuum to start the siphons but slow enough to allow the valve to remain closed long enough to allow siphon WS-1 to empty cell W-1 before the influent water again enters through tube IT.

The only calibration necessary can be made by collecting the water from each water siphon tube (WS-1A to 5A) during normal operation. The volumes in W-1 through W-5 are adjusted as necessary by raising or lowering the depths to which the siphon tubes extend into the cell. The WS-1 to 5 tubes should be glued to the outside of the water cells so that they are rigid, but they should be cut off approximately at the top level of the cell dividers and then an adjustable extension added to furnish the desired length. The cell ends of the WS-1 to 5 tubes should be exactly parallel to the water surface in the cell so that the siphon breaks abruptly. The delivery volume of the W-6 cell will vary with the flow rate through tube IT. Therefore, it can be calibrated only after the desired flow rate has been set. The height of the siphon tube WS-6 is then adjusted accordingly.

Tube sizes and other specifications are included in Table 1.

Figure 3, from Mount and Warner (1965), is reproduced here for convenience in constructing the needle valve (NV1 in Figure 2A). For the water delivery system described in this paper, inlet and outlet tubes of ⅜-inch I. D., and a needle made of 13-mm glass tubing are suggested. The glass rod should be about 6 mm in diameter. A string, a pulley, and a small plastic cup filled with sand make a suitable counterbalance weight should it be necessary to substitute for the valve spring. The tension of the valve spring should be

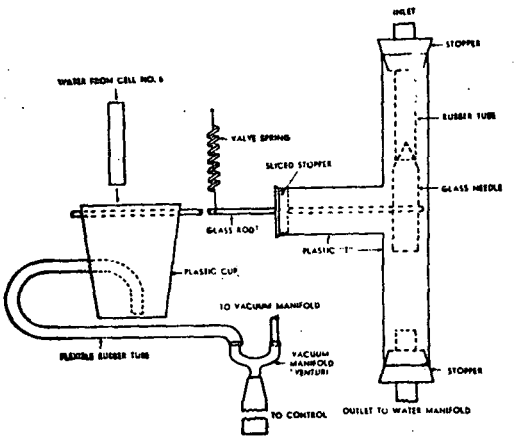

FIGURE 3.—Detail of the main water valve and vacuum venturi.

adjusted so that the valve closes quickly as water enters the valve bucket (VaBu), but yet does not reopen before WS-1A is empty. It is advisable to install a device such as an oblong wire loop around the glass rod near the valve bucket. This wire is adjusted so that the full weight of the water rests at the bottom of the wire loop when the valve is closed. This protects against breaking of the glass rod at the point at which the valve spring attaches. This loop must not inhibit full opening of the valve. The U-shaped vacuum manifold venturi, valve venturi (VaV) in Figure 2B, is now replaced by a glass "T" connector which operates more efficiently. It is suggested that a mechanical or electrical counter be incorporated to the movement of the valve arm. The counter records the number of cycles and daily determinations of the flow rate can be made for further confidence in the water delivery system.

When the delivery system becomes dirty due to algal or other growth or organic material, it can be cleaned by dissolving granular calcium hypochlorite (such as that used for swimming pools) and adding it into the W-1 cell as water is entering the system. The water from the system must be diverted from the holding tanks to avoid killing the organisms. After all water cells are filled it is best to stop the water flow into the system for several minutes for more complete cleaning. It is suggested that the system be allowed to run

for 1–2 hours (with the water diverted from the holding tanks) and that sodium thiosulfate be added sufficient to neutralize any remaining chlorine. If the water is sufficiently hard, a calcium precipitate may coat the glass in the delivery system, in which case the above procedure with a 10% nitric acid solution is recommended. The acid or chlorine appears to have no effect on the silicone rubber glue used in assembly of the system.

ADAPTATIONS

The water delivery system is an extremely versatile device. If more than six water cells are desired the system could be constructed with additional cells by an appropriate increase in width. In addition, any number of cells, other than the one operating the water valve and valve venturi, can be removed from operation by clamping the appropriate vacuum line(s) (Va-1 to 5). More than one water cell may also be used to deliver water to an individual holding tank. If it is desired to deliver different volumes of water at the same time, the length of the extensions of the water siphons (WS) into the individual water cells can be adjusted to provide more or less than 500 ml.

For applications that would require greater maximum flow rates than that of the system described (approximately 250 ml/min), an increase in the depth of the system from 2 to 4 inches would double the potential flow rate. If this is done water delivery tubes of increased size are recommended.

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APPENDIX 3

BIOMONITORING INDUSTRIAL EFFLUENTS

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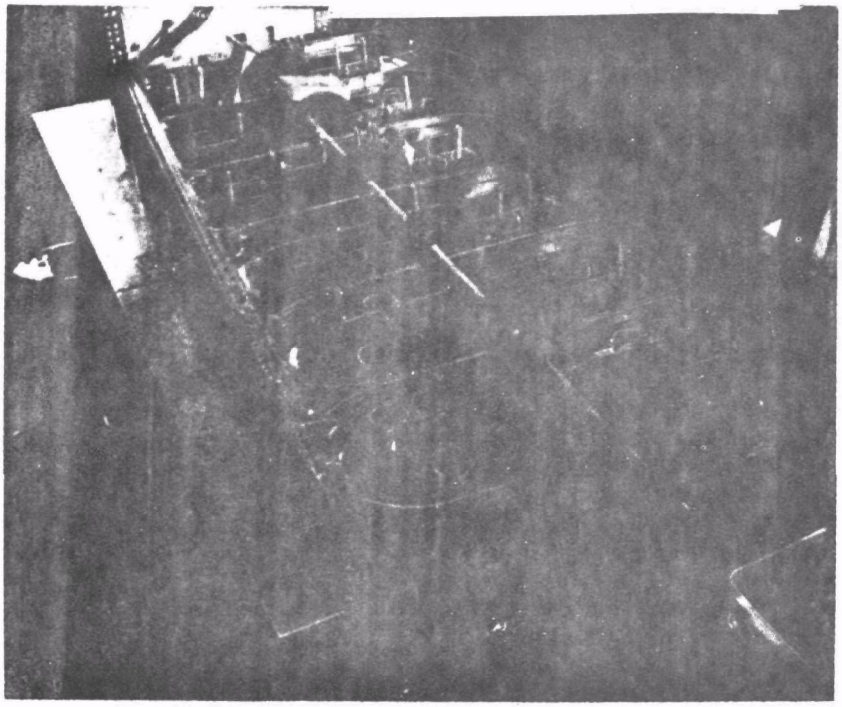
BIOMONITORING INDUSTRIAL EFFLUENTS

by Herbert W. Jackson and William A. Brungs, Jr.

The Toxicity of Industrial Effluents Can Be Evaluated By Continuously Flowing Samples Through Test Aquariums.



Apparatus used in studying effects of toxicants in water on aquatic life. In the study shown, paddlewheels in the exposure chambers circulate water over fish eggs. Toxicant is added by means of a serial dilution apparatus.

Plant operating personnel need to know the general quality of an effluent being discharged at a fairly constant rate. They also must be warned if a slug of toxic material is released to the receiving water. For example, many of the fish kills resulting from the release of slugs of highly toxic substances could have been prevented had these slugs been detected before the effluent left the plant.

Conventional bioassay procedures can evaluate only single samples taken at particular times. Continuous-flow bioassays of single grab samples over a long period of time can be very useful, but do not solve the problem of transient variations. A technique that does permit exercising continuous surveillance over the toxicity of an effluent is biomonitoring, a concept similar to the one advanced by Henderson and Pickering[1] for water supplies.

The Concept

Some progressive plants have met this need to determine effluent quality by installing aquariums in which fish are exposed to the plant effluent on a continuous flow-through basis. A "satisfactory" effluent quality is determined by the survival of the fish. Any deleterious change or effect is evidenced either by the death of the fish or a change in their behavior. This is biomonitoring.

Conventional bioassays[2,3,4] can provide important information about the actual toxicity of batches of the effluent in terms of TL_m's (that concentration which will kill half of the test animals in some stipulated period of time) and, if sufficient samples are tested, about the range of variation. This is a relatively slow process and would be prohibitive on an hourly or even a shift basis. Bioassays should be run from time to time to ascertain the exact toxicity of a waste even though it is being monitored as outlined below. Such tests also provide essential guidance in setting up appropriate dilutions for continuous monitoring.

The following procedures refer only to toxic wastes having a relatively rapid action. Wastes such as cadmium which have long delayed cumulative effects at low concentrations[5], oxygen-demanding wastes, radioactive wastes, and others would either be inappropriate or would not elicit a recognizable reaction soon enough to be of use in the following context.

While the procedures described here

are for use with a final effluent, they might also be applied to process wastes within the plant.

Objectives of Biomonitoring

Three basic objectives of biomonitoring are:

(A) To demonstrate the continuous suitability of an effluent for aquatic life provided slow-acting or cumulative toxins are not involved;

(B) To detect change (usually deleterious) in the biological acceptability of the effluent itself:

(C) To detect change in the effect of the effluent on the biota of the receiving water.

The continuous testing of an undiluted effluent (Objective A) is usually accomplished by leading a small stream of the effluent through an aquarium. This aquarium may be located in a public lobby to enhance public relations, or it may be in the plant for operational use only. This is a relatively simple and direct approach and needs no elaboration.

Objectives B and C are intrinsically more difficult to accomplish. By definition it is assumed that wastes requiring biomonitoring may exhibit acute toxicity; hence to achieve Objectives B and C, the effluent will probably require some dilution to support aquatic life.

Equipment and Flow Plans

A single basic design of exposure tanks and flow plan can be used to accomplish Objective B or C. With the exception of a simple suggestion for proportioning flow of effluent to dilution water, engineering devices for accomplishing the various needs outlined are not discussed. (See references 6, 7, and 8.)

Dimensions and arrangement should be adapted to local circumstances. Special care should be used to ensure that all surfaces that come in contact with the waste or the dilution water are constructed of nontoxic and noncorrosive materials. This precaution is particularly necessary for marine waters, where bimetalic contacts are very dangerous. An experienced aquatic biologist should be consulted in the preparation of plans. Settling in the tanks will be minimized if fish are used as the test organisms since their movements will keep tank contents well mixed except for heavy solids.

Exposure tanks (Figure 1) should be large enough (10 to 20 gallons) that the test organisms can live normally under plant conditions[8]. The larger sizes are more stable, but also require

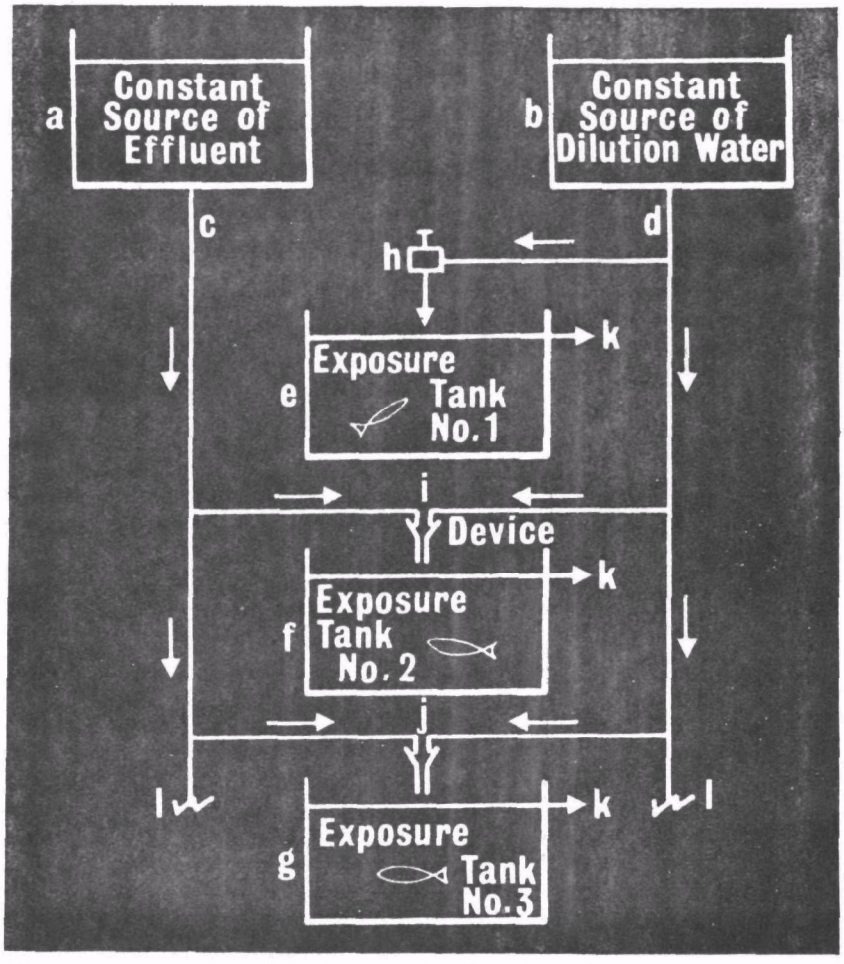


Figure 1 — Schematic flow plan for objectives B or C.

larger supplies of effluent and dilution water. Simple construction will facilitate feeding, cleaning, and disease control. "Eye-appeal" is not necessary unless public relations are involved, but scrupulous sanitation is essential as in all long-term animal culture[9,10]. Tanks should be situated in a lighted and well-ventilated room, but not exposed to direct sunlight. Ambient room temperatures are generally satisfactory, but should not be permitted to go above or below limits based on local biological experience.

Inlet and overflow should be similar in all tanks so that conditions will be the same except for the quality of the water. The total flow of water through the tank should be adjusted so that the hydraulic retention times are equal, whether the total flow is coming from one source or two. There are no standards for ideal hydraulic detention time, but generally it is advisable to exchange the volume of each tank at least once each shift, and preferably more often.

Tank No. 1 (Figure 1) contains only unadulterated dilution water to establish that the test animals will live in it. This tank is the control or "reference" to which the other tanks are compared. Tank 2 and 3 (more may be added at point 1) contain mixtures of effluent and dilution water. If the experimental animals die or show distress in these tanks, a change for the worse in the characteristics of the effluent being monitored is indicated.

Note that Tanks 2 and 3 are connected to both the effluent supply line c and to d, the dilution water supply line; i and j are mixing or proportioning devices set to predetermined amounts. In contrast, Tank 1 is connected only to line d (the dilution water); h is an adjustment valve or device to regulate the flow. All tanks overflow to the sewer through opening k, which should be screened to prevent escape of test animals and clogging in outlet pipe.

Effluent Supply and Dilution

The supply of effluent should be constant and controllable. The prime requisite is that it be fresh so that changes may be detected at the earliest possible moment. A constant-head

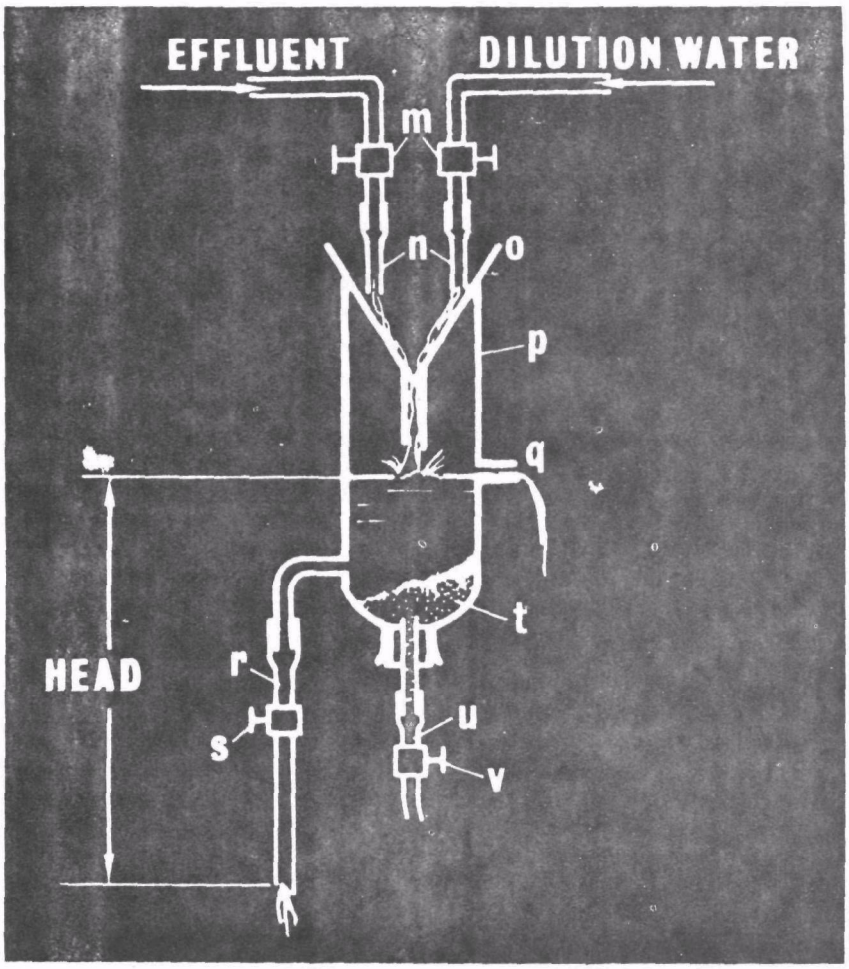


Figure 2 — Suggested plan for proportioning flow between effluent and dilution water. (See i and j Figure 1.)

reservoir is suggested as a relatively simple device to achieve this, or a simple tap on a waste line under pressure might be adequate.

The source and quality of the dilution water is the most important single factor in the system because this is the scale or standard by which the toxicity of the effluent is assayed.

Any available biologically acceptable water may be used to detect simple change in the toxicity of the effluent itself (Objective B). Biological acceptability in this case is determined simply by whether the test animals will live in it under plant conditions. Another consideration should be constancy of chemical and physical characteristics. Consideration might be given, under certain circumstances, to a standardized water prepared in batches.

To test the effect of the effluent on the receiving water (Objective C), the dilution water obviously must be the receiving water. In most cases the water varies from time to time as run-off water washes in different materials from the surface of the land, or other plants release various wastes. Plan C (Objective C) automatically evaluates the toxicity of the effluent when discharged into this changing situation. Thus it is important that Plan C dilution water should be obtained in such a way that none of the waste to be tested has been swirled to the point of removal by an eddy or backwater. This water should contain all of the components present down to the outfall being monitored, but none of the effluent itself.

In a stream situation, dilution water can be taken well upstream from the waste discharge. A pipeline with a continuous flow is ideal, since slugs of material from upstream sources that might modify the toxicity of the effluent being monitored would be taken into the monitoring system and quickly distributed to the exposure tanks. Because such systems are notoriously subject to "problems," batch transportation of control water may have to be employed. In this case, the intervals between refills should be as short as possible to ensure that the water in the monitoring system represents that in the stream as closely as possible. In lakes, estuarine, or coastal situations, batch supply may be the only practical solution.

If the receiving water is already continuously toxic to aquatic life, it is unsuitable for use in the system. Under such circumstances Plan C would be unworkable and the only recourse would be Plan A or B. Treated water such as drinking water from a city or industrial plant should never be used for any of these plans, even if dechlorinated, because the chemicals used in treatment may react with the waste being monitored. It is not unusual for even dechlorinated treated water to be actually toxic to the experimental animals.

Proportioning Flow

The plan (Figure 2) by which effluent and dilution water are proportioned to the test tanks (Tanks 2 and 3) determines what the system will accomplish. A suggested plan for proportioning flow between effluent and dilution water (i and j) in Figure 1 is given in Figure 2. The control valves (m) are shown as if on a rigid pipe to contain heavy pressure. If pressure is low as from a nearby low-head reservoir, laboratory-type pinch clamps on rubber tubes (n) could serve the same purpose. In case of clogging jet nozzles may give less trouble than valves. Sedimentation chambers ahead of the valves (m) might also be useful.

Rubber tubes (n) can be momentarily diverted to catch flow in an appropriate sampling device such as a graduated cylinder. Based on the time required to discharge some standard volume, flow could be proportioned to any desired ratio (for example, one part effluent to two parts dilution water).

After the effluent and dilution water are mixed in and pass through the funnel (o), the mixture enters an inverted polyethylene bottle (p) with the bottom removed. This bottle is equipped with an outlet (q) to discharge excess water to waste. This device maintains a constant head discharge to the tank through tube r, final control is by a valve (s). Design of the discharge mechanism should be identical for all tanks. If it is necessary to lead the final discharge to tanks in varying positions, a siphon-breaking device should be included at the end of

tube r to avoid modifying flows.

The still well (t) to collect precipitates is optional. Material that would settle out here would be kept from settling out in the tanks. The sediment can be periodically removed through drain u by releasing valve v. The removal of such material, as well as the use of a similar trap in the effluent line ahead of valve m (where indicated by suspended solids in the effluent), is a policy matter that should be resolved in the project-design statement.

Flow Plan for Objective B

If the system is to be operated to detect only serious detrimental change in the effluent itself, Tanks 2 and 3 should contain mixtures in such proportions of effluent and dilution water as to permit the test animals to live as long as the effluent is normal.

One possible combination would be to adjust the mixing mechanism at i (Figure 1) to admit such a proportion of effluent that the test animals in Tank No. 1 could barely survive. The slightest increase in toxicity of the effluent would then immediately be made evident by the death of the test animals and appropriate remedial action could be taken. The mixing mechanism j in Tank No. 3 might be adjusted to provide a greater margin of safety, for example 1/2 or 1/10th the toxicity of Tank 2. If Tank 2 animals then died, but Tank 3 animals survived, it would presumably indicate only a moderate increase in toxicity.

Flow Plan for Objective C

Monitoring the effect of the effluent on the receiving water, from the point of view of protecting aquatic life, is ideal, but may also be very difficult. The reference tank (e, Figure 1) receives water fresh from the receiving body. This water is free of any trace of the effluent being monitored, but contains all substances, natural and artificial, presently in the receiving water. These components may change from time to time, and one of these changes may increase the toxic effect of the effluent (synergism).

Thus the death of animals in the strongest test tank, but not in the reference tank, may be the result of an increase in the toxicity of the plant waste or of a synergistic reaction of the effluent with a material in the receiving water. No matter what the cause, the death itself serves as a warning and immediate action can be taken on the discharge to protect aquatic life.

On the other hand, if a slug of strongly toxic matter enters the receiving water from some outside source, the deaths of the animals in the reference Tank 1 (and probably also those in exposure Tanks 2 and 3) would show that the "fish kill" presumably in progress in nature was not the result of the effluent. A parallel installation under Plan B might demonstrate no change in the waste being monitored.

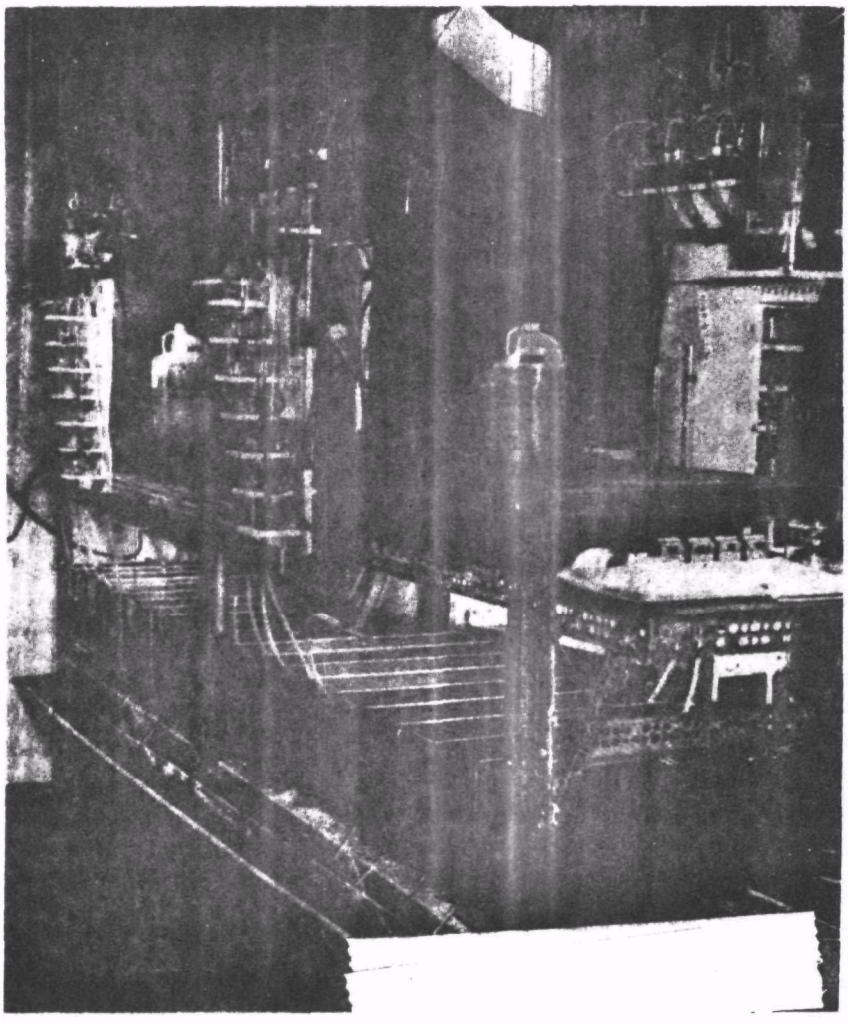
Dilution Systems

Various dilution systems might be employed under Plan C. One possible system is to simply test two or more constant proportions of effluent against the (changeable) receiving water, and then to observe the actual portions of waste entering the receiving water. If the highest known ratio of flow of effluent to stream is, for example, 1 to 10, and the strongest concentration in the monitor system is 1 to 10, then as long as the fish in the 1-to-10 mixture live, no damage would be expected in the stream especially at high flows.

If the stream flow should fall below its base level, or the waste flow increase, damage might be expected, and preparations could be made for remedial action. If the above conditions were constant, and dead fish began to appear in the tank with the highest concentration of effluent (No. 2, for example), this might indicate a rise in the toxicity of the plant effluent itself.

A different approach is to proportion the mixure to stimulate the actual mixture taking place in the receiving water. For this purpose if the location were on a flowing stream, the flow of both the stream and final effluent must be known. Periodic adjustments might be made by hand, or by automatic equipment involving telemetry of both effluent and stream flow.

Serial-dilution apparatus used for continuous delivery of various concentrations of materials in water.



Test Animals

No universal recommendations can be made about test animals to be used although many suggestions are available[1,2,4]. Irwin[11] investigated the suitability of 57 species of freshwater fishes for this purpose. Briefly, they should be of local importance, they must be a type that can be maintained in good health in the laboratory in the dilution water to be used, and enough must be employed so that reasonable statistical reliability is assured (for example, 10 per tank).

Fish are usually employed as test animals, although there is no reason not to use any other organisms that can be successfully kept alive in the test tanks[12]. A prime consideration for public relations purposes might be the local importance of the organisms selected, for example, oysters, shrimp, or mummichogs in coastal locations, or young bluegills, trout, or minnows in inland areas. The number and size of test animals used may have to be determined by experience. Such factors as temperature and oxygen content of the tanks will affect the number of experimental animals that can be maintained.

Continuous availability is also important. It is always wise not to change the test species after a program has been established, since the reactions of different species to the waste being monitored may not be the same[11]. Whatever species is selected, it should be one that is either available on a year-round basis, or one that can be stockpiled at times of abundance and successfully kept until needed.

Animals in the exposure tanks should be fed the same as those in the stock tanks. The same kind of food in the same ratio of food to weight of test fish should be added to each tank at the same intervals. Unnatural acceptance of food in exposure tank may indicate a measure of distress, even in the absence of mortality.

Special Considerations

Oxygen determinations should be run occasionally to ensure that any deleterious symptoms are the result of the effluent and not of oxygen deficiency. Total oxygen demand by fishes is more nearly a function of the total weight of fish than the number. Two or three 6-inch fishes might demand as much or more oxygen than a dozen or more 2-inch fishes.

Actual minimum acceptable levels will also depend on the temperature and type of fish used: Carp or *Tilapia* might endure a minimum of 2 ppm of oxygen at 90°F, where trout or salmon would require at least 5 ppm at 60°F.

Long-continued exposure to low level concentrations in tanks may result in cumulative intoxication or acclimatization. In most cases these effects can probably be best counteracted by periodic renewal of the test fishes, for example: At 60-day intervals (as it is reported[13] that at 60 days either acclimatization or increased sensitivity may modify toxicity).

When obtaining stocks of test animals from receiving waters, these same factors should be borne in mind. Fish or other organisms taken from below the outfall might have acquired some immunity or sensitivity to the effluent being tested. Those taken from well above the outfall probably have not, unless they have recently migrated upstream.

Fish of a species normally present in the receiving stream, but imported from some other (unpolluted) source, will presumably exhibit a completely unconditioned response. It is also possible that the effluent being monitored is one to which acclimatization is so slow or slight as to be negligible under the conditions of this test.

Generally, since the aquatic life to be protected is that already present in the stream, the most logical source of fish is the stream itself. Under operating conditions, however, it is not always practicable to collect the experimental animals from this source and imports from another area may be necessary.

Selection of Dilutions

The "critical range" of toxicity may be defined as the range between the highest concentration that kills no test animals and the lowest concentration that kills all. The TL_m of the conventional bioassay[3] is in the middle portion of this range. In general, as a test is prolonged, the critical range is narrowed until a level of relative stability is reached. The slope and magnitude of the curve thus represented are functions of the toxicant, species used, and environmental conditions.

When an effluent is to be biomonitored under Plan B, the selection of appropriate dilutions might be based on the above concept of "critical range." If a "tight" control is desired, the highest concentration might be established near the TL_m. When a batch of test animals is first placed in such a dilution, approximately half of them may (by design) be expected to die. The survivors, however, would constitute a rigorous control as *any* increase in toxicity would be expected to kill the remaining animals in order of susceptibility until, as the top of the critical range is reached, all would be dead.

A somewhat less stringent control would be effected with a dilution near the lower end of the critical range. It is not generally practicable to determine the lower end of the critical range precisely, but a reasonable estimate can often be made. If the critical range in question happens to be relatively wide, a moderate increase in effluent toxicity would kill only some of the test animals. If the critical range is very narrow, there is little choice between a dilution set at the lower end and one at the TL_m.

In any large population of test animals kept in an exposure tank over an extended period of time, an occasional animal may be expected to die. The mortality of significance then is not the occasional individual death, but the sudden death of 25%, 50%, or 100% of the test animals. When this happens, biomonitoring has sounded the alarm to take appropriate action to detoxify the effluent or to divert it from the receiving stream until it is again normal. ■

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APPENDIX 4

CONTINUOUS-FLOW FISH BIOASSAY APPARATUS FOR MUNICIPAL AND INDUSTRIAL EFFLUENTS

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February 1971

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SERL Report No. 71-3

CONTINUOUS-FLOW FISH BIOASSAY APPARATUS FOR MUNICIPAL AND INDUSTRIAL EFFLUENTS SERL REPORT NO. 71-3

Appendix A consists of SERL Report No. 71-3 published in February, 1971. This report describes the development, construction and operation of the continuous-flow fish bioassay apparatus used throughout this study.

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I. INTRODUCTION

There are several advantages to continuous-flow bioassay techniques for evaluating toxicity of municipal and industrial effluents over the traditional "batch" procedures as outlined in the Twelfth Edition of Standard Methods [1]. In 1965 Mount and Warner [2] described a serial dilution apparatus for continuous-flow assays. Subsequently, Mount and Brungs [3] described a proportional diluting device providing a repeated dosing to assay vessels, which approximates continuous flow. Both devices were intended for "clean water" systems dosed with small portions of highly toxic substances. They were not intended to dilute partially-treated wastewaters of relatively low toxicity. The design of a continuous-flow fish bioassay apparatus applicable to treated or untreated domestic or industrial wastes was the objective of the work described in this report.

Standard Agreement S-1956 between the California State Department of Fish and Game and The Regents of the University of California charged the University's Sanitary Engineering Research Laboratory with cooperating with the State in the design of a continuous-flow fish bioassay apparatus. In fulfillment of this agreement SERL was to:

a. Prepare a list of design criteria which the bioassay apparatus must meet to satisfy its needs in evaluating advanced waste treatment.

b. Meet with the State Departments of Fish and Game (DFG) and Water Resources (DWR) to select the criteria which the apparatus must meet for the needs of the State.

c. Prepare designs of alternative assay systems which will meet the selected criteria.

d. Meet with the Departments of Fish and Game and Water Resources representatives to select the most suitable design.

e. Assist in the construction and evaluation of a prototype of the selected design.

Items a, b, and c were covered at a meeting held at Nimbus, California on April 16, 1970. It was decided at that meeting that Item c could not be performed efficiently without first obtaining some operating experience with preliminary designs. Thus, Items c and e were treated as one task which had to be accomplished before proposing the final design of the bioassay apparatus. This report is concerned primarily with the design selected and placed into use by SERL and DFG during subsequent studies of the San Francisco Bay-Delta system.

Criteria for the design of the continuous or pulsating flow bioassay apparatus included:

a. The apparatus must accurately and reliably deliver preset dilutions of wastewater to assay vessels at a desired rate of flow.

b. It should be sufficiently reliable to operate satisfactorily for 24 hours unattended and up to 120 hours without shutdown.

c. The apparatus should be of lightweight modular construction for ease of portability.

d. Construction should be of nontoxic materials which are resistant to corrosion and capable of being easily cleaned mechanically or with chemical cleaning agents.

e. The apparatus should be capable of diluting primary domestic wastewater effluents as well as wastewaters subjected to more extensive treatment processes.

f. It should be flexible enough to provide a range of dilutions from 100% wastewater to less than 10%.

g. It should be possible to control the assay vessel liquid detention time at six hours or less and down to two hours if desired.

II. THE DILUTING APPARATUS

The diluting apparatus which was determined to give the best performance according to the design criteria is shown in detail on Figure A-1. The working principle of this device is similar to that described by Mount and Brungs [3] in that a pulsating flow from individual proportioning chambers is used to feed the bioassay vessels. Figure A-2 is a photograph of the assembled bioassay apparatus in operation.

The Mount and Brungs apparatus has only clean water introduced which is hydraulically controlled and shut off when siphons are flowing. A portion of the flow has toxicant added, then is rediluted with the remaining water. The design described in this report accommodates separate wastewater and dilution water feed with electrical control. Continuous flow of wastewater in this diluter prevents occurrence of suspended solids problems in a shut-off valve. Fail-safe features prevent operation in case of dilution or wastewater feed failure. The electrical control system adds flexibility and reliability of operation, especially in assay vessel detention time establishment.

OPERATING PRINCIPLE

The diluter (Figure A-1) consists of two proportioning boxes, one for dilution water and one for wastewater. Five proportioning chambers within each box operate on the fill-and-draw principle, each discharging a preset volume once per diluter cycle. To achieve a diluted wastewater flow to an assay vessel the discharge of two proportioning chambers, one dilution water and one wastewater, is combined. Four diluted wastewater streams flow from four pairs of chambers. The single remaining chamber in each box discharges directly to an assay vessel, resulting in one vessel receiving 100% wastewater and one vessel receiving only dilution water which serves as a control during the bioassay test. These are assay vessels one and six, receiving discharges from W1 and D6 proportioning chambers, respectively.

The wastewater concentration in the flow to vessels two, three, four, and five is determined by the "working volumes" of the pairs of dilution chambers discharging to each one. The working volume is established by the chamber dimensions and its siphon depth (see later section entitled PROPORTIONING BOXES). The flow rates (and assay vessel detention times) are fixed by the dilution chamber working volumes and diluter cycle frequency.

A dosing sequence is initiated when all proportioning chambers in both proportioning boxes are filled. They are filled sequentially by overflow from the previous (uphill) chamber with the waste or dilution water introduced to one end of the proportioning box. When all chambers

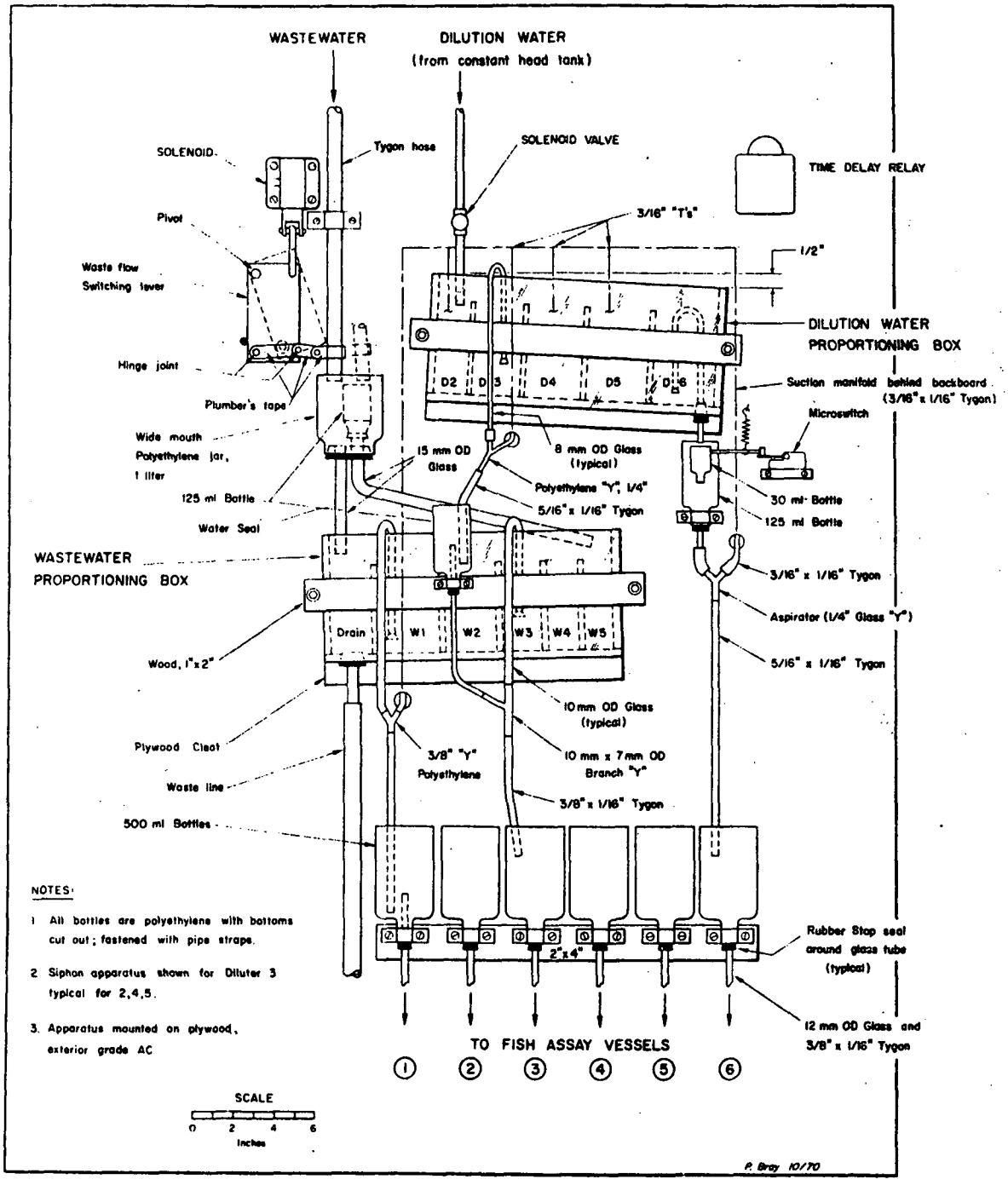


FIGURE A-1. PROPORTIONAL DILUTER

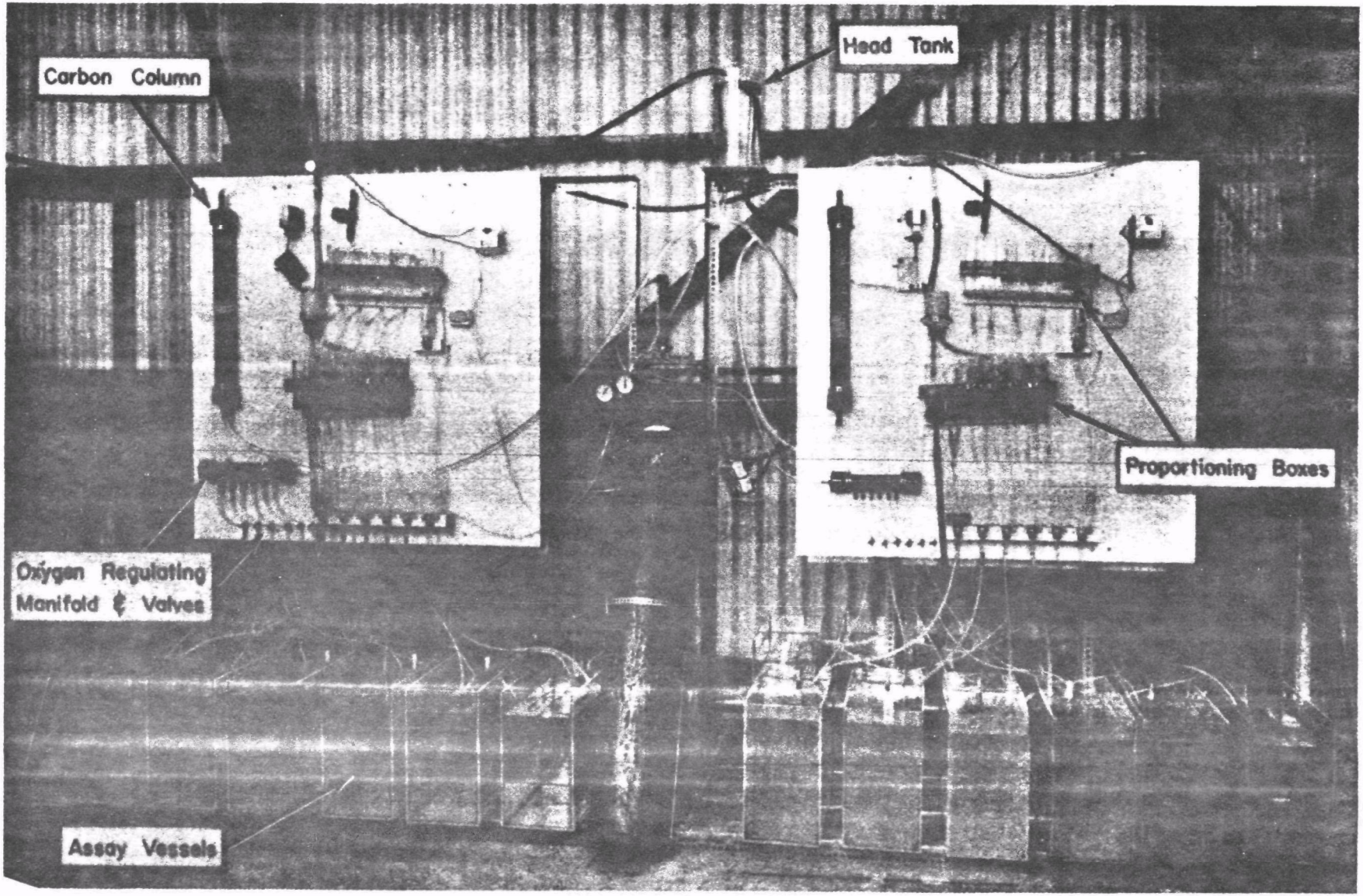


FIGURE A-2. FOUR CONTINUOUS FLOW FISH BIOASSAY UNITS IN OPERATION AT SERL

of the wastewater proportioning box are full, W1 overflows to waste. When all chambers of the dilution water proportioning box have been filled, the siphon tube in D6 begins to operate. The water flowing from the D6 siphon fills the inverted 30-mℓ plastic bottle, depressing the micro switch lever which shuts off both wastewater and dilution water flows (see section ELECTRICAL CONTROL). The overflow and draining flow from the inverted 30-mℓ bottle (the bottom is cut out and the neck plugged except for a small drain hole) is captured in the 125-mℓ bottle and discharged through the aspirator and on to fish assay vessel number six.

The flow through the aspirator creates a suction on the manifold running above the dilution water proportioning box water level (and behind the backboard). The manifold is connected by Y's to the siphons from W1 and D2 through D5 proportioning chambers. The siphon for each of these chambers is necessarily submerged and the discharge tube from each Y is led to a water seal such that a vacuum can be drawn and thus initiate the attached siphons (D2 through D5 and W1). The water seals provided consist of inverted 125-mℓ polyethylene bottles with the bottoms cut out. The outlet tubes (glass tubing) extend up into the bottle through rubber stoppers thus providing a small reservoir to act as a water seal (or trap) for the siphon discharge tubes above.

Overflow from the water seals serving the D2 through D5 siphons passes through a second set of Y's which act as aspirators to start the siphons from W2 through W5. Five hundred-mℓ polyethylene bottles with bottoms removed act as funnels and mixing vessels (and a water seal for W1) for the flow passing to the six assay vessels.

All of the proportioning box sections are drained to the depth of the siphons during each cycle. Then each is refilled with wastewater or water for the next cycle, this being initiated by a return of the microswitch to the closed position.

WASTEWATER AND DILUTION WATER DELIVERY

Wastewater is delivered continuously to the dilution apparatus where it is used to fill the wastewater proportioning box chambers or is bypassed to waste, according to the position of the solenoid. When actuated, the solenoid pulls the waste flow switching lever to direct the waste flow to the wastewater proportioning box (cf. Figure A-1). When deenergized the solenoid drops the lever by gravity (or spring return if gravity return is not sufficiently fast) to allow the wastewater to bypass to the drain portion of the wastewater proportioning box. The bypassed flow, as well as overflow from W1, may pass to waste or be recycled to a holding tank if the wastewater to be bioassayed is hauled in and in short supply.

Dilution water may be obtained from various sources, according to the objectives of the assay. When a noncorrosive, suspended solids free water is used, a shut-off type of solenoid valve of corrosion-resistant and nontoxic materials can be used to control the flow, as is shown in Figure A-1. The flow should come from a constant-head tank to minimize pressure fluctuation. If more corrosive (e.g., saline) water is to be used for the bioassay, a bypass system for dilution water similar to that shown for wastewater in Figure A-1 can be used. A head tank would be less necessary for a bypass system and water conservation could be practiced by returning bypassed (and head tank overflow) water to a holding vessel. This would be desirable in instances where dilution water is in short supply or must be transported a considerable distance.

ELECTRICAL CONTROL

The control of wastewater and dilution water flows to the respective proportioning boxes is accomplished by the two solenoids shown in Figure A-1. Figure A-3 is a more complete schematic of the electrical control system, showing the specific components used in the prototype diluter constructed at SERL. Components include two solenoids for wastewater and dilution water control, a microswitch, and a time delay relay.

The electrical cycle is initiated when power is turned on or the microswitch returns to its normally closed position at the end of a dilution cycle. This provides power, through the normally closed (NC) contacts, to the time delay relay. The microswitch achieves its NC state when the 30-mℓ bottle in the D6 discharge empties (i.e., D6 siphon ceases to flow). A small spring is necessary for adjustment of the critical balance of the microswitch so it moves to the depressed condition when D6 siphon is flowing and returns to normal after flow ceases and the bottle drains.

After the time delay relay (TDR) is energized, a time delay period is initiated. After a preset time the normally open contact is closed which actuates the solenoids, allowing liquid flow to the proportioning boxes. The TDR used for the SERL prototypes is shown in Figure A-3, along with two alternates. Solenoid and solenoid valve models indicated are illustrations of workable alternates. Other alternates are equally acceptable for these applications as long as the time delay for closing the circuit to the solenoids is adjustable.

After the proportioning boxes are filled, the D6 siphon begins to flow, filling the 30-mℓ bottle which breaks the microswitch contact. This breaks the circuit to the TDR and the solenoids, shutting off flow to the proportioning boxes. The TDR automatically resets so that after D6 empties the next cycle begins.

The setting on the TDR establishes the desired hydraulic detention time in the assay vessels (time required to fill the empty vessel to its overflow point). Knowing the vessel volume and volume of discharge to each vessel per diluter cycle (500 mℓ for this system),

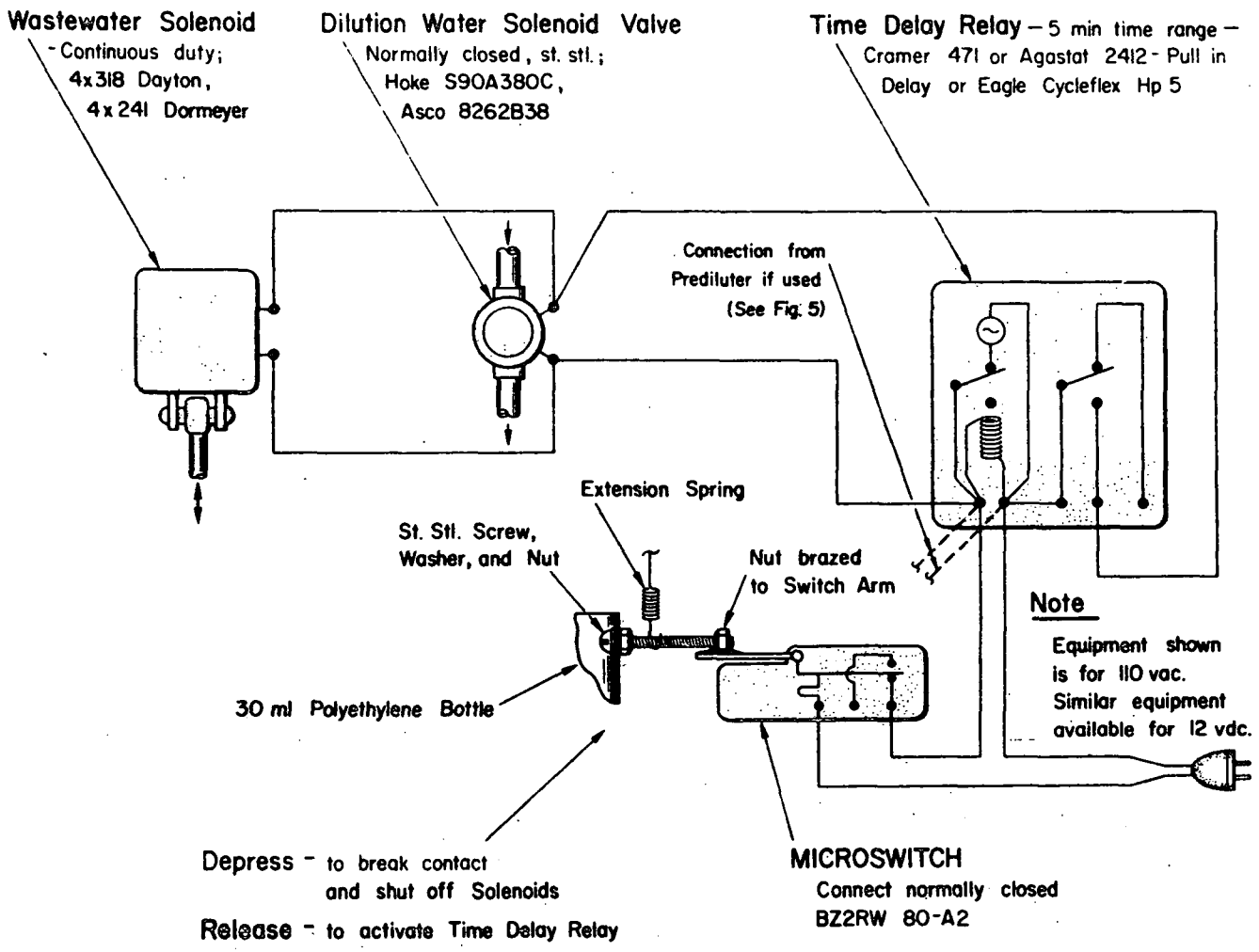


FIGURE A-3. ELECTRICAL DIAGRAM

the frequency of diluter cycling can be computed. Then the fill and discharge time for the diluter proportioning boxes (the time from solenoid actuation to microswitch return to normal after the dilution chambers empty) is determined. The TDR is set to delay the difference between the desired cycle time and fill-empty time. As as example:

Fish assay vessel volume, 30 ℓ
Volume delivered per diluter cycle, 500 mℓ
Desired vessel detention time, 6 hr
Therefore:

$$\frac{30\ \ell}{0.5\ \ell} = 60 \text{ cycles per detention time}$$

$$\frac{6 \text{ hr} \times 60 \text{ min/hr}}{60 \text{ cycles/detention time}} = 6 \text{ min/cycle}$$

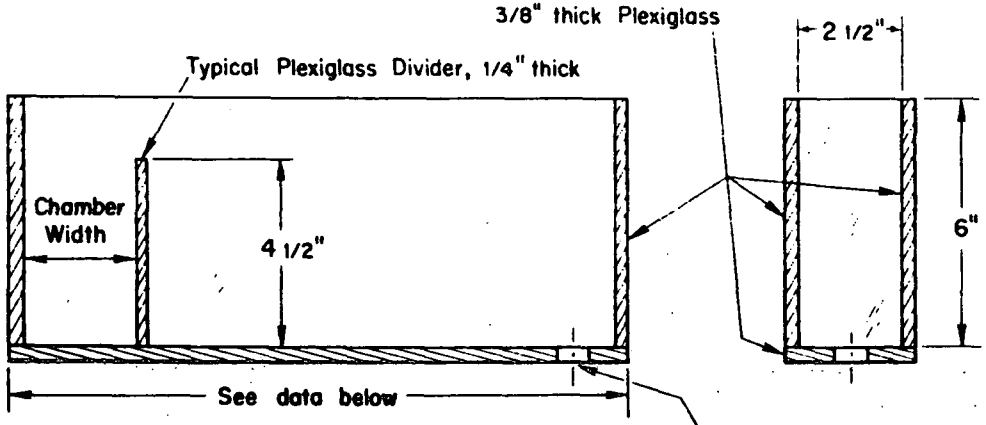
or 1 cycle of the diluter each 6 minutes. If the time (as measured with all systems in operation) for fill and discharge of the diluter is 3-1/2 minutes, the TDR must be set for 2-1/2 minutes to achieve the 6-min cycle time.

PROPORTIONING BOXES

The proportioning boxes for the wastewater and dilution water were designed to deliver dilutions ranging from 100, 50, 25, 12, and 6% wastewater to 100, 80, 64, 51, and 41% wastewater. This range of dilutions and other intermediate dilution ranges can be achieved by adjusting the depth of the siphon in each proportioning chamber. Total delivery (wastewater plus dilution water) to each assay vessel is designed to be 500 mℓ per cycle.

Details of construction of the plexiglass boxes are shown in Figure A-4. The boxes constructed at SERL had 3/8-in. outside walls and bottom with 1/4-in. interior dividers. The plexiglass pieces can be readily precut and assembled using acrylic solvent cement. Plexiglass can be cut with a table saw using a sharp, fine tooth blade. Solvent-cemented joints are most satisfactory when sawed edges are sanded smooth and polished surfaces are sanded lightly to give them texture prior to application of the solvent. The solvent application should be continuous until the plexiglass surface becomes gelatinous, then the pieces joined, aligned and held firmly in place for at least a one-hour curing time. Application of acrylic cement along the joint during curing will help prevent bubble formation. Leaks may be stopped using an acrylic cement which contains acrylic monomer.

A detail of the individual siphons is also shown in Figure A-4. Wood strips across the front of each proportioning box (see Figure A-1) serve the dual purpose of retaining the proportioning boxes on the mounting board and providing mounting strips for the siphons, which are held by short loops of Tygon tubing. The siphon depth into each



DILUTION WATER PROPORTIONING BOX

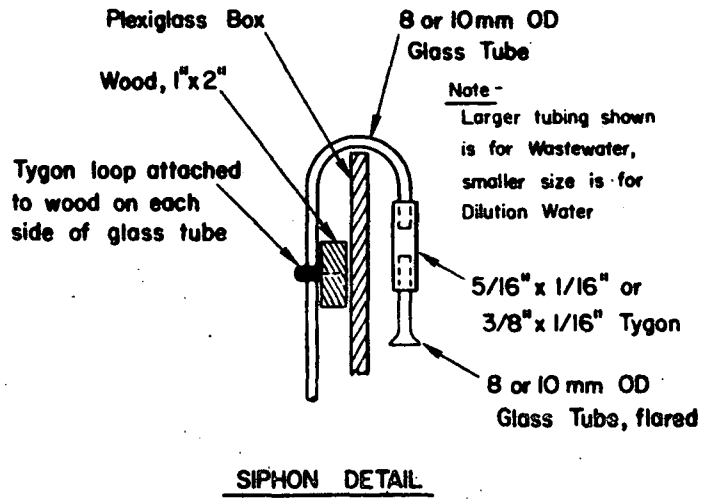
5 Chambers	Width, in.
D2	1 3/4
D3	2 3/8
D4	2 5/8
D5	2 7/8
D6	3 1/8

4 Dividers at 1/4" each,
2 ends at 3/8" each.
Total length = 14 1/2"

WASTEWATER PROPORTIONING BOX

6 Chambers	Width, in.
Drain	1 3/4
W1	3
W2	2 1/2
W3	2
W4	1 3/4
W5	1 1/2

5 Dividers at 1/4" each,
2 ends at 3/8" each.
Total length = 14 1/2"



SIPHON DETAIL

FIGURE A-4. DILUTER PROPORTIONING BOXES AND SIPHON DETAIL

chamber can be adjusted with different lengths of Tygon tubing between the flared and bent glass tubes. Fine adjustment can be achieved by changing the depth of glass tube insertion into the Tygon tubing. The flared intake provides each siphon with a more reproducible end point to the siphoning action and thus more consistent volumes.

DILUTION ADJUSTMENT

Selection of a particular dilution sequence must be made for each waste bioassay. Municipal wastewaters following primary treatment may have a toxicity such that the median lethal concentration (LC-50, TL-50, or TLm) is in the range of 20 to 80%.* A dilution range including the final LC-50 must be chosen. Industrial wastes are much more variable in toxicity and a special knowledge of the application or a trial and error selection must be made in these applications. Treatment may of course alter the acute toxicity of wastewater effluents.

The established practice is to select a dilution series with logarithmically equivalent spacings between dilutions [1, 4]. Having selected the dilutions to be used, the volume required from each proportioning chamber (working volume) can be computed. Subsequently, knowing the volume per unit depth for each chamber, siphon depths are established. Dilution adjustment can be made with reasonable accuracy by measuring the depth of the siphon in each proportioning chamber. Table A-1 contains chamber volumes per unit depth for proportioning boxes constructed to the dimensions in Figure A-4. Percentage of waste with working volumes and siphon depths is shown for three logarithmic series of wastewater concentration. Actual dilutions achieved are computed from measurement of dilution water delivery and total delivery during operation.

FAIL-SAFE FEATURES

The diluter shown in Figure A-1 contains fail-safe features. If the dilution water supply is interrupted, the dilution water siphons and the wastewater siphons will not function. As neither wastewater nor dilution water will be delivered to the assay vessels, the bioassays will become "static" until the malfunction is detected and rectified.

A reduction in the rate of dilution water flow will result in extended fill time for the dilution water proportioning box. This will in turn result in a longer cycle time and increased detention periods in the assay vessels. However, the dilutions will remain constant.

If electrical failure occurs, neither dilution nor wastewater solenoids can be activated, therefore no water or wastewater is

*Values in this range have been experienced for continuous flow 96-hour bioassays of domestic wastewater utilizing the Golden Shiner. Tap water was used as dilution water.

TABLE A-1

DILUTER ADJUSTMENTS FOR THREE LOGARITHMIC CONCENTRATION SERIES

Assay Vessel	1	2		3		4		5		6 control
Proportioning Chamber	W1	D2	W2	D3	W3	D4	W4	D5	W5	D6
Chamber Volume, mℓ/cm depth	48.4	28.2	40.3	38.3	32.2	42.4	28.2	46.3	24.2	50.4
Percent Waste	100	50		25		12		6		0
Working Volume, mℓ	500	250	250	375	125	438	62	469	31	500
Siphon Depth, cm[a]	10.3	8.9	6.2	9.8	3.9	10.3	2.2	10.1	1.3	9.9
Percent Waste	100	67		45		30		20		0
Working Volume, mℓ	500	165	335	275	225	350	150	400	100	500
Siphon Depth, cm	10.3	5.9	8.1	7.2	7.0	8.3	5.3	8.6	4.1	9.9
Percent Waste	100	80		64		51		41		
Working Volume, mℓ	500	100	400	180	320	244	256	295	205	500
Siphon Depth, cm	10.3	3.6	9.9	4.7	10.0	5.8	9.1	6.4	8.5	9.9

[a]Siphon Depth = Distance from beginning to end water surface during a drain cycle of diluters. Approximately: depth to the bottom of tube below the top of the box dividers.

delivered. Once again the bioassays become "static" tests until the power is restored.

If the wastewater supply fails, the W1 proportioning chamber will not receive wastewater. Each time a siphon empties a dilution chamber, especially with the flared siphons as shown in Figure A-4, it draws the water surface to slightly below the end of the siphon. This creates an air break that stops the siphon from flowing. The air break in chamber W1, when the chamber is not refilled, prevents a vacuum from being drawn in the manifold connecting siphons W1, D2, D3, D4, and D5 to the aspirator. Therefore, with a wastewater supply failure, even though D6 fills and siphons, the aspirator fails to start the other siphons and all assay vessels become "static" units except vessel 6 (the control vessel) which has a slightly increased flow and decreased detention time.

A reduction in the wastewater flow rate, such that the wastewater proportioning chambers are not full when the dilution water proportioning box is full and ready to discharge, results in a reduced flow to vessel 1 if W1 receives sufficient liquid to close the air break. If the air break in W1 does not close, vessels 1 through 5 do not receive flow during that cycle. This latter will occur when W1 receives no waste by the time D6 fills and starts to siphon, shutting off the waste flow to the wastewater proportioning box. None of the siphons will start due to the air break in W1. The wastewater proportioning box will continue to fill during subsequent fill cycles of D6 until W1 receives wastewater and then all siphons operate.

One deviation from fail-safe operation should be noted. If the waste flow fails after only a small amount has entered the W1 proportioning chamber such that the water surface is still well below the top, the aspirator suction may not be sufficient to start the siphon and empty W1. This will allow all dilution water siphons to start on subsequent cycles although no wastewater is flowing. A more efficient aspirator may alleviate this problem.

PREDILTUION FOR MORE TOXIC WASTES

Wastewaters which exhibit greater toxicity than can be accurately determined with the proportional diluter described herein can be "prediluted" prior to testing. An apparatus for predilution on a continuous basis for use with the proportional diluter system is shown in Figure A-5. A pulsating flow principle with positive volumetric dilution and wastewater control as used here is similar to that employed for the individual dilutions in the proportional diluter. The predilution electrical control system is tied into the one for diluter control only for power. It is connected so the power is off and predilution flows do not operate while the diluter siphons are working. It may be connected to prevent flow to the prediluter while the diluter is filling or siphoning.

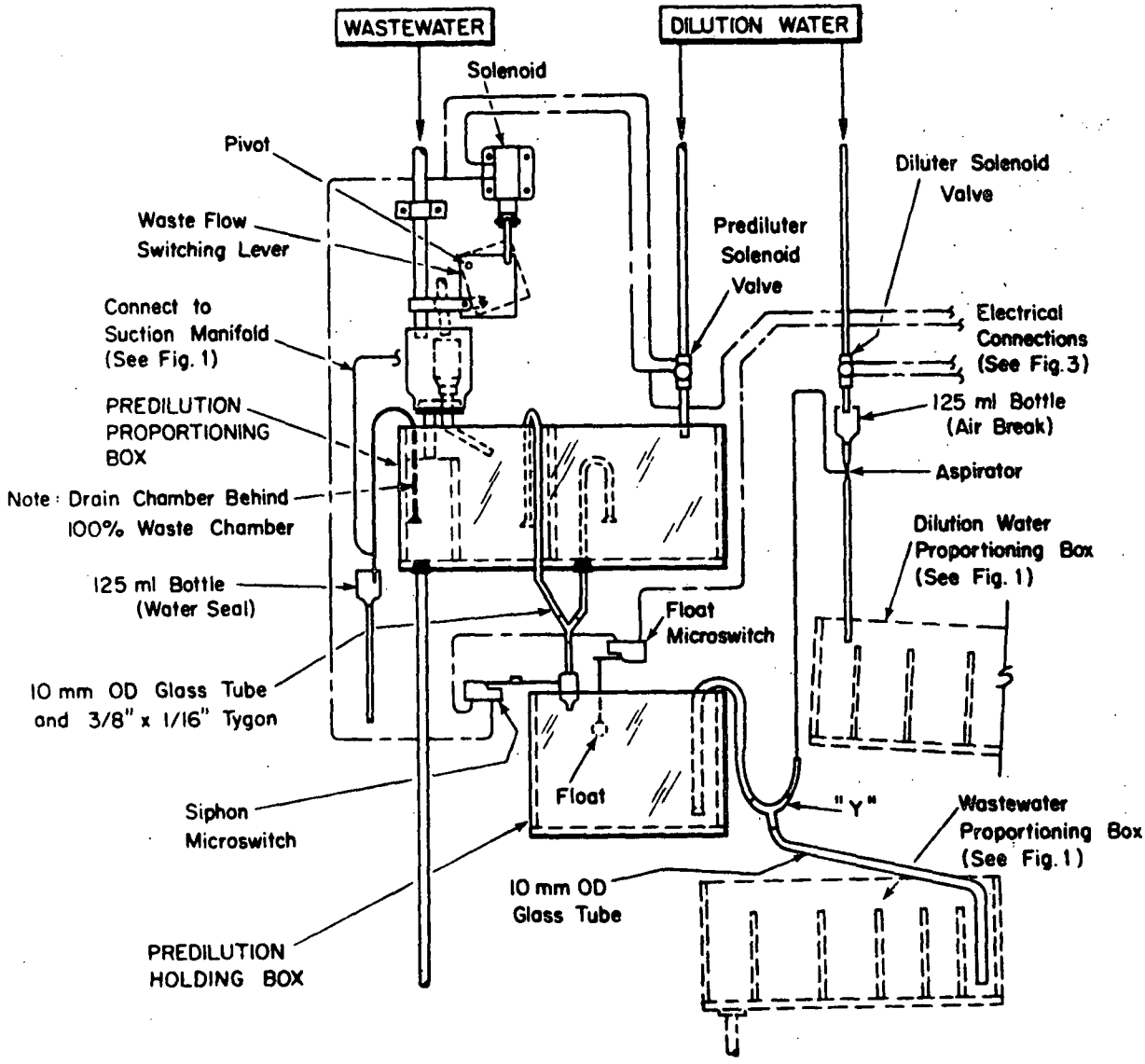


FIGURE A-5. PREDILUTION SYSTEM

In Figure 5 the two microswitches are wired in series using the normally closed contacts. When power is applied with all prediluter boxes empty (siphon microswitch up and float microswitch down normally), the solenoid valve for dilution water and solenoid for waste flow switching will be actuated. This will cause the predilution proportioning boxes to fill with wastewater and dilution water. The wastewater flow must be sufficient so its compartment fills first and overflows.

When the dilution water box fills to the top of its siphon, siphoning commences. The siphoning dilution water shuts off both solenoids by depressing the siphon microswitch, and starts the siphon from the wastewater box. When the predilution proportioning boxes empty the siphon microswitch reengages, but the float attached to the float microswitch is adjusted so it keeps the circuit broken until the predilution holding box is emptied.

The solenoid valve for dilution water delivery to the dilution water proportioning box (see Figure A-1) is connected to an aspirator for use with the predilution apparatus. The aspirator starts the siphon from the predilution holding box to the wastewater proportioning box when the normal diluter cycle starts filling the dilution water proportioning box (refer to section on WASTEWATER AND DILUTION WATER DELIVERY). The line from the predilution box siphon to the wastewater proportioning box must be extended down into chamber W5 below the siphon level from that chamber to ensure that a vacuum can be drawn by the aspirator to empty the predilution box.

The chamber dimensions and siphon depths in the predilution proportioning box determine the predilution achieved from this appurtenance to the basic diluter. About 2000 mℓ total delivery per cycle is needed to assure adequate flow to the wastewater proportioning box. A box 4 in. wide by 6 in. deep with 4-1/2-in. high wastewater overflow is satisfactory with 4-in. long wastewater chamber and 7-in. long dilution water chamber. This provides a predilution range of 10% to 50% wastewater. The predilution holding box must have an operating capacity equal to the total delivery per cycle. A box 4 in. wide by 8 in. long by 6 in. deep provides a 2-liter working capacity.

The fail-safe features of the basic diluter can be retained by providing a small chamber in the prediluter proportioning box between the wastewater and overflow chambers. (On Figure A-5 they are shown adjacent to each other perpendicular to the page.) This additional chamber contains a siphon to waste (through a water seal) and is started by a main diluter manifold connection (refer to Figure A-1). If waste flow should stop, the main diluter siphons would not start due to the air break in this redundant siphon.

OTHER DILUTER DESIGNS

In arriving at the present design as the most appropriate, several alternatives were considered. For example, proportioning with a battery of small pumps was discussed and rejected because of

the difficulty of incorporating fail-safe features, due to the problem of achieving constant flows with variable discharge heads and the relatively large initial expense which would be involved. There are undoubtedly applications where proportioning with timer-controlled pumps would be acceptable or possibly even preferable.

A design was constructed in which orifices discharged vertically upward from tubes leading from constant head tanks for the waste and dilution waters. The orifices could be adjusted to alter the head and the flows from a wastewater and a dilution water orifice combined for a particular dilution. Tests with the apparatus showed that the low flows required were extremely difficult to obtain consistently, that very minute head variations in the constant head tank changed the flows significantly, and that suspended solids accumulation in the aperture changed flows appreciably. In addition, the necessity for a head tank is undesirable for wastewater because of solids precipitation.

Another design of similar principle was also tested. This design incorporated flow tubes leading directly from the sides of constant head tanks for each waste and dilution water. The tubes had lips over which the liquid flowed in proportion to its height (adjustable) with respect to the tank liquid level. Once again solids accumulation at the discharge point, where they dried and decreased discharge with time, was the basis for rejection. Solids also accumulated in the constant head vessel and fail-safe features could not be incorporated. This design might be applicable for suspended solids free wastes as it is easily adjustable. Two constant head tanks in series would be desirable to control variations in discharge.

The design chosen was superior to all other designs considered especially in the consistency of delivery of the preset dilution volumes and the ease with which the delivery could be checked once it was established. Periodic visual inspection of a single diluter cycle ascertains whether it is (and has been) working correctly and if a full working volume is delivered from each proportioning chamber each cycle.

III. CONTINUOUS FLOW BIOASSAY APPARATUS

The complete apparatus for bioassay testing includes the diluter described above plus the assay vessels, a means for furnishing waste and dilution water, and other appurtenances. Figure A-2 shows 4 bioassay setups operating on 4 separate waste streams. The diluters are mounted on plywood (exterior grade, painted). A single diluter can be mounted on a 30-in. wide by 4-ft high plywood piece. A dilution apparatus including diluter and prediluter required a 3-ft wide by 5-1/2-ft high piece. Two slotted angle frames are supporting 2 diluters each, back-to-back. The 6 fish assay vessels for each diluter are located at ground level.

Cost of materials for the apparatus shown in Figure A-2 was about $800. Three to four man weeks were necessary for assembling the units. The assay vessels used by SERL are constructed of 1/4-in. plexiglass with "welded" corners and have PVC pipe tees for overflows. Figure A-6 shows the dimensions of these vessels. The lower end of the tee is screened (nylon screen) to prevent fish escape.

Since domestic wastewater with varying degrees of treatment may be assayed, supplemental oxygen is required to maintain adequate dissolved oxygen in each assay vessel. Cylinder oxygen has been used quite successfully and the apparatus for its administration can be seen in Figure A-2. The oxygen from the cylinder is delivered through a pressure regulating valve and a needle valve and metered through a rotameter to a manifold (lower left corners of each diluter). From the manifold, oxygen is regulated to each individual assay vessel through aquarium air valves, and released at the bottom of the vessels through air stones. Aeration with pure oxygen reduces the gas flow necessary to maintain a given DO and thereby reduces the likelihood of stripping volatiles from solution in the assay vessel.

For the bioassay tests in progress and shown in Figure A-2, tap water was used as dilution water. The tap water was passed through a column of activated carbon to assure that no chlorine was present. The columns containing the carbon can be seen fastened vertically on the left side of the diluters.

OPERATING PROCEDURE

Bioassays run by SERL with the diluters, in connection with the 1970 Bay-Delta project, used wastewater pumped directly from the effluent stream of various treatment processes. The desired dilution range was selected (no prediluters have been placed in operation to date) and the siphon depths set to the nearest 0.1 cm measured from the top of the dilution box dividers. A total delivery per cycle of 500 mℓ was used and a total cycle time of 6 min accomplishes a 6-hr detention time in the 30-ℓ assay vessels. This is the maximum detention

time reported to be recommended in the forthcoming thirteenth edition of Standard Methods [4].

The fish assay vessels were essentially "completely mixed" due to the combined effects of the periodic discharges of wastewater/dilution water and the oxygen bubbling through the tanks. This provided a dampening effect on transients which may have occurred in the waste stream. A shorter detention time would increase the effect of concentration transients, although wastewater that has passed through major treatment structures will have undergone some dampening of peaking transients.

Normally 20 to 30 fish were used per assay vessel with weights of 1 to 4 g per fish. The daily flow through each vessel usually exceeded 3 liters per gram of fish, easily meeting or exceeding recommended flows [1, 4].

Administration of the bioassay tests generally followed recommendations made in Standard Methods [1], but with some modifications based on the work of Sprague [4].

OPERATING EXPERIENCE

Operation of 4 continuous flow fish bioassay apparatus on a nearly continuous basis has been very satisfactory. Wastes assayed have included municipal primary effluent as well as secondary and tertiary effluents. Periodic (each one or two weeks) flushing of proportioning boxes with tap water under pressure has been sufficient for operation of up to 8 consecutive assays each of 1-week duration. Assay vessels have been drained and flushed at the same intervals. No bioassay run has been terminated due to diluter failure.

Solids and grease accumulation in small amounts on the interior of proportioning chambers has given the appearance of fouling but closer inspection has revealed that the transparent construction materials fostered an illusion.

The diluters have proven to be very consistent in proportioning flows to the assay vessels. Small deviations may occur due to the siphoning action and sequence of siphoning from the individual dilution chambers. This is the result of a flow from one chamber to another existing at the time chamber D6 begins to siphon. Even though the solenoids shut off the feed, the water level in each chamber is slightly above the dividers causing the first siphons which start operating to pass slightly more liquid during that cycle due to overflow from adjacent chambers. The rate of waste flow to the diluter will also affect slightly the dilutions achieved as the flow rate between chambers determines the depth of each dilution chamber above the dividers at the start of siphoning.

Two diluters operated for 7 weeks at SERL without dilution adjustment were checked weekly for performance. During these checks the total delivery per cycle and delivery per cycle from the

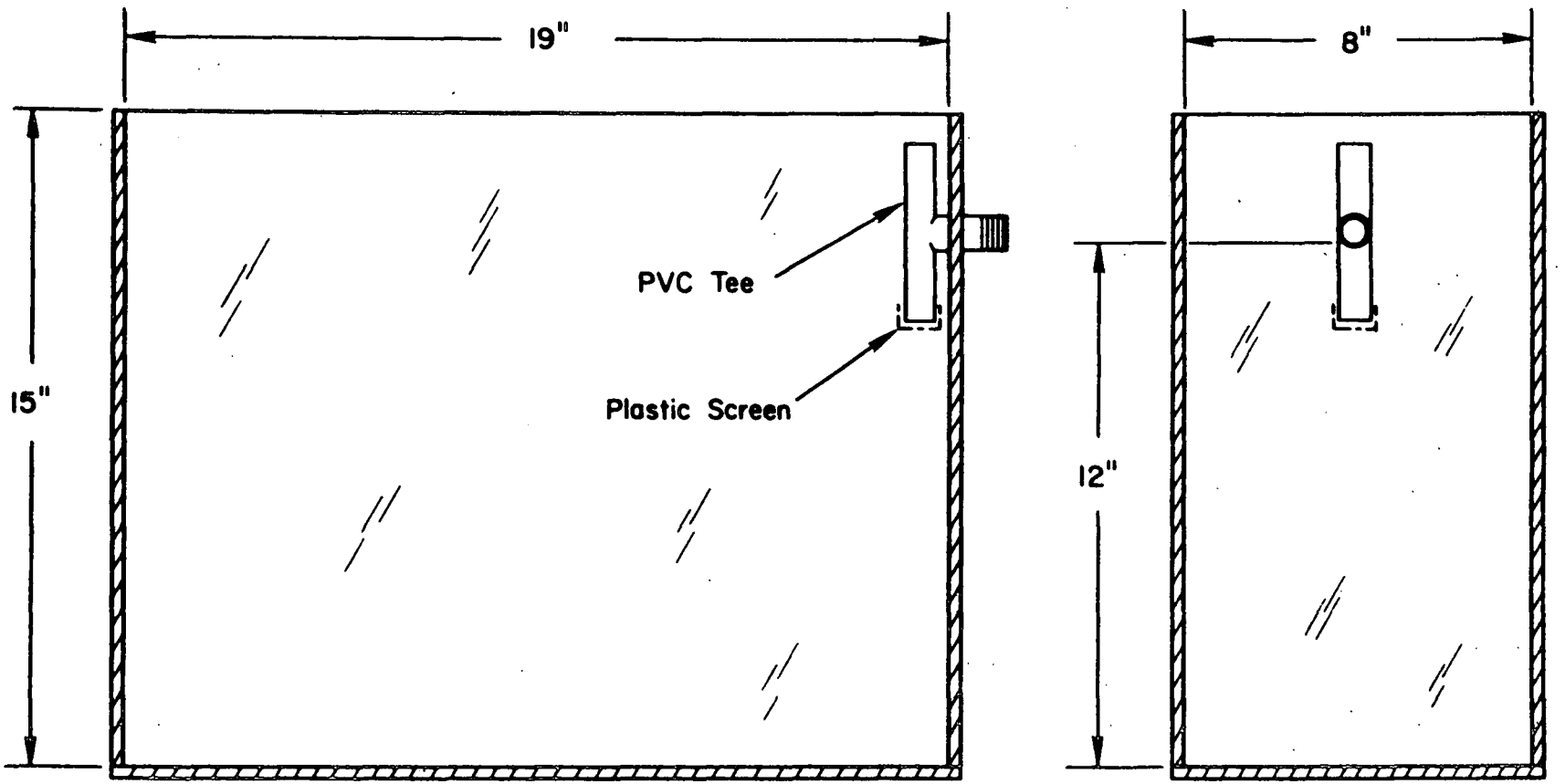


FIGURE A-6. 30-LITER ASSAY VESSEL

dilution water proportioning chamber were measured and the waste percentage computed. Table A-2 shows the waste percentages measured each week and the mean, standard deviation, and coefficient of variation* for each dilution over the 7-week period. Diluter A operated with primary effluent while diluter B operated with treated wastewater.

From Table A-2 no trend in standard deviation can be seen although the coefficient of variation increased as the percentage wastewater in the dilution decreased. This indicates the errors are largely attributable to the siphoning deviations mentioned earlier in this section. The average standard deviation of the 8 dilutions was 0.58%.

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*Standard deviation x 100, divided by the mean.

TABLE A-2

DILUTER PERFORMANCE FOR SEVEN WEEKS
OF OPERATION WITHOUT ADJUSTMENT

Week	Percent Waste Delivered to Assay Vessel							
	Diluter A				Diluter B			
	A2	A3	A4	A5	B2	B3	B4	B5
1	64.3	36.4	19.1	14.1	81.3	68.1	59.0	46.2
2	64.1	37.3	21.0	14.3	82.1	67.9	59.0	47.0
3	64.1	37.7	21.4	13.7	81.4	68.0	58.8	46.0
4	65.0	37.0	21.3	14.3	81.4	68.1	57.9	45.7
5	63.6	36.3	21.5	13.0	81.2	67.3	59.3	45.6
6	63.1	36.3	20.8	13.3	81.1	67.4	58.6	45.7
7	63.0	35.0	20.5	13.6	81.4	67.0	59.3	45.6
Mean	63.9	36.6	20.8	13.8	81.4	67.7	58.8	46.0
Standard Deviation	0.71	0.88	0.83	0.50	0.32	0.45	0.49	0.51
Coefficient of Variation, %	1.1	2.4	4.0	3.7	0.4	0.7	0.8	1.1

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