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REGIONAL COPPER-NICKEL STUDY

AQUATIC TOXICOLOGY PROJECT

OPERATIONS MANUAL

September 26, 1977

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Aquatic Toxicology Project

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David T. Lind

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## 1. INTRODUCTION

The purpose of the copper-nickel aquatic toxicology project is to help predict the toxic effects of discharges from copper-nickel mining and processing operations in northeastern Minnesota on local aquatic organisms.

The aquatic toxicology project staff is conducting acute and chronic toxicity bioassays\* of heavy metals expected to be present in these discharges, using Lake Superior water and surface waters of the copper-nickel study area as dilution waters. Our principal test organisms are the fathead minnow, Pimephales promelas, and the cladoceran Daphnia pulicaria because we can culture these species easily in the laboratory. We are also working with local species on a more limited basis.

From our acute and chronic toxicity experiments, from surveys of the aquatic toxicology literature, and from information gathered under other projects with the Copper-Nickel Study, we hope to shed light on:

1) the relative toxicity of different components likely to be found in mining and processing discharges to a given species, 2) the relative sensitivity of different species to a given toxic component, 3) the toxic effects of combinations of these components, and 4) the relationship of receiving water characteristics to metal toxicity.

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\*For a general discussion of bioassay techniques and interpretation of results, see APHA et al. (1975).

## 2. WATER SUPPLY SYSTEMS

### 2.1. Experiments in Lake Superior Water

Apparatus for conducting toxicity experiments in Lake Superior water is housed at the U.S. Environmental Protection Agency's Environmental Research Laboratory in Duluth, Minnesota (ERL-Duluth). The laboratory water supply is drawn from an intake 335m off the mouth of the Lester River and 20m deep, and is pumped to experimental systems through iron and PVC pipe. A thermostatically-controlled flow of heated water in stainless steel coils raises the temperature of incoming lake water to the desired experimental temperature, which is continuously monitored.

### 2.2. Experiments in Other Surface Waters

Apparatus for conducting toxicity experiments in waters other than Lake Superior is housed in a mobile laboratory, the specifications for which are given in the Appendix. Water for experiments is pumped from 0.3m to 0.7m beneath the surface of the lake or stream to the experimental system through polyethylene and PVC pipe. Incoming water is strained through a polyethylene inlet chamber with 3mm openings and further strained through a 40 mesh stainless steel screen. The water then flows into a 100-liter polyethylene mixing tank in the laboratory where its temperature is raised to the desired level by thermostatically-controlled electric immersion heaters. An immersion pump circulates the water in the tank, and a recorder monitors its temperature. All pumps which supply water for experiments are constructed of stainless steel and non-toxic plastics.

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### 3. TEST ANIMAL CULTURES

#### 3.1 Fathead Minnow

##### 3.1.1. Origin

The fathead minnows (Pimephales promelas Rafinesque) used in most of our experiments belong to a strain originating from culture ponds at the Newtown Fish Farm of the Ohio Division of Wildlife. This strain has been cultured since 1969 at ERL-Duluth. We set up a separate culture unit at ERL-Duluth in November, 1976, using fish of the Newtown-Duluth strain.

##### 3.1.2. Culture System and Procedures

Adult fathead minnows (three males and four females) are kept in four 25-liter aquaria constructed of window glass panes and silicone rubber cement. Approximately 300 ml/min of Lake Superior water at  $25 \pm 1^{\circ}$  C flows into each aquarium through the system described above, trickling down a 30cm-long spillway above each aquarium which alleviates air supersaturation resulting from heating. Water overflows from each aquarium through a standpipe.

The adult fish are fed pesticide-free commercial fish pellets once per day, and frozen adult brine shrimp once per day. Each aquarium with adult fish contains five arch-shaped stainless steel spawning substrates coated with sand (Benoit and Carlson, 1977). Eggs are deposited on the undersides of the substrates.

Egg-bearing substrates are collected three times weekly and placed in a separate aquarium identical to those described above, and also supplied with 300 ml/min of Lake Superior water at  $25 \pm 1^{\circ}$  C. All substrates collected in a given week are placed in the same aquarium. Dead embryos and fungus growth are removed daily from egg-bearing substrates.

Substrates are removed from the aquarium when hatching is complete (about four days). Each week newly-spawned eggs are placed in a fresh aquarium so that a separate cohort of fish in a separate aquarium is produced from each week's spawning.

Young fish are fed slightly in excess of consumption according to the schedule given in Table 1. During the sixth week after spawning, the number of fish in a given cohort is reduced to approximately 150 by removing the largest and smallest fish. A cohort is used in experiments during the ninth week.

Table 1. Feeding regime for fathead minnow culture.

Week 1:	hard-boiled egg yolk suspension*
Week 2:	hard-boiled egg yolk suspension San Francisco Bay brine shrimp nauplii Zeigler Bros. trout fry starter granules
Weeks 3-7:	San Francisco Bay brine shrimp nauplii Zeigler Bros. trout fry starter granules
Week 8:	frozen adult brine shrimp Zeigler Bros. #1 trout pellets
Adult fish:	frozen adult brine shrimp Zeigler Bros. #2 trout pellets

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\*Egg yolk suspension is fed twice per day. All other foods are fed once per day.



All aquaria are illuminated for 16 hours each day by Vita-Lite fluorescent tubes providing from 40 to 80 foot-candles at the air-water interface.

### 3.2. Daphnia pulicaria

#### 3.2.1. Origin

Daphnia pulicaria Forbes used in our experiments originated from a collection made in June, 1974, by ERL-Duluth personnel from Island Lake, T52N, R15W, St. Louis County, Minnesota. The animals were identified by Dr. Byron Torke of the University of Wisconsin-Milwaukee. D. pulicaria is often confused with D. pulex since the two species are morphologically quite similar (Brandlova, et al., 1972).

A culture was started at ERL-Duluth with D. pulicaria from the June, 1974, collection. In November, 1976, we began a separate culture of D. pulicaria with animals taken from the laboratory's culture.

#### 3.2.2. Culture System and Procedures

The Daphnia are cultured in two-liter glass battery jars filled with Lake Superior water which has been strained through 105 mesh stainless steel bolting cloth and allowed to equilibrate to room conditions for 24 hours before use. Water temperature is maintained at  $18 \pm 1^{\circ}$  C by regulating room temperature. Culture jars are illuminated for 16 hours each day by Vita-Lite and Gro-Lux fluorescent tubes providing a light intensity of 120 foot-candles at the air-water interface.

Approximately 50 animals of mixed age are transferred weekly (using a widemouth medicine dropper) into each of five culture jars. Two ml of food (Table 2) is added to each culture jar when the Daphnia are transferred and again after three or four days. Weekly transfer into fresh jars keeps the culture in an exponential population growth phase and prevents male production, formation of ehippia (resting eggs), and population "crashes", as well as controlling bacteria and algae growth. Week-old and two-week-old cultures are kept in case of poor Daphnia production in fresh cultures.

Twenty-four hours before starting a bioassay with Daphnia, adults with large eggs are transferred from the newest culture jars containing sufficient numbers of adults into fresh jars containing food and test water. The young produced during the following 24 hours are transferred into intermediate beakers containing test water with no food, and then into test solutions.

Table 2. Preparation of Food for Daphnia pulicaria.

Ingredients: 0.5g Cerophyl (powdered dehydrated cereal grass leaves)  
10g Zeigler Bros. trout fry starter granules  
Lake Superior Water

Procedure: Mix a suspension of Cerophyl, trout granules, and water, diluting to 400ml. Pour suspension into blender. Rinse mixing flask with 100ml of water and pour into blender. Blend at high speed for 5 minutes. Strain homogenate through 105 mesh stainless steel bolting cloth. Rinse blender with 50ml water and pour rinse water through bolting cloth into homogenate. Keep refrigerated.

#### 4. EXPERIMENTAL SYSTEMS AND PROCEDURES

##### 4.1. Acute Bioassays With Fish

##### 4.1.1. Exposure System

A flow-through exposure system is used for all acute bioassays with fish. A proportional toxicant diluter similar to that described by Mount and Brungs (1967) is used to maintain five different toxicant concentrations and a control in a set of exposure chambers. When necessary, test water is vigorously aerated before flowing into the diluter to alleviate air supersaturation caused by heating to test temperature.

Stock solutions of toxic heavy metals are prepared by dissolving the hydrated sulfate (reagent grade) in distilled water and adding one drop of concentrated hydrochloric acid to each liter of stock solution. The stock solution is dispensed in the diluter by the metering apparatus described by Mount and Brungs (1967). Successive dilutions of the toxicant solution delivered by the diluter are 70, 49, 34, and 23 percent as concentrated as the highest level.

The five concentrations and the control supplied by the diluter are each dispensed into a flow-splitting chamber (Benoit and Puglisi, 1973) which divides the discharge of each concentration in half and delivers each half to a separate exposure chamber. With duplicate exposure chambers for each of the five treatment concentrations and the control, a total of twelve chambers are supplied by the diluter.

Each exposure chamber is cylindrical, molded of linear polyethylene, has an inside diameter of 21.5cm, and contains 6 liters of test solution. As fresh solution is added by the diluter, each chamber overflows through a glass tube inserted in its wall. The diluter delivers one chamber volume per chamber per hour, which is sufficient to replace 90 percent of the solution in each chamber every 2.5 hours (Sprague, 1969). Exposure chambers are illuminated for 16 hours each day by Vita-Lite fluorescent tubes, providing a light intensity of 20 to 40 foot-candles at the air-water interface.

#### 4.1.2. Experimental Procedure

Test fish are randomly assigned to exposure chambers 24 hours before introduction of toxicant solution to the diluter. For bioassays conducted in water other than Lake Superior water, the fish are first acclimated to the test water at test temperature in a 40-liter polyethylene holding tank for 6 days. Test fish are not fed after being placed in exposure chambers, since food particles might interact with metal ions.

To begin an experiment, toxicant stock solution is introduced to the metering apparatus described above. All exposure chambers are immediately drained to  $\frac{1}{4}$  their capacity and then allowed to fill normally so that toxicant concentrations in the chambers will quickly adjust to the concentrations in the incoming water.

Mortality in each exposure chamber, and total length of each dead fish, is recorded at 24-hour intervals after the experiment is begun. A fish is considered dead when all movement ceases and it fails to respond to gentle mechanical prodding.

The experiment is terminated after 96 hours, or after mortality has ceased. Continuing experiments until cessation of mortality is of value in comparing modes of action of different toxicants. Survivors in each exposure chamber are anesthetized with tricaine methanesulfonate, blotted on paper toweling, weighed as a group to the nearest milligram, and their total individual lengths measured to the nearest millimeter.

#### 4.1.3. Monitoring Experimental Conditions

All temperature measurements and all water samples for chemical analysis are taken from the center of each exposure chamber.

Test water temperature is measured daily to the nearest  $0.1^{\circ}$  C with a precision-grade partial-immersion mercury thermometer. Water temperature controls are adjusted if temperatures in all exposure chambers vary in the same direction from the desired temperature by at least  $0.2^{\circ}$  C.

pH of the water in each exposure chamber is measured daily to the nearest 0.01 unit with a glass electrode, calomel reference electrode, and digital or expanded-scale analog meter, standardized with commercial buffers. Procedures outlined in APHA et al. (1975) and in manufacturers' instructions are followed in making pH measurements.

Dissolved oxygen concentration in each chamber is measured daily to the nearest 0.1 mg/liter using a polarographic membrane electrode and analog meter calibrated against the azide modification of the Winkler iodometric method (APHA et al., 1975).

Total alkalinity of the test water is measured potentiometrically once in each experiment in one control chamber to the nearest mg/liter as  $\text{CaCO}_3$ , according to procedures described in APHA et al. (1975).

Hardness of the test water is measured titrimetrically once in each experiment in one control chamber to the nearest mg/liter as  $\text{CaCO}_3$ , according to procedures described in APHA, et al. (1975). A dry mixture of Eriochrome Black T and sodium chloride is used as the endpoint indicator.

Specific conductance of the test water is measured once in each experiment in one control chamber to the nearest 2 percent (at full meter scale) with a platinum-electrode conductance cell and temperature-compensated bridge with five-range analog readout. The conductance apparatus is calibrated periodically against solutions prepared with dried NaCl crystals and deionized distilled water.

A sample of test water is withdrawn from one control chamber once in each experiment, chilled, and shipped to the Minnesota Department of Health (MDH), Analytical Services Laboratory in Minneapolis, Minnesota for total organic carbon analysis by the combustion-infrared method (APHA, et al., 1975).

An aliquot of test water is withdrawn daily from each exposure chamber and acidified to a concentration of 0.15 percent by volume ultrapure  $\text{HNO}_3$ . A composite sample from each exposure chamber is shipped to the MDH laboratory for atomic absorption spectrophotometric analysis of the total concentration of the metal being studied in the toxicity experiment.

Periodically, test solution samples are withdrawn from three exposure chambers representing the highest, middle, and lowest treatment levels and filtered for metal analysis according to the following procedure: first, a 47-mm Millipore membrane filter with 0.45 micron mean pore size

is loaded into an acid-rinsed Millipore aseptic filtration unit. Vacuum is applied to the lower chamber, and 100 ml of 0.3 percent  $\text{HNO}_3$  (grade suitable for mercury determination) is passed through the filter, followed by 100 ml of deionized distilled water. The lower chamber is emptied, a 100-ml sample bottle is placed beneath the filter head, and the unit is reassembled. A 200 ml sample of test solution is withdrawn from an exposure chamber. The first 100 ml are filtered into the bottle in the filtration unit. The bottle is removed, acidified, and capped. The other 100 ml are poured into another sample bottle and acidified, and the bottle is capped. Both samples are analyzed for total metal concentration by the MDH laboratory.

#### 4.1.4. Analysis of Data

Median lethal concentrations of toxicants ( $\text{LC}_{50}$ 's) are calculated from mortality data by two methods. The first method is an iterative maximum-likelihood regression technique using the logarithmic transformation on toxicant concentration and the probit transformation on percentage mortality (Finney, 1971). The second method uses a nonparametric statistic, the trimmed Spearman-Kärber estimator, to predict the  $\text{LC}_{50}$ . This estimator has been shown to be more robust than the commonly-used maximum-likelihood estimator from the standpoint of its accuracy and precision, its lower sensitivity to an anomalous organism response, and its ability to produce a calculable estimate under a variety of conditions (Hamilton, et al., 1977).

## 4.2. Chronic Bioassays With Fish

### 4.2.1. Exposure System

All chronic toxicity experiments with fish employ a flow-through exposure system. A proportional toxicant diluter similar to that described by Mount and Brungs (1967) maintains five different toxicant concentrations and a control in a set of exposure chambers. Test water is sand-filtered, and, when necessary, is aerated vigorously before flowing into the diluter to alleviate air supersaturation caused by heating to test temperature.

Toxicant stock solutions are prepared and dispensed as described in a previous section. Successive dilutions of the toxicant solution delivered by the diluter are 50, 25, 12, and 6 percent as concentrated as the highest level.

Flow-splitting chambers, as described in a previous section, divide the flow of each concentration in half and allow for duplicate exposure tanks for each of the five treatment concentrations and the control. Each tank is constructed of glass and silicone rubber cement, measuring 42.5 X 25.0 X 15.0 cm high, and is divided lengthwise into two 12.5-cm-wide chambers. Water depth in each chamber is maintained at 12.0 cm by a glass standpipe in one end, giving the chamber a capacity of 6.4 liters. The diluter delivers one chamber volume per chamber per hour, which is sufficient to replace 90 percent of the solution in each chamber every 2.5 hours. Exposure chambers are illuminated for 16 hours each day by Gro-Lux fluorescent tubes, providing a light intensity of 5 to 15 foot-candles at the air-water interface.



#### 4.2.2. Experimental Procedure

The experiment is started with viable undamaged fish eggs between 24 and 48 hours after fertilization. Fifty eggs are randomly assigned to each exposure chamber and placed in screen-bottom cups which are continuously oscillated in the chamber to circulate test water past the eggs. When all the eggs in a given cup have hatched, 25 undeformed larvae are transferred from the cup to the chamber proper. Percentage hatch of undeformed larvae is recorded, and deformed larvae are considered dead.

The young fish are fed twice daily with brine shrimp nauplii, which are collected after incubation of San Francisco Bay brine shrimp eggs for 24 hours at 26 to 27°C. All exposure chambers receive an equal amount of food, which is slightly in excess of the amount consumed in any chamber. Excess food and feces are siphoned from all chambers on alternate days as soon as the young fish become large enough to see clearly.

After 30 days, all fish are removed from the chambers and preserved in 10 percent formalin. The fish are later weighed individually to the nearest milligram after being blotted on paper toweling, and measured to the nearest millimeter.

#### 4.2.3. Monitoring Experimental Conditions

All water samples for chemical analysis are taken in a screened-off area near the outlet of each pair of test chambers to avoid removing any fish. Temperature is measured in the center of one of the two chambers. Temperature is measured daily; pH, dissolved oxygen, total alkalinity,

and hardness are measured weekly. Weekly composite toxicant samples are taken, and periodic sample filtrations for "dissolved" metal analysis are performed with three treatments (highest, middle, lowest). All chemical analyses follow procedures described in Section 4.1.3.

#### 4.2.4. Analysis of Data

Percentage egg hatch, percentage fish survival, mean fish weight, and biomass (number of survivors X mean weight) in each chamber are subjected, with appropriate transformations, to nested 1-way ANOVA. Treatment means are compared with the control mean by Dunnett's two-tailed procedure (Steel and Torrie, 1960) to determine which toxicant concentrations have deleterious (or beneficial) effects on these parameters.

#### 4.3. Acute Bioassays With *Daphnia pulicaria*

##### 4.3.1. Exposure System

Acute toxicity bioassays with *Daphnia pulicaria* are performed in static (unrenewed) test solutions. Exposure chambers are 250-ml glass beakers, and two to four replicate beakers are employed per treatment level.

The toxicant stock solution and the series of test solutions are prepared from test water which has been strained through 105 mesh stainless steel screen and allowed to equilibrate to the test temperature of  $18 \pm 1^{\circ}$  C. Appropriate volumes of stock solution are made up to 1 liter to create the desired toxicant concentrations. Each beaker receives 200 ml of test solution, and the test solutions are allowed to equilibrate to laboratory conditions for 24 hours before the experiment is started.

Test temperature is controlled either by regulation of ambient air temperature or by bathing the test beakers in water regulated at test temperature. Glass panes are placed over the test beakers to retard evaporation. Test beakers are illuminated for 16 hours each day by Gro-Lux and Vita-Lite fluorescent tubes, providing a light intensity of 130 foot-candles at the air-water interface.

#### 4.3.2. Experimental Procedure

Five Daphnia pulex, zero to 24 hours old, are randomly transferred to each test beaker to begin the experiment. No food is added to test solutions. After 48 hours, the number of survivors in each test beaker is recorded.

#### 4.3.3. Monitoring Experimental Conditions

Temperature is measured in one replicate of each treatment and the control each day of the experiment. pH is measured in an extra replicate of each treatment and the control (none of which receive Daphnia) at the beginning of the experiment, and in one regular replicate. Dissolved oxygen, total alkalinity, hardness, and specific conductance are measured in one replicate of the control at the end of the experiment. A sample for metal analysis is taken from an extra replicate of three treatments (highest, middle, and lowest) at the beginning of the experiment, and from one regular replicate of each treatment and the control at the end of the experiment. Sample filtrations for "dissolved" metal analysis are also performed periodically on three treatments (highest, middle, and lowest) at the end of the experiment. All chemical analyses follow procedures described in Section 4.1.3.

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Median lethal concentrations of toxicants (LC50's) are calculated from mortality data by the two methods described in Section 4.1.4.

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## Toxicology, Appendix

### MOBILE LABORATORY SPECIFICATIONS

The following is a list of specifications for a trailer to be used by the Minnesota Pollution Control Agency to conduct aquatic toxicity bioassays in water from Minnesota lakes and streams.

#### Trailer Construction

The chassis shall be of heavy-duty construction and formed of box-tubing longitudinal members and I-beam or formed steel cross members welded into an integral unit. The trailer shall be equipped with tandem axles with minimum six-ply tires on each wheel. A spare tire and rim shall be provided, but shall not be attached by any device to the trailer itself. All four wheels shall have electric brakes. Total payload capacity of the trailer shall be a minimum of two tons. The chassis shall be constructed in such a manner as to allow the trailer body to rest entirely above the wheels, thereby eliminating interior wheel wells. The trailer shall be constructed with a standard A-frame hitch assembly for a 2" or 2-5/16" ball, and shall have a dolly wheel for supporting the front of the trailer when detached from the towing vehicle. A switch shall be provided to engage the trailer brakes in case of accidental breakaway in transit.

All chassis and body lighting shall conform to Federal specifications and shall include 12-volt clearance lights, tail lights, stop lights, and directional lights. The electrical connector shall be of standard design and shall carry leads for electric brake controls.

The body framework shall be constructed of vertical steel posts and steel roof supports. All corner posts and door headers shall be steel and welded in place.

The trailer exterior shall be white pre-finished and double-crimped aluminum with fully weathertight joints. The roof shall be one-piece aluminum.

Insulation shall be 1" styrofoam sheets in ceiling and walls, and a minimum of ½" under the floor. The entire underside of the trailer shall be undercoated.

Exterior dimensions of the trailer body shall be 18' long by 8' wide.

Interior height shall be a minimum of 7' at its lowest point.

#### Trailer Equipment

The trailer shall be furnished with two 30-pound LP gas tanks mounted on the front A-frame, and all necessary valves and fuel lines. Four leveling jacks shall be furnished to allow stationary leveling of the trailer, and adequate bracing or brackets for the jacks shall be provided.

The trailer door shall be centered in the rear wall and hinged on the road side, with a window having an area of not less than 1 square foot, shall open outward, and shall not be less than 36" wide by 6'6" high. A retractable step shall be provided. The door lock shall be furnished with a minimum of two keys, and the door shall be equipped to accommodate a padlock.

The floor covering shall be one-piece, water-resistant, and capable of withstanding heavy foot traffic. Walls shall be covered with vinyl-coated paneling. The ceiling shall be white, with a washable surface.

Two powered roof-top vents and one powered side wall exhaust fan shall be installed as shown in the attached drawings. Wiring to the roof-top vents shall be of sufficient gauge, and roof cut-outs and supports of sufficient strength to permit future use of air conditioners in these locations. One roof-top air conditioner with minimum 13,000 BTU capacity shall be installed in the location shown in the attached drawings.

An LP gas forced air furnace with an enclosure shall be installed on the front wall as shown in the attached drawings. Furnace capacity shall be a minimum of 21,000 BTU. A thermostat shall be installed on the curb side wall.

One window with an area of at least 450 square inches shall be provided at the front of the trailer, and shall be of one-piece design to facilitate removal in an emergency.

An electrical distribution box with a minimum of six circuit breakers shall be installed on the rear wall, curb side, and connected to electric distribution circuits as shown in the attached drawings.

A 230-volt power inlet, two 230-volt turnlock power outlets with weatherproof plates and outlet caps, and two 115-volt turnlock outlets with weatherproof plates and caps shall be installed on the exterior rear curb



side of the trailer. The outlets shall have their own breakers, and shall be protected with suitable ground fault interrupters. The exterior outlets shall be inside a weather-protective hood.

A minimum of six 115-volt 3-pin duplex outlets shall be provided and wired as shown in the attached drawings. Outlets on the road side wall shall have weatherproof plates and outlet caps.

Six double-tube, 4' fluorescent ceiling fixtures shall be installed as shown, and shall be equipped with diffusers which remain in place during transit. A 24-hour timer with a manual switch override, equivalent to Dayton No. 2E021, shall be installed beneath the electrical distribution box to control the ceiling fixtures only.

A drain with faceplate shall be installed near the center of the trailer floor to facilitate drainage of accidental water spills. The drain shall be constructed in a manner that allows water to drain without allowing air to enter, and shall not protrude from the floor in such a manner as to create a hazard to operating personnel.

An overhead cabinet with two doors shall be installed on the front wall as shown in the attached drawings. Both doors shall be hinged at the top and shall include devices to hold them open. Positive latches shall be provided to prevent the doors from opening while the trailer is in transit.

A counter and cabinets shall be installed along the curb side wall as shown in the attached drawings. A stainless steel sink shall be installed as shown, and shall measure approximately 14" by 16" by 8" deep. A high rise

hot and cold water swivel faucet with shut-off valves shall be installed, and the cold water line shall run to an external connection. The hot water line and drain shall be left unconnected.

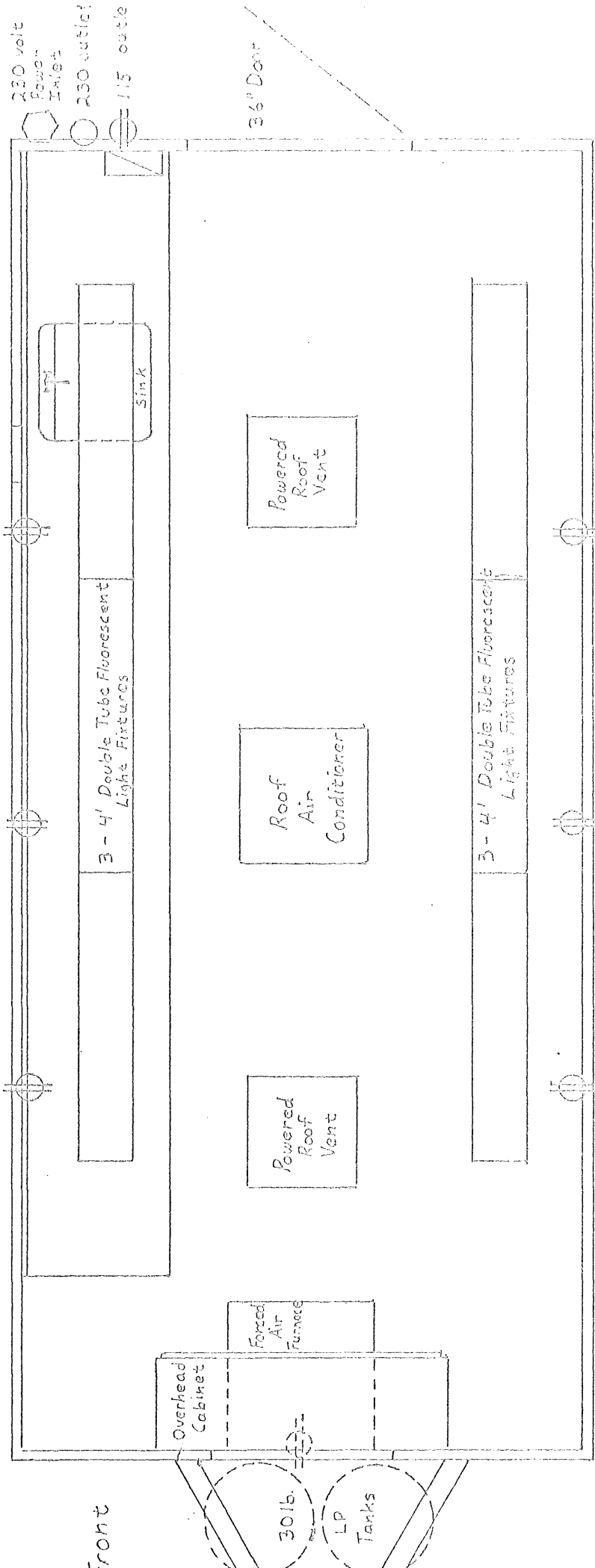
A 4-cubic-foot refrigerator with a freezer compartment and a door lock or positive latch shall be installed in the space shown. The refrigerator shall be powered by 115 volts AC and connected to the trailer electrical system.

Two steel file cabinets shall be installed in the spaces shown, and each shall include one letter-size file drawer, two drawers 5" to 6" deep, and a pull-out writing shelf. All drawers shall have full suspension, and shall have a latching or locking device to prevent opening in transit.

Each remaining under-counter cabinet shall have a latching door and three adjustable shelves which can be moved in increments not less than  $\frac{1}{2}$ " or more than 2", and will not collapse in transit. The cabinet beneath the sink shall contain no shelves.

A toespace shall be provided beneath all cabinets. Open areas beneath the counter are to be used as knee space.

The counter top, splash board, and cabinet doors shall be covered with material which will resist 50 percent concentrations of laboratory acids and bases and is of constant color and composition throughout its thickness.

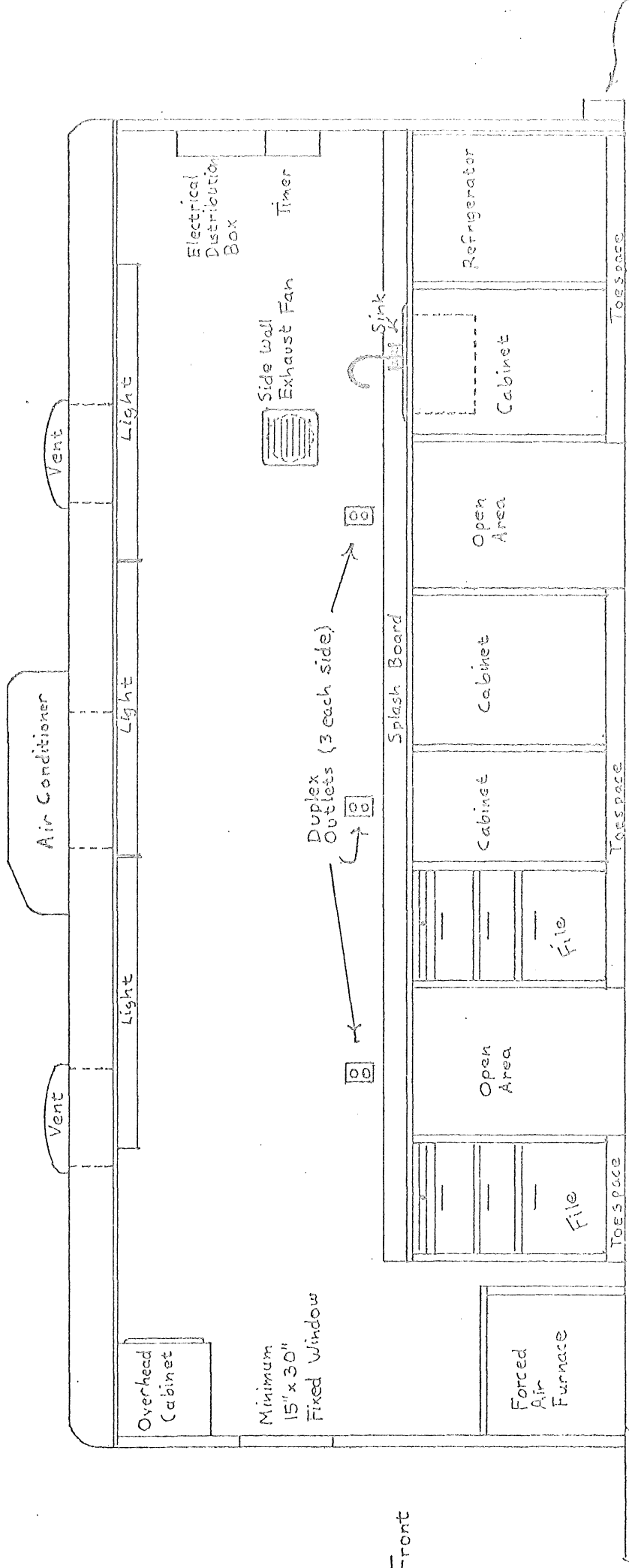


Scale: 1/2" = 1'

FLOOR

⊕ 115 V. AC Duplex Outlets

BIOASSAY TRAILER - POLLUTION CONTROL AGENCY



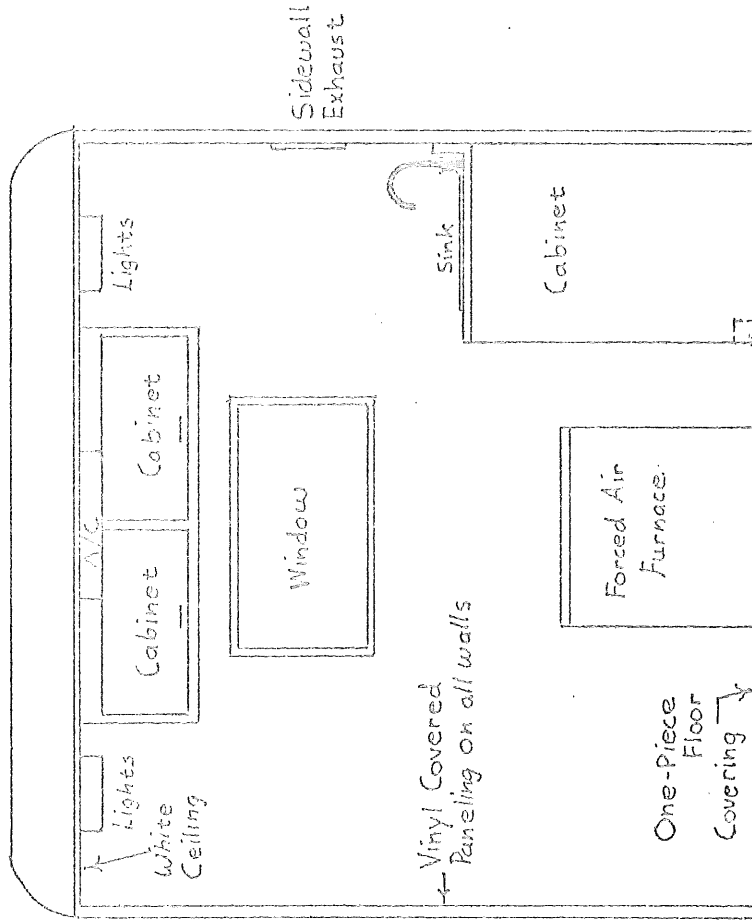
Weather-Protective Outlet Housing

Scale: 1/2" = 1'

CURB SIDE WALL

BIOASSAY TRAILER - POLLUTION CONTROL AGENCY

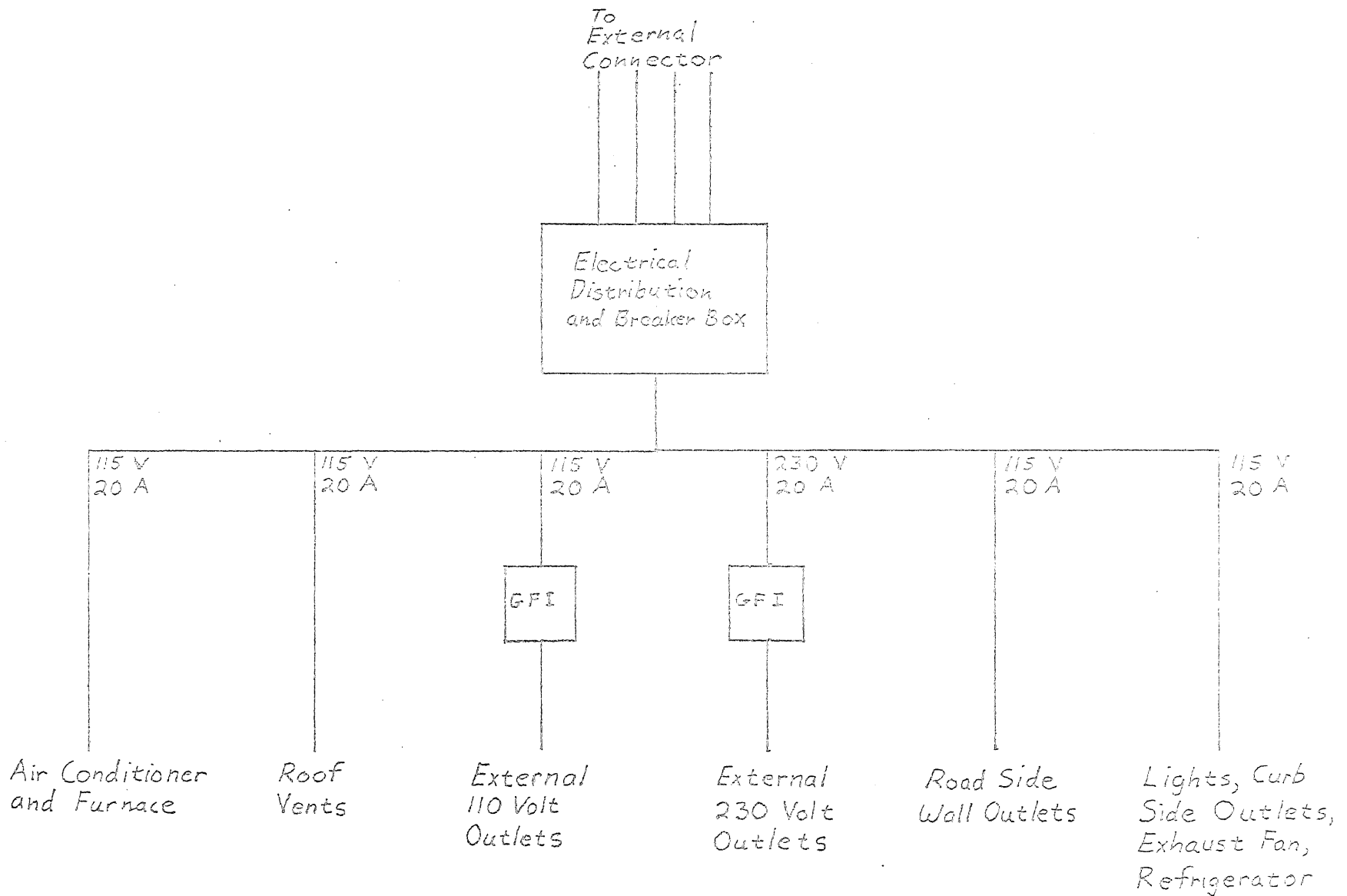
Front



Scale: 1/2" = 1'

FRONT WALL

BIOASSAY TRAILER - POLLUTION CONTROL AGENCY



WIRING

BIOASSAY TRAILER - POLLUTION CONTROL AGENCY