

## Acute Cadmium Toxicity Studies Upon Nine Species of Aquatic Insects

ROBERT W. CLUBB, ARDEN R. GAUFIN  
AND JAMES L. LORDS

*Department of Biology, University of Utah,  
Salt Lake City, Utah 84112*

Received September 5, 1974

Continuous-flow bioassays were employed to determine 96-hour median tolerance limits (TL<sub>m</sub>), for the stonefly, *Pteronarcella badia* (Hagen) (TL<sub>m</sub> was 18.0 mg Cd/l) and the mayfly, *Ephemerella grandis grandis* Eaton (TL<sub>m</sub> was 28.0 mg Cd/l). Ninety-six hours TL<sub>m</sub> values for other species of aquatic insects tested were not determined, since these species were relatively insensitive to cadmium.

Insects exposed for four days in cadmium-containing water, then placed in tap water, show a linear rate of cadmium loss. This loss may lower or prevent mortality under ideal conditions.

### INTRODUCTION

The need for water quality criteria with which to establish water pollution standards is necessary for the protection of aquatic life. The development of these criteria should have as a first consideration, acute toxicity studies, since such studies indicate individual species sensitivity as well as lethal concentrations. Such information may then be used as a basis for long-term studies, thus establishing ultimate concentration limits to preserve aquatic life. Further information is needed concerning effects of a "slug" of a pollutant. Accidental spills in a stream environment would result in a rapid increase of a pollutant (slug) followed by normal stream conditions. The effects of a sudden discharge of cadmium in a stream environment are unknown to the authors.

Cadmium is generally regarded as a toxic heavy metal. The widespread distribution in the environment can be related to increased technological use (Chemical Rubber Company, 1971). Several sources are responsible for cadmium in the aquatic environment. These include cadmium electroplating processes, which use approximately 50% of the cadmium produced each year in the United States, long-life battery industries (McCaul 1971), and runoff from agriculture areas fertilized with phosphate (approximately 1.8 ppm Cd) and superphosphate (approximately 8.9 ppm Cd) fertilizers (Schroeder *et al.*, 1967).

Cadmium toxicity studies on aquatic organisms have been largely confined to fish. Doudoroff and Katz (July, 1953), Pickering and Henderson (1965), and others, have summarized much of the information on the toxicity of metals. These data indicate a wide variability in the toxicity of cadmium to fish, dependent upon: the species, the chemical characteristics of the water, and the anion of the metal salt. In 96-hour median tolerance limit (TL<sub>m</sub>) studies on Fathead

Minnows, values ranged from 0.63 mg Cd/l in soft water (20 mg/L), to 73.5 mg Cd/L in hard water (360 mg/L) (Pickering and Henderson 1965). Goldfish were killed in 8.7 to 18 hours with 0.001 ppm Cd, alkalinity in this case was not stated (Schroeder *et al.*, 1967).

Warnick and Bell (1969) studied the toxicity of several heavy metals on three species of aquatic insects and found for cadmium that; the stonefly, *Acroneuria lycorias* had a 14-day TLM of 32.0 mg/liter; the mayfly, *Ephemerella subvaria* had a 96-hour TLM of 2.0 mg/liter; and the caddisfly, *Hydropsyche betteni* had a 10-day TLM of 32.0 mg/liter. They reported both the alkalinity and hardness of the water in these experiments to be approximately 50 mg/L, generally considered to be soft water.

The purpose of this study was: (1) to determine the acute toxicity of cadmium to nine species of aquatic insects, as a first step in determining criteria for the protection of these insects and (2) to determine the effects of a 4-day slug of cadmium followed by four days of tap water, as measured by cadmium concentrations in the water soluble and nonwater-soluble fractions of homogenized insects.

## METHODS

Continuous-flow bioassays were conducted in accordance with the methods described by the American Public Health Association (1971). These assays were used to determine acute toxicity and TLM values for nine species of aquatic insects. Tap water, thermostatically controlled, oxygen saturated, was cycled through a serial diluter (Mount and Warner, 1965) to introduce cadmium. The cadmium-containing water was then channeled into a glass ladder (Gaufin September, 1973) at three separate points. The ladder had three sections, each with a different cadmium concentration (Fig. 1).

Ninety-six hour TLM values were determined for *Pteronarcella badia* and *Ephemerella grandis grandis*. However, with other insects, a 96-hour TLM value was not determined, because these insects were relatively insensitive to cadmium. In those insensitive species, results are reported in terms of percent survival at a specific time and cadmium concentration. Cadmium concentrations were determined using atomic absorption spectrophotometry. Death was determined as physiological death, i.e., the point at which no response occurred when the insect was removed from the ladder and stimulated with forceps.

In order to be certain that the observed effect resulted from cadmium and not chloride, the anion used in this study, an experiment was conducted where all organisms were exposed for 22 days, to the amount of chloride ion, as NaCl, which equaled that amount added at the maximum cadmium concentration tested; no mortality was observed.

The test organisms used in this study were: the true flies (Diptera) *Atherix variegata* Walker, *Hexatoma* sp., *Holorusia* sp.; the mayfly (Ephemeroptera), *Ephemerella grandis grandis* Eaton; the stoneflies (Plecoptera), *Acroneuria pacifica* Banks, *Arcynopteryx signata* (Hagen), *Pteronarcella badia* (Hagen), *Pteronarcys californica* Newport; and the caddisfly (Tricoptera), *Brachycentrus americanus* (Banks). These organisms were chosen because of their abundance,

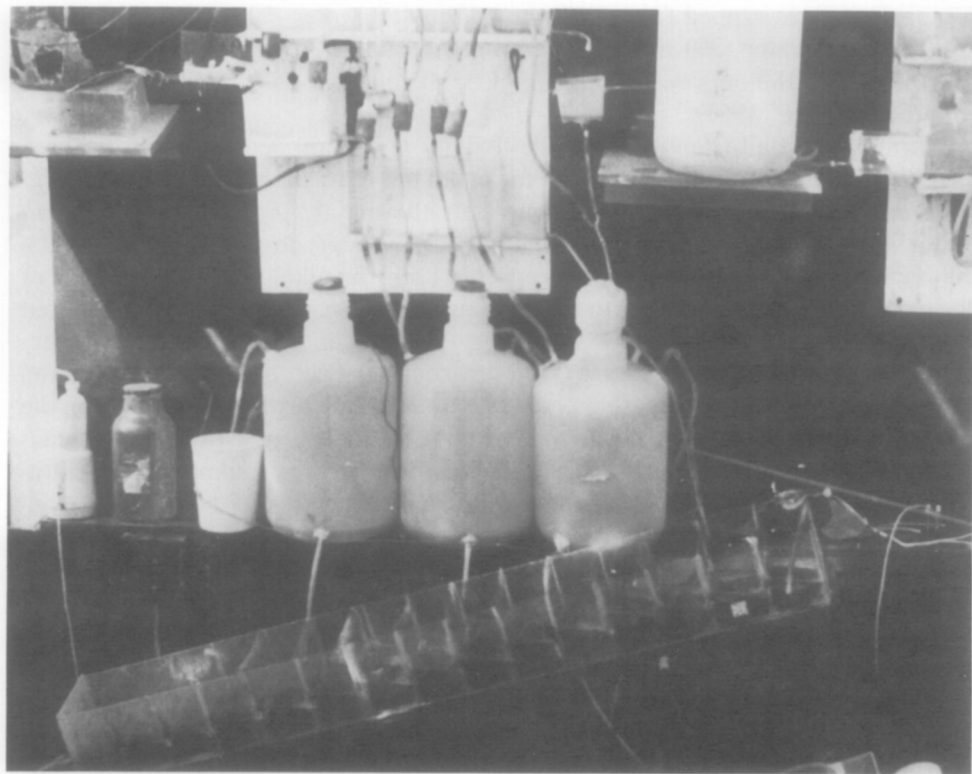


FIG. 1. Apparatus. Lower foreground: ladder. Center three bottles: flow control reservoirs. Upper center on white board: Mount Serial Diluter. Upper left, silver bottle: cadmium stock solution.

wide distribution and the importance of these species in the aquatic food chain. All organisms were collected from hard water ( $\geq 180$  mg/L) streams (Schraer, 1972). *Atherix*, *Hexatoma* and *Acroncuria* were collected from the Weber River near Peoa, UT. *Pteronarcys* was collected from the Provo River near the mouth of Provo Canyon, UT. All other organisms were collected from Thistle Creek, seven miles south of Thistle, UT. All organisms were acclimated to the ladder conditions for ten days prior to use in the bioassay.

Ten insects of each species were placed in each section of the cadmium-containing ladder and in one section of the control ladder. Daily mortality, cadmium concentration, dissolved oxygen, and temperature measurements were made.<sup>1</sup> After each bioassay, the ladder was rinsed with tap water for 2 hours, cleaned with 10% HCl, then rinsed again with tap water for an additional 2 hours. The diluter was started 24 hours prior to the start of a bioassay, to ensure stabilization of the cadmium concentration in the ladder. At this time, several leaves and small acid rinsed stones were placed in the ladder. Test organisms could use these as food and substrate.

<sup>1</sup> Dissolved oxygen concentrations were determined using an oxygen probe, verified weekly using the Winkler technique.

Cadmium slug experiments were conducted on *Pteronarcys californica* and *Pteronarcella badia*. Insects were placed in the ladder containing cadmium for 4 days. At this time the cadmium stock solution was disconnected from the diluter and the ladder was flushed with tap water until no detectable cadmium was observed. Tap water at the same flow rate as before was introduced into the ladder. Each day a sample of insects was removed from the ladder, rinsed in 2% HCl (to eliminate nonspecific binding), rinsed in distilled water, dried, weighed, homogenized in 0.001 M Hydroxymethyl Aminomethane (Tris) buffer, pH 7.0, with a mortar and pestle and centrifuged at 3000g for 10 minutes. The supernatant and sediment were collected, acidified to 5% HCl and heated at 100°C for 18 hours in sealed tubes. Cadmium concentrations were determined on the acidified samples against a 5% HCl blank.

Hill *et al.* (1963) reported zinc (200 ppm), copper ( $\geq 10$  ppm) and iron ( $\geq 50$  ppm) were antagonistic, in aggregate or singularly, to cadmium toxicity in chicks. Therefore, to estimate the effects these elements may have had in the bioassays, atomic absorption spectrophotometric analysis were made for the above ions. Iron and copper was not detectable, zinc was less than 1.0  $\mu\text{g/L}$ .

The dilution water had the following characteristics: temperature  $10 \pm 2^\circ\text{C}$ ; dissolved oxygen, (minimum) 6.0 mg/liter; alkalinity 240 mg/liter; pH 7.8. Average flow rate in the ladder was 25.0 liter/hour. Cadmium stock solutions were made using  $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$  in 1% HCl. The cadmium concentrations tested ranged from 1.0 to 42.5 mg/liter as  $\text{Cd}^{2+}$ . Bioassays were discarded if the cadmium concentration varied more than 0.5 mg/liter from the designated cadmium concentration. High concentrations were not used because of solubility limitations and diluter design.

TABLE I  
BIOASSAY DATA<sup>a</sup>

Species	Percent survival at 7 days		96-hour TLm (mg Cd/liter)
	Cadmium concentration mg/liter	Percent survival	
Diptera (true flies)			
<i>Atherix variegata</i>	10.0	100	
<i>Hexatoma</i> sp.	10.0	100	
<i>Holorusia</i> sp.	17.5	100	
Ephemeroptera (mayflies)			
<i>Ephemerella grandis grandis</i>	17.5	50	28.0
Plecoptera (stoneflies)			
<i>Acroneuria pacifica</i>	17.5	80	
<i>Arcynopteryx signata</i>	17.5	60	
<i>Pteronarcella badia</i>	17.5	30	18.0
<i>Pteronarcys californica</i>	14.0	100	
Tricoptera (caddisflies)			
<i>Brachycentrus americanus</i>	17.5	100	

<sup>a</sup> Dissolved oxygen concentration (minimum) 6.0 mg/l. Temperature  $10^\circ\text{C} \pm 2^\circ\text{C}$ ; Alkalinity 240 mg/l; Average flow rate 25.0 l/hr; Insects collected after November.

TABLE 2  
BIOASSAY DATA<sup>b</sup>

Percent survival at maximum cadmium concentration tested			
Species	mg Cd/liter	Days	Percent survival
Diptera (true flies)			
<i>Atherix variegata</i>	10.0	21	100
<i>Hexatoma</i> sp.	10.0	21	80
<i>Holorusia</i> sp.	42.5	7	50
Ephemeroptera (mayflies)			
<i>Ephemerella grandis grandis</i>	42.5	7	0
Plecoptera (stoneflies)			
<i>Acroneuria pacifica</i>	17.5	21	10
<i>Arcynopteryx signata</i>	17.5	21	10
<i>Pteronarcella badia</i>	42.5	7	0
<i>Pteronarcys californica</i>	14.0	14	100
Tricoptera (caddisflies)			
<i>Brachycentrus americanus</i>	42.5	7	100

<sup>b</sup> Dissolved oxygen concentration (minimum) 6.0 mg/liter; temperature 10°C ± 2°C; alkalinity 240 mg/liter; average flow rate 25.0 liter/hour; insects collected after November.

## RESULTS

Summaries of acute cadmium toxicity data to the nine insect species tested are given in Tables 1 and 2. *Pteronarcella* and *Ephemerella* were determined to be the most sensitive; *Brachycentrus*, *Pteronarcys* and *Atherix*, the least sensitive. Figures 2 and 3 represent 96-hour TLM data for *Pteronarcella* and *Ephemerella*. Both curves indicate no mortality in 96 hours at low cadmium concentrations (less than 4.0 mg/liter). The best fit for the data that explains the survival of *Pteronarcella* is an exponential curve,  $y = 1.14e^{-0.06x}$ , with a correlation coefficient<sup>2</sup> of 0.98. *Ephemerella*, however, had a linear curve,  $y = -2.24x + 1.13$ , which best explained these data; correlation coefficient in this case was 0.99.

The variation in cadmium toxicity to size classes of insects are given in Table 3. From these data, small, immature insects appear to be more sensitive to cadmium than larger, mature insects.

Figure 4 illustrates the rate of cadmium uptake and loss in a continuous slug study on *Pteronarcella* at 5.0 mg Cd/liter. Tables 4 and 5 lists the rates of cadmium uptake and loss in the supernatant, observed in *Pteronarcella* slug experiments at different cadmium concentrations. Results of slug experiment on *Pteronarcys* are given in Tables 6, 7, 8 and 9. Tables 6 and 7 indicate the rate of cadmium uptake in the supernatant and sediment, respectively. Correlation coefficients concerning

<sup>2</sup> Correlation coefficient ( $r$ ) determined by Least Square Fit.

$$\text{Exponentially } y = ae^{bx} \quad r = n\sum_{XY} - \sum_X \sum_Y / (n\sum_X^2 - (\sum_X)^2)(n\sum_Y^2 - (\sum_Y)^2)^{1/2}$$

$$\text{Linear } y = mx + b$$

$$r = \frac{\sum_{i=1}^n (X_i - \bar{X})(Y_i - \bar{Y})}{\left( \sum_{i=1}^n (X_i - \bar{X})^2 \sum_{i=1}^n (Y_i - \bar{Y})^2 \right)^{1/2}}$$

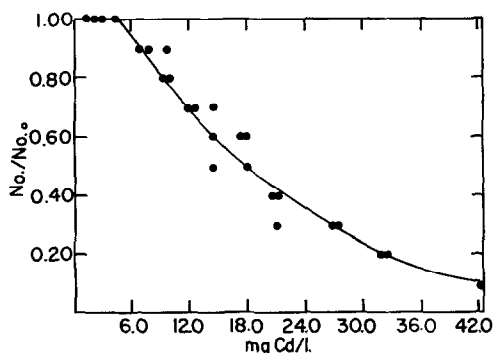


FIG. 2. Ninety-six hour median tolerance limit data for the stonefly, *Pteronarcella badia* (Hagen).

these data are given for day one through day four. Rate of cadmium loss, in *Pteronarcys*, in the supernatant and sediment are given in Tables 8 and 9. Correlation coefficients were determined for the entire four day period.

### DISCUSSION

Mortality rates consistently increased with increased cadmium concentrations in all species tested, except *Arcynopteryx*. Variability in the sensitivity of *Arcynopteryx* to cadmium was observed from sample to sample. Data concerning this organism are given for the maximum survival observed; therefore, no definitive statement may be made concerning the absolute sensitivity of this organism to cadmium.

Regardless of the cadmium concentration tested or the species of insect involved, no mortality was observed for at least 48 hours in those insects collected after November. However, with insects collected prior to November, mortality was observed after 24 hours; thus such insects appear to be more sensitive to cadmium. This would suggest that insects in the early stages of development are more sensitive to cadmium.

Long-term studies indicate the incidence of molting is reduced in those insects subjected to cadmium compared to controls. *Pteronarcys* and *Arcynopteryx*

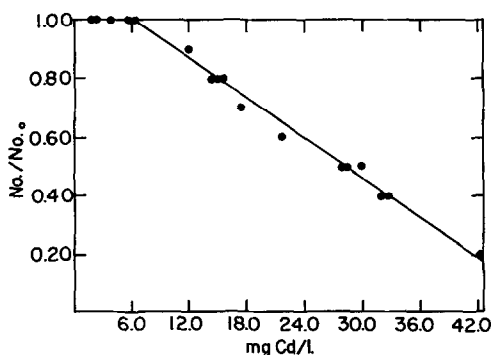


FIG. 3. Ninety-six hour median tolerance limit data for the mayfly, *Ephemerella grandis grandis* Eaton.

TABLE 3  
 CADMIUM TOXICITY FOR DIFFERENT SIZE CLASSES OF THREE INSECT SPECIES

Species	Collected before November			Collected after November		
	Average weight of insect	Cadmium concentration	Percent survival @ 2 weeks	Average weight of insect	Cadmium concentration	Percent survival @ 3 weeks
<i>Acroneuria pacifica</i>	133.4 mg	2.5 mg/liter	50	231.1 mg	6.0 mg/liter	60
<i>Pteronarcella badia</i>	30.6 mg	4.0 mg/liter	40	83.0 mg	6.0 mg/liter	60
<i>Arcynopteryx signata</i>	75.4 mg	2.5 mg/liter	60	129.1 mg	8.0 mg/liter	60

were the only insects to exhibit successful emergence; however, the emergence of these insects occurred within the first two days of the experiment. It would appear from these observations, that insects may not exhibit physiological death, but are ecologically dead, since the ability to molt or emerge was inhibited.

### PULSE

Both *Pteronarcys* and *Pteronarcella* show a rapid increase in the amount of cadmium adsorbed, in both the supernatant and sediment following 24 hours of treatment in water containing cadmium. In successive days the rate of adsorption decreased in both fractions. When tap water was exchanged for the cadmium-containing water, a linear decrease in cadmium was observed in the supernatant and sediment.

The rate of adsorption and the rate of loss in *Pteronarcys* were conducted in

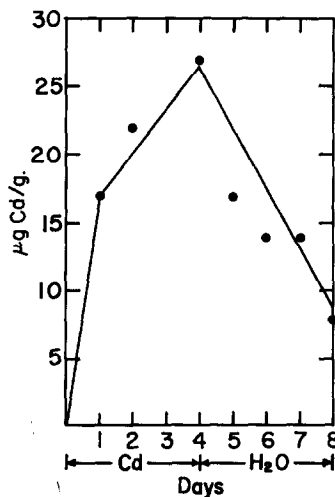


FIG. 4. Cadmium uptake and loss in the supernatant of *Pteronarcella badia* (Hagen), when exposed to 5.0 mg Cd/liter for 4 days, then placed in tap water for an additional four days. Correlation coefficient for each case  $\geq 0.93$ .

TABLE 4  
THE RATE OF CADMIUM UPTAKE IN THE SUPERNATANT OF *Pteronarcella badia*

Cadmium concentration	Rate of uptake first 24 hours $\mu\text{g/g/day}$	Rate of uptake during days 1-4 $\mu\text{g/g/day}$	Correlation coefficient days 1-4
9.0 mg/liter	19.0	-0.005	0.26

TABLE 5  
THE RATE OF CADMIUM LOSS IN THE SUPERNATANT OF *Pteronarcella badia* AFTER FOUR DAYS OF EXPOSURE TO CADMIUM, WHEN PLACED IN TAP WATER

Cadmium concentration	Rate of loss $\mu\text{g/g/day}$	Correlation coefficient
3.0 mg/liter	-0.75	0.99

TABLE 6  
THE RATE OF CADMIUM UPTAKE IN THE SUPERNATANT OF *Pteronarcys californica*

Cadmium concentration	Rate of uptake first 24 hours $\mu\text{g/g/day}$	Rate of uptake during days 1-4 $\mu\text{g/g/day}$	Correlation coefficient days 1-4
5.0 mg/liter	1.90	-0.01	0.48
3.0 mg/liter	1.45	-0.06	0.51

TABLE 7  
THE RATE OF CADMIUM UPTAKE IN THE 3000g SEDIMENT OF *Pteronarcys californica*

Cadmium concentration	Rate of uptake first 24 hours $\mu\text{g/g/day}$	Rate of uptake during days 1-4 $\mu\text{g/g/day}$	Correlation coefficient
5.0 mg/liter	320.0	84.0	0.98
3.0 mg/liter	220.0	64.0	0.66

TABLE 8  
THE RATE OF CADMIUM LOSS IN THE SUPERNATANT OF *Pteronarcys californica* AFTER 4 DAYS OF EXPOSURE TO CADMIUM, WHEN PLACED IN TAP WATER

Cadmium concentration	Rate of loss $\mu\text{g/g/day}$	Correlation coefficient
5.0 mg/liter	-1.64	0.99
3.0 mg/liter	-0.49	0.95

TABLE 9  
THE RATE OF CADMIUM LOSS IN THE 3000g SEDIMENT OF *Pteronarcys californica*  
AFTER 4 DAYS OF EXPOSURE TO CADMIUM, WHEN PLACED IN TAP WATER

Cadmium concentration	Rate of loss $\mu\text{g/g/day}$	Correlation coefficient
5.0 mg/liter	-46.0	0.98
3.0 mg/liter	-15.5	0.98

separate experiments, during February, 1972 and June, 1973, respectively. Although the absolute values differed, all data revealed similar results. Variation in the absolute values may be attributed to the time differential in sampling or variation in size class of insects used in the different experiments.

The rate of adsorption and loss in *Pteronarcys* were conducted in a continuous experiment at 5.0 mg/liter. Rates of adsorption and loss at other cadmium concentrations were conducted in separate experiments throughout 1973.

These data suggest the following; insects may recover from an accidental cadmium spill in a stream environment, if: (1) the insects were mature, (2) lethal cadmium concentrations were not attained, (3) rate of cadmium loss does not change significantly beyond four days, and (4) other physical-chemical parameters of the environment are not altered.

#### SUMMARY

Four conclusions may be drawn from these data: (1) There was an observed variability in cadmium toxicity in all the aquatic insects tested. (2) *Pteronarcys* was the most sensitive to cadmium of the insects tested. (3) The stage in the life history of the organism effects the sensitivity of that organism to cadmium. (4) Insects subjected to an accidental cadmium spill may recover from the detrimental effects of cadmium.

In the establishment of water quality criteria for aquatic organisms, with regard to cadmium toxicity, acute toxicity studies are only the first step. Chronic exposure studies as well as pulse experiments are necessary to determine the toxicity of a pollutant. In addition, these parameters should be studied at all stages of the life history of the organism. Synergistic and antagonistic studies among common variables such as dissolved oxygen, pH, temperature, alkalinity are also needed. Results of such studies may establish firm standards which will permit the growth, survival, and reproduction of all aquatic life.

#### ACKNOWLEDGMENT

This research was sponsored in part by E.P.A. Training Grant No. W. P. 242-04 S1 T900141.

#### REFERENCES

- American Public Health Association, Inc. (1971). "Standard Methods for the Examination of Water and Waste Water" 13th Edition. American Public Health Assoc., New York, 874 pp.
- Chemical Rubber Company (CRC) Press (1971). "Cadmium in the Environment" (L. Friberg, M. Piscator, and G. Nordberg, Eds.). CRC, Cleveland, OH 166 pp.
- P. Doudoroff, and M. Katz (1953). Critical review of literature on the toxicity of industrial wastes and their components to fish. II. The metals as salts. *Sewage and Industrial Wastes* 25, 802-839.

- A. R. Gaufin (1973). Water quality requirements of aquatic insects. Ecological Research Series, EPA-660/3-73-004. 89 pp.
- C. H. Hill, G. Matrone, W. L. Payne, and C. W. Barber (1963). *In vitro* Interactions of Cadmium with Copper, Zinc, and Iron. *J. Nutr.* **80**, 227-235.
- J. McCaull (1971). Building a shorter life. *Environment* **13**, 3-41.
- D. I. Mount, and R. E. Warner (1965). A Serial-dilution apparatus for continuous delivery of various concentrations of materials in water. U.S. Public Health Service, Environmental Health Series, WSPC, PHS Publication 999-WP-23.
- Q. H. Pickering, and C. Henderson (1965). The acute toxicity of some heavy metals to different species of warm water fishes. *Proc. 19th Ind. Waste Conf., Purdue Univ.* **35**, 578-591.
- D. W. Schraer (1972). The effects of heated water on homeostasis, survival and emergence of selected aquatic insect fauna. Doctoral Dissertation, University of Utah. 188 pp.
- H. A. Schroeder, A. P. Nasen, I. H. Tipton, and J. J. Balassa (1967). Essential trace metals in man: Zinc, relation to environmental cadmium. *J. Chronic Disease* **20**, 179-210.
- S. L. Warnick, and H. L. Bell (1969). The acute toxicity of some heavy metals to different species of aquatic insects. *J. Water Poll. Cont. Fed.* **41**, 280-284.