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I am submitting herewith a thesis written by Richard L. Sayrs, Jr. entitled "Lethal and Sublethal Effects of Cadmium on Daphnia (Crustacea : Cladocera)." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

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LETHAL AND SUBLETHAL EFFECTS OF CADMIUM ON DAPHNIA (CRUSTACEA: CLADOCERA)

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee

Richard L. Sayrs, Jr.

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ABSTRACT

Acute and chronic toxicity studies were performed to determine the effects of cadmium on *Daphnia pulex*, Leydig 1860 emend. Richard 1896, *D. magna* Straus 1820, and *D. parvula*, Fordyce 1901, three cladocerans commonly found in fresh water. Test specimens were exposed to several series of concentrations of cadmium sulfate for 96 hours in the acute toxicity studies. The 24-, 48-, 72- and 96-hour 50 percent lethal doses (LD50's) were calculated by probit analysis using a computer program. Mortality was defined as immobilization. The 96-hour LD50's for *D. pulex* ranged from 0.213 to 0.064 mg/liter. The 96-hour LD50 could not be determined for *D. parvula*; however, the 72-hour LD50 was 0.183 mg/liter. The 96-hour LD50 for *D. magna* was 0.086 mg/liter. All LD50's were reported in terms of the salt concentration.

Daphnia pulex was selected for long-term (chronic) study and was exposed to two concentrations of cadmium sulfate (0.012 and 0.025 mg/liter) at two temperatures (15.5 and 19.5 C). Molting, hatching of young, number of young, and mortality were observed every six hours for four weeks. Molting appeared to be a critical time for *Daphnia* exposed to cadmium sulfate. Dosed animals showed significantly higher mortality rates and produced 50 percent fewer young than the controls. The experimental temperatures did not alter the toxicity of cadmium.

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CHAPTER I

INTRODUCTION

General

The discharge of heavy metals by industry represents a serious water pollution problem due to the toxic properties of these metals and their potentially adverse effects on the aquatic environment. Studies of the toxicity of heavy metals to freshwater organisms have dealt largely with fish. There have been relatively few toxicity studies on aquatic invertebrates. It has been shown, however, that some zooplankton are more sensitive to heavy metal cations than fish (Anderson, 1950; Freeman and Fowler, 1953; Biesinger and Christensen, 1972). Since zooplankton provide a major source of food for many species of fish, elimination of zooplankton may result in a decrease in numbers of zooplanktivorous fish at heavy metal concentrations which may not directly affect the fish. The present study was undertaken to determine the effect of one of the heavy metals, cadmium, on three species of the cladoceran genus *Daphnia*.

Cadmium

Cadmium is a soft bluish-white metal with an atomic weight of 112.4 and an atomic number of 48. Cadmium occurs naturally in small quantities associated with zinc ores such as sphalerite. It forms a number of salts of which the sulfate is the most common. Electroplating accounts for about 60 percent of its commercial use, although it is extensively used in the manufacture of bearing alloys. The sulfide salt is also used as a yellow pigment in paint. The major source of cadmium in the aquatic environment is the effluents from cadmium electroplating plants.

Cadmium and solutions of its compounds are extremely toxic. The presence of cadmium at concentrations exceeding the U. S. Public Health Service guideline of 0.01 ppm. was reported in a survey of the freshwater systems of the United States (Kopp and Kroner, 1968).

Test Animals

Species of the cladoceran genus *Daphnia* have several advantages as test organisms for bioassay. Most are large, 1.0 to 2.5 mm in length, and may be observed without the aid of a microscope. *Daphnia* reproduce parthenogenetically during favorable conditions; therefore, clones can be established with resultant reduction in genetic variability. Newly hatched young can reach adulthood in a relatively short time, 6 to 11 days at 20 and 15 C, respectively (Robertson, 1971). Molting and egg laying are easily observed without the use of microscopic techniques. *Daphnia* are filter feeders and thus are relatively easy to feed.

The life cycle of the typical cladoceran consists of reproduction by parthenogenesis throughout most of the life span until unfavorable conditions (lack of food, high temperatures, buildup of metabolic waste) induce the appearance of males, with resultant production of fertilized eggs. The fertilized eggs are larger and only a few are produced in a single clutch. In *Daphnia*, the walls of the brood chamber are transformed into a protective capsule (ephippium) that is cast off at the next molt. The ephippia either float to the surface or sink to the bottom and can withstand drying and freezing until favorable environmental conditions return at which time the ephippial eggs hatch.

Terminology

A wide variety of terms and symbols have been used in various kinds of toxicity work. The concentration lethal to or affecting 50 percent of the test population has been called LD50 (median lethal dose), LC50 (median lethal concentration) and TLM (median tolerance limit). LD50 will be used in this paper except when citing other work.

The incipient lethal level, lethal threshold concentration, ultimate median tolerance limit, and incipient LD50 are terms which have been used to describe the concentration of toxicant "beyond which 50 percent of the population cannot live for an indefinite time" (Fry, 1947). The incipient LD50 will be used in this paper.

Immobility, inability to swim when stimulated by a small probe, rather than death was chosen for the end point in these studies because it was more reliably determined.

CHAPTER II

LITERATURE REVIEW

Research involving the effects of cadmium on aquatic organisms has generally been performed on fish. Toxicity of cadmium to fish was extensively reviewed by Douodorff and Katz (1953); Rudolfs, et al. (1950) and McKee and Wolf (1963). Methodology in bioassay research was well covered in the papers of Sprague (1969, 1970, 1971) which also included analysis and interpretation of results. However, information on the toxicity of cadmium to aquatic invertebrates, especially the freshwater Crustacea, is very limited.

Warnick and Bell (1969) studied the acute toxicity of heavy metal salts to three species of aquatic insect larvae (*Acroneuria lycorias*, a stonefly; *Ephemerella subvaria*, a mayfly; and *Hydropsyche betteni*, a caddis fly). The mayfly was the most sensitive insect tested to all metals used. The other two species of insect larvae were less sensitive than the mayfly and many species of fish. The 96-hour LC50 for *E. subvaria* was 2.0 mg/liter cadmium.

Clubb (1974) studied the biochemical and physiological aspects of cadmium toxicity on nine species of aquatic insects. The stonefly, *Pteronarcella badia* and the mayfly *Ephemerella grandis* were the most sensitive of the nine species tested. The 96-hour TLM's were 18.0 and 28.0 mg/liter cadmium, respectively. Long term studies showed that molting and emergence were reduced at sublethal concentrations of cadmium. Clubb (1974) also found a decrease in cadmium toxicity with a decrease in oxygen concentration.

The acute toxicity bioassays of Thorp and Lake (1974) using cadmium sulfate at 15 C resulted in 96-hour LD50's of 0.04 mg/liter cadmium for the amphipod *Austrochiltania subtenuis*; 0.06 mg/liter cadmium for the shrimp *Paratya tasmaniensis*; and 0.84 mg/liter cadmium for the mayfly nymph *Atalophlebia australis*. Thorpe and Lake (1974) also reported seasonal differences in the toxicity of cadmium to the shrimp *P. tasmaniensis*.

Rehwoldt, et al. (1973) reported ranges of LC50's of heavy metal ions to a variety of benthic invertebrates. Cadmium LC50's ranged from a low of 0.07 ppm for the amphipod *Gammarus* sp. to a high of 11.0 ppm for an unidentified damsel fly. An interesting result of the work was that although the eggs of the gastropod *Amnicola* sp. were less sensitive to copper, zinc, nickel, mercury and chromium than the adults, they were more sensitive to cadmium than were the adults. The LC50 for the eggs was 3.8-5.1 ppm cadmium and 8.4-10.1 ppm cadmium for the adults.

Three species of zooplankton were tested for the acute toxicity of various metals including cadmium, copper, lead, zinc, and mercury by Baudouin and Scoppa (1974). The 48-hour LC50's with 95 percent confidence limits were reported. The 48-hour cadmium LC50 for *Cyclops abyssorum* was 2.5 mg/liter cadmium, while that for *Eudiaptomus padanus* and *Daphnia hyalina* was 0.50 mg/liter cadmium and 0.005 mg/liter cadmium, respectively. <u>Daphnia hyalina was 100 times more sensitive to cadmium than was E. padanus</u> and 500 times more sensitive to cadmium than *C. abyssorum*.

Anderson (1950) established threshold concentrations of 25 cations toxic to *Daphnia magna*. Threshold concentrations were estimated from concentration-immobilization time curves for exposures of 64 hours. Molting was a particularly sensitive stage for *D. magna* exposed to certain of the cations including cadmium. The incipient LD50 was 0.0026 ppm as cadmium chloride.

Bringmann and Kuhn (1959) reported a 48-hour LC50 of 0.10 mg/liter cadmium for *Daphnia magna* in a study of the toxic effect of many pollutants to bacteria, algae, and crustacea in River Havel water.

Sporn, et al. (1970) reported an LD50 of 0.1 mg/liter of cadmium chloride to *Daphnia magna*.

The effects of cadmium on survival, growth, and reproduction in *Daphnia magna* were studied by Biesinger and Christensen (1972). In exposures lasting three weeks, reproduction was reduced by 16 percent at a concentration of 0.0005 mg/liter cadmium in one generation. A decrease in the growth rate was found at concentrations which allowed survival but impaired reproduction.

CHAPTER III

MATERIALS AND METHODS

General

Acute toxicity studies and long-term studies of the effects of cadmium were conducted under laboratory conditions. *Daphnia pulex* Leydig 1860 emend. Richard 1896, collected from a small temporary pond in Jefferson County, Tennessee, were selected for use in both the acute toxicity studies and the long-term toxicity studies. They were cultured for six weeks in the laboratory prior to testing. *Daphnia magna* Straus 1920 were originally purchased from a commercial biological supply firm and were cultured for approximately five months prior to use as test organisms. *Daphnia parvula* Fordyce 1901 were originally collected from Fort Loudoun Reservoir and cultured for several months prior to use. The three cultures of *Daphnia* were fed a combination of Fleischmann's active dry yeast and an algal culture of *Scenedesmus* sp.

All glassware utilized during the study was prepared by soaking in dilute chromic acid, followed by washing in Alconox detergent. The glassware was thoroughly rinsed in tap water and then thoroughly rinsed three times in distilled water.

Culture water and test media were prepared by boiling Knoxville, Tennessee city water for one hour to remove all traces of chlorine. This water was allowed to stand at room temperature for 24 hours and then aerated.

Chemical analyses of the culture water were performed prior to the start of the experiments using the procedures described in the Hach Chemical Company Manual. The following parameters were analyzed: alkalinity, calcium hardness, total hardness, chlorine, iron, ammonia nitrogen, nitrate nitrogen, nitrite nitrogen, and total phosphate. The pH of the culture water was measured using a Beckman model-72 pH meter. All toxicity studies were performed at 15.5 $\pm$ 1 C and/or 19.5 $\pm$ 1 C in Precision Scientific incubators.

Acute Toxicity Studies

A total of eight acute toxicity studies were conducted using the three species of *Daphnia*, two temperature regimes, and various doses of cadmium sulfate salt ($3CdSO_4 \cdot 8H_2O$). *Daphnia pulex* was exposed to the series of concentrations of cadmium sulfate shown in Table I, Experiments 1, 2, 5, and 8. Experiments 1, 2, 3, and 7 were conducted at 15.5 C while Experiments 5, 8, and 10 were performed at a temperature of 19.5 C. *Daphnia parvula* was tested at 15.5 C only and was exposed to the series of concentrations of cadmium in Experiments 3 and 7 (Table I). *Daphnia magna* was only tested at 19.5 C at the concentrations shown in Table I, Experiment 10. The concentration for each dose was prepared by adding appropriate volumes of a stock solution of cadmium sulfate to the culture water in a one liter volumetric flask and diluting to one liter with additional culture water. This water was then called the test medium. The stock solution was prepared by adding 0.1250 grams of A.C.S. reagent

TABLE I

Doses[1] of Cadmium Sulfate Used in the Acute Toxicity Experiments of Three Species of *Daphnia*

Dose	Exp. 1	Exp. 2	Exp. 3	Exp. 5	Exp. 7	Exp. 8	Exp. 10
1	2.000	0.500	0.625	0.500	0.625	0.750	2.500
2	1.000	0.375	0.500	0.438	0.500	0.625	2.000
3	0.500	0.250	0.250	0.375	0.250	0.500	1.000
4	0.250	0.125	0.125	0.312	0.125	0.375	0.750
5	0.188	0.094	0.094	0.250	0.094	0.250	0.500
6	0.125	0.062	0.062	0.188	0.062	0.125	0.250
7	0.062	0.031	0.031	0.125	0.031	0.004	0.125
8				0.094		0.062	0.062
9						0.031	

[1]All values in mg/liter as $3CdSO_4 \cdot 8H_2O$

grade cadmium sulfate salt to culture water and diluting to one liter in a volumetric flask.

Adult females with eggs were randomly selected from the appropriate laboratory culture and placed in a 500 ml beaker which contained a rinse solution of the test medium. A minimum of 40 females were selected for each dose and a minimum of 40 but up to 100 females were selected for the control. The *Daphnia* were transferred using a separate 150 ml Kahn Serum Transfer pipette for each dose including the control. After all the test animals had been transferred to the rinse containers, they were transferred one by one to 300 ml specimen dishes which were used for the toxicity tests. Ten *Daphnia* were placed in each test container. Test animals were not fed during the acute toxicity studies in order to minimize ingestion of cadmium. The acute toxicity studies were always performed at the same temperature at which the *Daphnia* had been cultured. In addition, the rinse media and test media were also maintained at the test temperature.

Observations were made each 24-hour period for 96 hours. The number of immobile *Daphnia* in each container was recorded. In addition, observations were made on the hatching of young, molting and erratic swimming behavior.

LD50's were calculated using probit analysis as discussed in Finney (1962). Results were corrected for mortality in the controls using Abbott's formula (Finney, 1962). Probit analyses were performed using a computer program written by Richard J. Daum and Wallace Killcreas at the Entomology Research Division of Mississippi State University.

Long-Term Studies Using Specimens of Unknown Age

Adult female *Daphnia pulex* with eggs were randomly selected from a laboratory culture of the appropriate temperature (15.5 C and 19.5 C) and tested individually at 0.012 mg/liter cadmium sulfate. The dose selected for this experiment was approximately half the 96-hour LD10 as estimated from Experiment No. 2. It was felt that this concentration was low enough to allow survival in the test animals over the long exposure period.

After all the *Daphnia* had been selected, they were placed in a rinse solution to prevent dilution of the test media and transferred individually to 8-dram shell vials. The shell vials were 25 x 95 mm and contained approximately 30 ml of test media. Controls were also selected randomly from the appropriate laboratory culture, placed in an intermediate rinse of culture water and then transferred individually to 8-dram shell vials.

Long-Term Studies using Specimens of Known Age

Zero to six-hour old young were selected from the controls of the unknown age experimental animals and were tested at two temperatures (15.5 C and 19.5 C) and two doses (0.025 and 0.012 mg/liter cadmium sulfate). The specimens were selected by first placing all the young which had hatched during the previous six hours in a 500 ml beaker of culture water. Test animals for this experiment were randomly selected and placed in a rinse solution and then transferred to 8-dram shell vials. Controls were handled the same way as the test animals.

Experimental animals and controls were fed one or two small grains of Fleischmann's active dry yeast and one ml of a green culture of *Scenedesmus* sp. daily. Each vial was cleaned daily and new test media or culture water was added to make up for that lost while cleaning.

Observations

Adult females and newly hatched *Daphnia pulex* from both experiments were observed every 6 hours for 4 weeks. The vials were checked in the same sequence each observation period in an attempt to make the time interval between observations as regular as possible. Molting, egg laying, hatching, death, and any signs of distress were recorded. In addition, the number and sex of the young were observed and recorded. Instar time, development time and length of life were calculated from the above observations. Instar time was calculated as the time interval between successive molts. Development time was calculated as the time interval between the appearance of eggs in the brood chamber and their release as young from the brood chamber. Since observations were made at six hour intervals, instar time and development time were estimated as the time interval between the observation of the first event (egg laying or molting) and the observation of the second event. The actual occurrence of an event could have happened any time between the observation period before the event and the observation in which the event was observed. Therefore, the estimates for instar and development time are within plus or minus six hours of the actual development or instar time.

CHAPTER IV

RESULTS AND DISCUSSION

Water Analyses

Chemical analyses of the dechlorinated tap water used in the experiments are summarized in Table II. The results are within the normal range of most of the rivers, reservoirs, and ponds in the lowlands of eastern Tennessee. The data in Table II compare favorably with the results of chemical analyses of the pond water from which the *Daphnia pulex* were originally obtained (Robertson, 1971).

Daphnia pulex was reported to live in water with a pH range of 6.1 to 10.3 by Davis and Ozburn (1969), though they suggest that a population would not thrive outside the range of 7.0 to 8.7. The pH was measured periodically during the experiments and ranged between 7.5 and 7.6.

Acute Toxicity Studies

Several preliminary experiments with various concentrations of cadimum sulfate resulted in the selection of a range of 2.0 to 0.312 mg/liter cadmium sulfate as test ranges for the acute toxicity studies. Three species of *Daphnia* were tested at two temperatures. The 24-, 48-, 72-, and 96-hour LD50's were calculated from the percent mortality and concentrations of cadmium sulfate using a computer program. The LD50 values and their 95 percent confidence intervals shown in Table III show the low concentrations of cadmium sulfate to which *Daphnia* were susceptible. The 96-hour LD50's ranged from 0.064 to 0.213 mg/liter as

TABLE II

Results of Chemical Analyses of Dechlorinated Knoxville, Tennessee City Water

Parameter	mg/liter
Alkalinity	90.4
Calcium hardness	81.3
Hardness total	121.0
Chlorine	0.0
Iron	0.06
Ammonia nitrogen	0.33
Nitrate nitrogen	0.65
Nitrite nitrogen	0.0
Phosphate total	0.67

TABLE III

LD50 Values* and 95 Percent Confidence Limits for the Acute Toxicity Experiments

	24 Hr. LD50	48 Hr. LD50	72 Hr. LD50	96 Hr. LD50
<u>Daphnia pulex</u>				
Exp. No. 1 15.5 C	0.4130 (0.324-0.530)	0.128 (0.042-0.214)	0.070 (0.037-0.100)	0.064 (0.023-0.096)
Exp. No. 2 15.5 C	N.S.	0.765 (0.464-2.347)	0.225 (0.176-0.302)	0.129 (0.102-0.158)
Exp. No. 5 19.5 C	N.S.	N.S.	0.267 (0.205-0.336)	0.083 (0.031-0.123)
Exp. No. 8 19.5 C	N.S.	4.150 (1.414-489.73)	0.560 (0.409-0.927)	0.213 (0.153-0.267)

TABLE III (Continued)

	24 Hr. LD50	48 Hr. LD50	72 Hr. LD50	96 Hr. LD50
Daphnia parvula				
Exp. No. 3 15.5 C	N.S.	N.S.	0.183 (0.103-0.244)	**
Exp. No. 7 15.5 C	N.S.	0.550 (0.278-2.284)	**	**
Daphnia magna				
Exp. No. 10 19.5 C	N.S.	0.535 (0.463-0.618)	0.156 (0.123-0.198)	0.086 (0.059-0.112)

* = All values expressed as mg/liter cadmium sulfate.
** = High mortality in the controls did not allow fitting a regression equation to those data.
N.S. = Nonsignificant regression.
( ) = 95 percent confidence limits.

cadmium sulfate. Anderson (1950) found that the 64-hour LC50 was less than 0.026 ppm cadmium chloride for Daphnia magna. This was considerably lower than the 0.086 mg/liter reported here. Baudouin and Scoppa (1974) reported 48-hour LC50 for Daphnia hyalina of 0.011 mg/liter as cadmium sulfate which is also much lower than the values found during these studies. Many investigators have reported that hard water with high calcium concentrations reduce the sensitivity of aquatic animals to zinc (Cairns and Scheier, 1957 and Lloyd, 1960). Pickering and Henderson (1965) found that cadmium was more toxic in soft water than in hard water due to the greater solubility of cadmium in low hardness water. Solubility of the cadmium sulfate in the present investigations was not a problem because of the low concentrations of cadmium used.

Due to high mortality in the controls, only two LD50's could be computed for Daphnia parvula. The 48-hour LD50 for D. parvula was 0.550 mg/liter cadmium sulfate while the 96-hour LD50 from another experiment was 0.183 mg/liter, (Table III). The steepness of the slopes of the two regression lines (Figure 1) for this species indicated that D. parvula may have a narrow range of tolerance to cadmium.

The 48-, 72-, and 96-hour LD50's for Daphnia magna (Table III) were 0.535, 0.156, and 0.086 mg/liter cadmium sulfate, respectively. Inspection of the regression lines (Figure 2) and confidence limits (Table III) indicated that the incipient LD50 may not have been reached at 96-hours

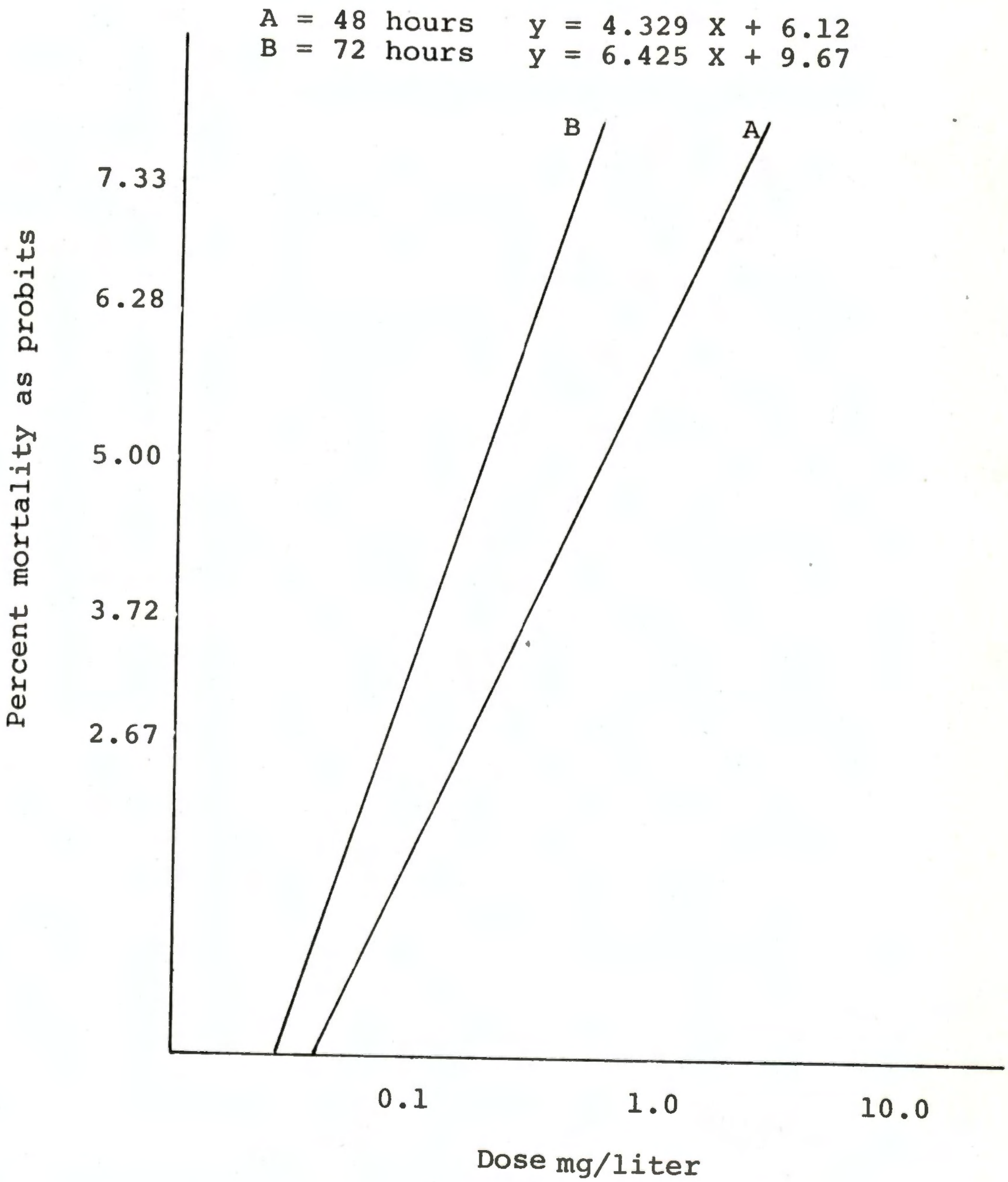

Figure 1. A comparison of 48- and 72-hour percent mortality of Daphnia parvula exposed to cadmium sulfate (Exp. No. 7, 15.5 C).

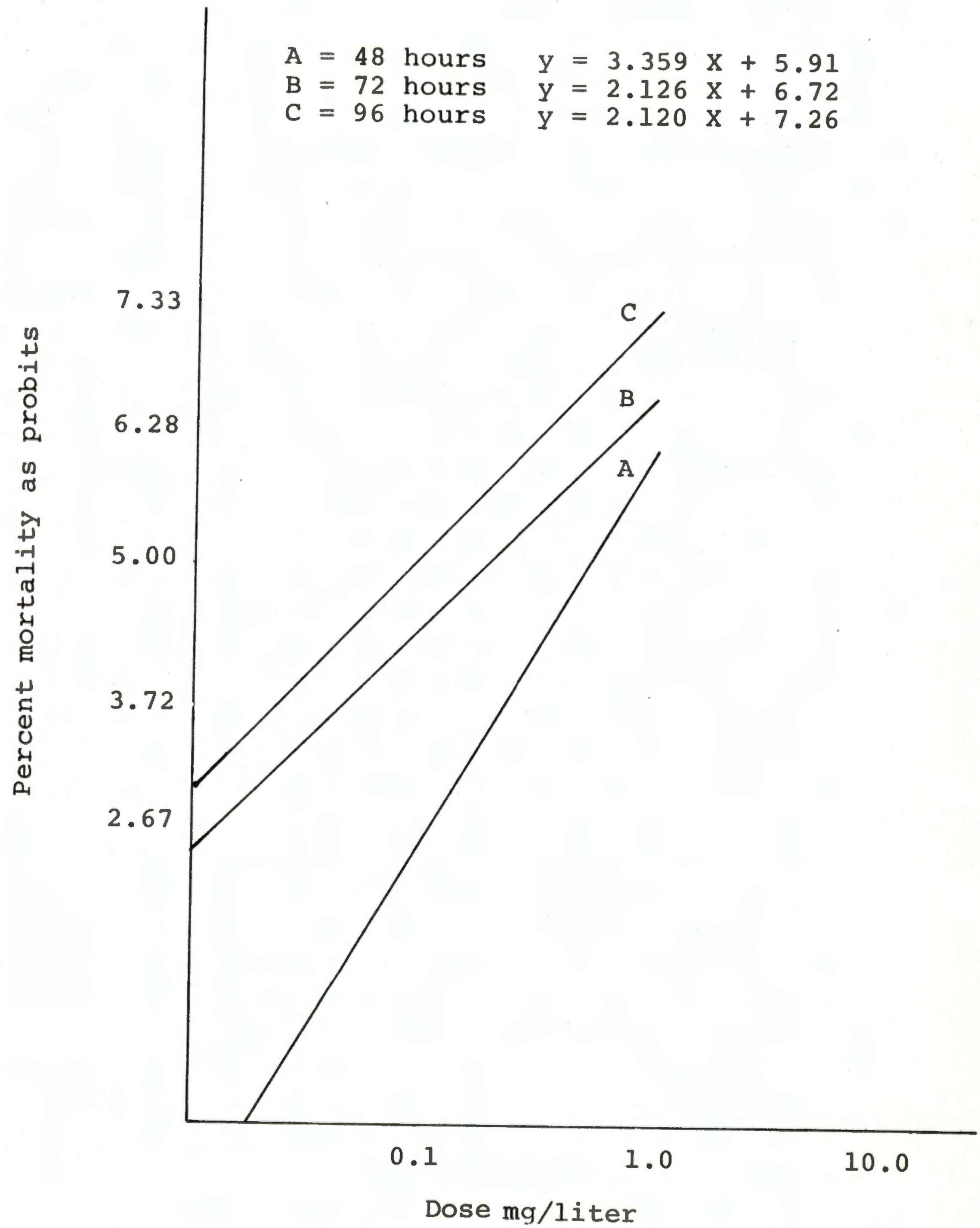

Figure 2. A comparison of 48-, 72-, and 96-hour percent mortality of Daphnia magna exposed to cadmium sulfate (Exp. No. 10, 19.5 C).

as suggested by Sprague (1969). However, the slopes were not as steep as those found with D. parvula indicating that D. magna may have a wider range of tolerance than the former species.

Daphnia pulex was tested at two temperatures and although the highest LD50 measured was at 19.5 C (Exp. No. 8), the results of the other experiments (No.'s 1 and 5) were not consistent enough to conclude that cadmium was less toxic at 19.5 C than at 15.5 C. Cairns and Scheier (1957) have shown that a 10 C increase in water temperature does not increase the toxicity of zinc to the bluegill sunfish, but Powers (1920) reported that a 10 C rise may halve the survival rate for fish when subjected to concentrations of heavy metal ions. Rehwoldt et al. (1973) concluded that there were no significant differences between the toxicity of cadmium at 28 C and 15 C to their test species.

Figures 3 through 6 show the cadmium sulfate LD50 regression lines for Daphnia pulex tested at the two temperatures. The 72- and 96-hour LD50's were not significantly different as determined by comparing the 95 percent confidence limits (Table III, p. 16) for Experiment 1. The 96-hour LD50 in Experiment 1 may therefore estimate the incipient LD50 as suggested by Sprague (1969). Comparing the confidence limits of the 72- and 96-hour LD50's in experiments 2 through 8 (Table III) indicated that the 96-hour LD50's in these experiments are not good estimates of the incipient LD50.

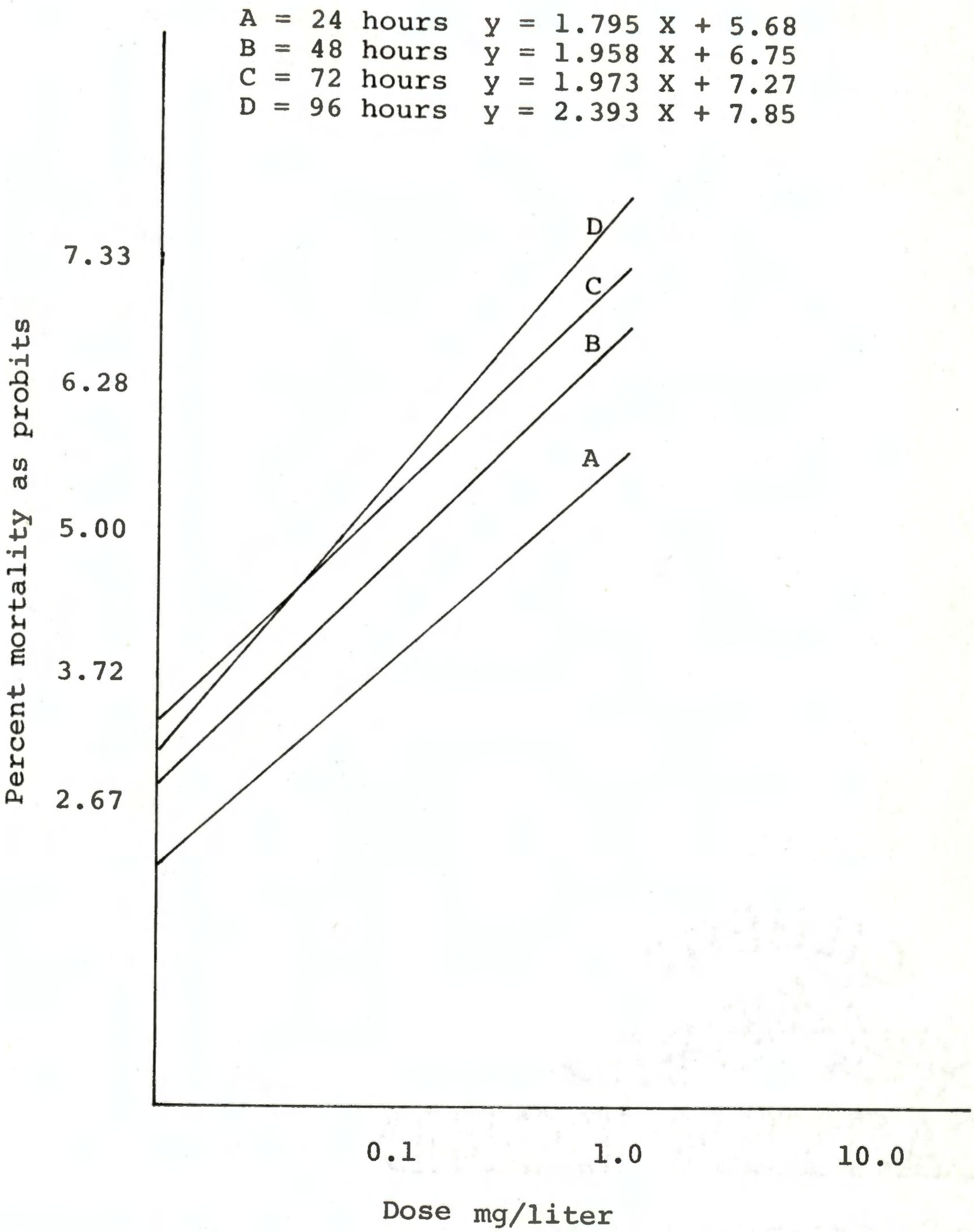

Figure 3. A comparison of 24-, 48-, 72-, and 96-hour percent mortality of Daphnia pulex exposed to cadmium sulfate (Exp. No. 1, 15.5 C).

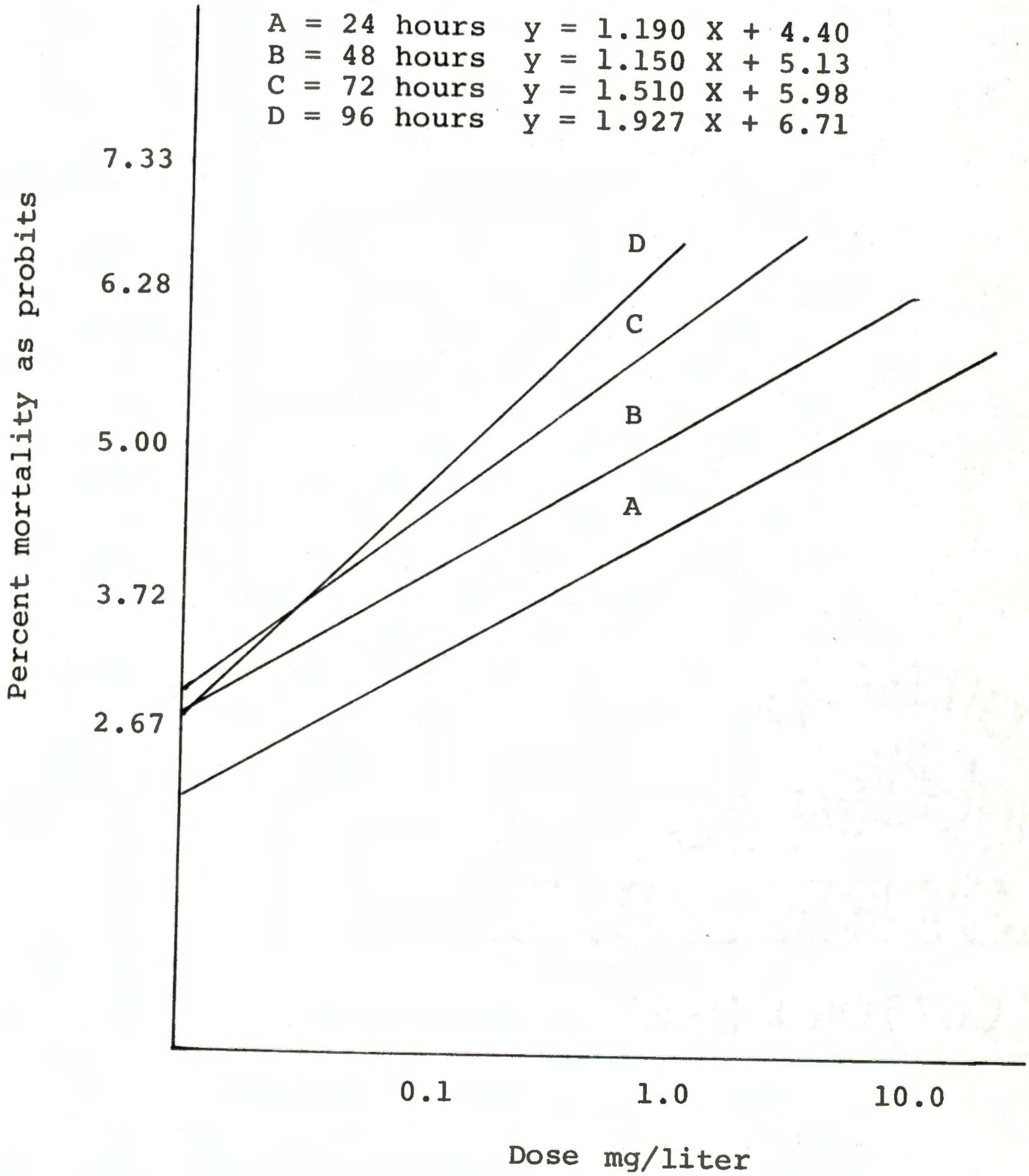

Figure 4. A comparison of 24-, 48-, 72-, and 96-hour percent mortality of Daphnia pulex exposed to cadmium sulfate (Exp. No. 2, 15.5 C).

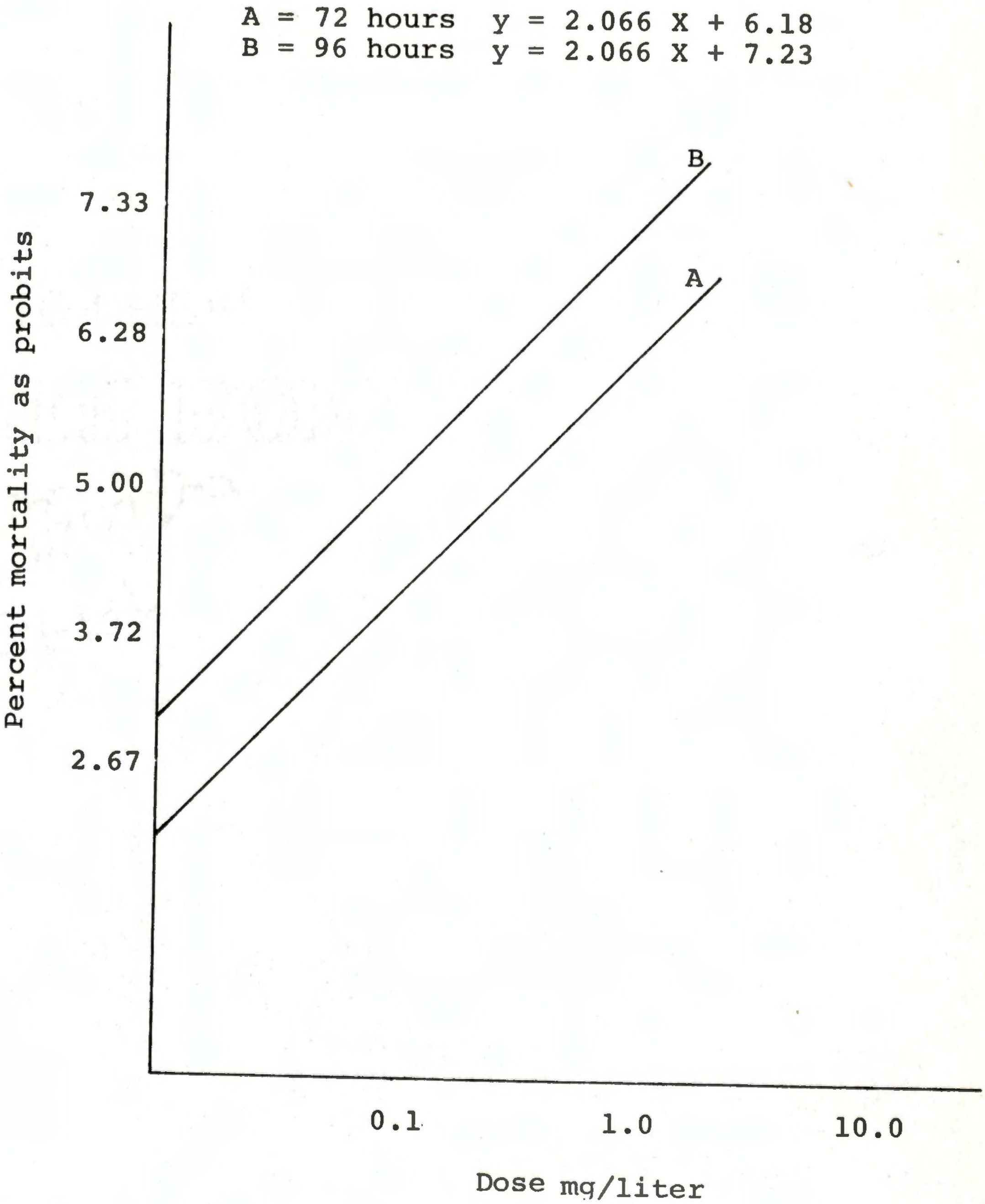

Figure 5. A comparison of 72- and 96-hour percent mortality of Daphnia pulex exposed to cadmium sulfate (Exp. No. 5, 19.5 C).

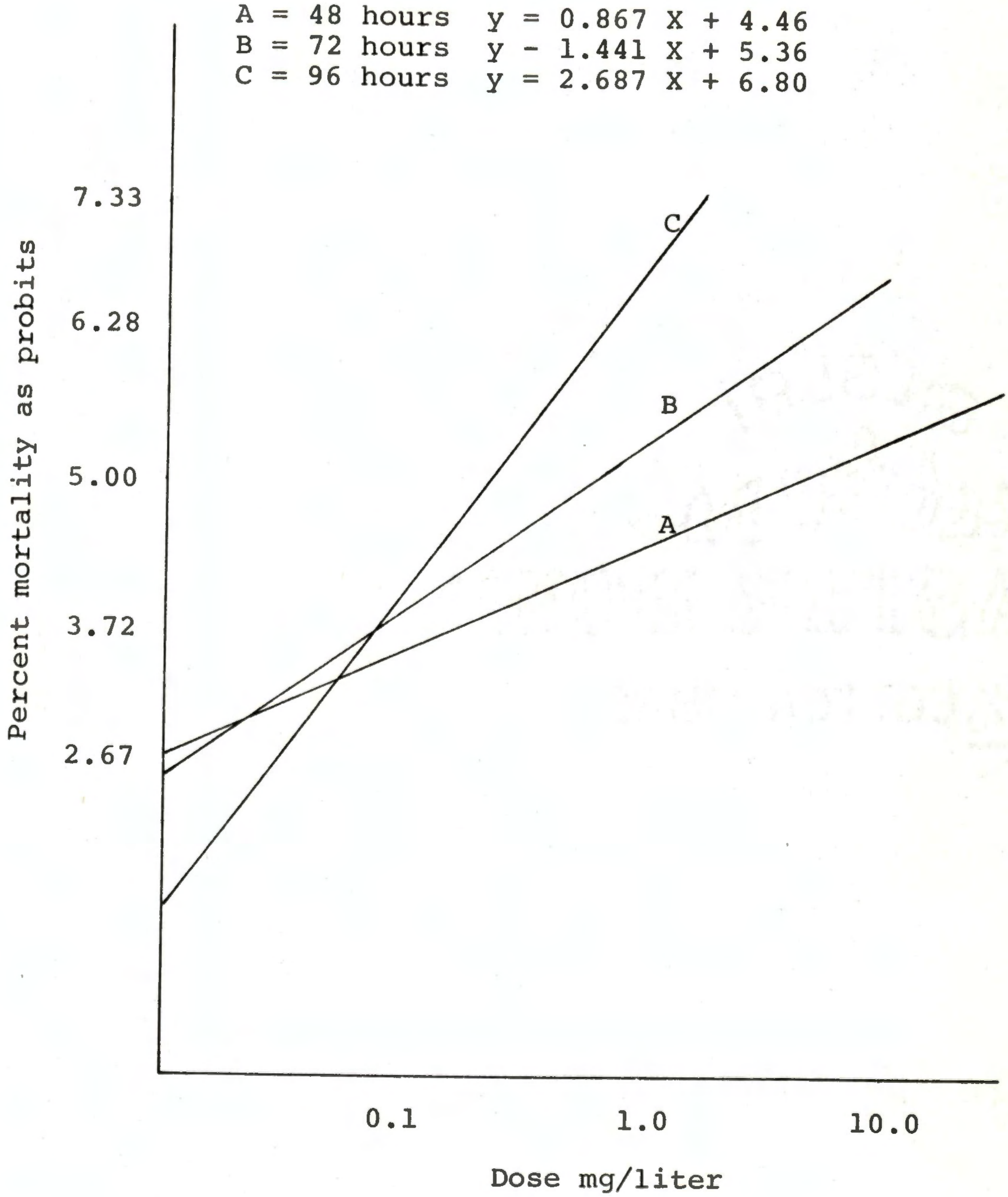

Figure 6. A comparison of 48-, 72-, and 96-hour percent mortality of Daphnia pulex exposed to cadmium sulfate (Exp. No. 8, 19.5 C).

A comparison of Figures 3 and 4 with Figures 5 and 6 did not reveal any major difference between the toxicity curves for D. pulex tested at 15.5 C and 19.5 C. Experiment No. 8 (Figure 6) was the only experiment in which the slope of the regression lines changed considerably between the 48-, 72-, and 96-hour tests.

Long-term Studies

Observations of individual *Daphnia pulex* in shell vials yielded several qualitative measures of the lethal and sublethal effects of cadmium sulfate. It was observed that animals subjected to doses of cadmium sulfate often became immobile during or right after they had either released young or molted. In contrast, the controls would usually live for several days after releasing young or molting before they became immobile. Dosed animals were often observed having trouble molting and would usually become immobile before the next observation period. Becker (1973) noted that *D. pulex* exposed to concentrations of zinc often had difficulty in molting, but did not usually become immobile for several days following molting.

Test animals often molted several times without ever laying eggs. They also often molted with the eggs or embryos still in the brood chamber. Cadmium sulfate dosed animals experienced long delays between hatching and molting and between molting and laying of eggs. Young were often hatched prematurely and eggs were sometimes reabsorbed or kicked out of the brood chamber.

Long-term Studies using Specimens of Unknown Age

Adult *Daphnia pulex* of unknown age were tested at two temperatures (15.5 C and 19.5 C) and one dose (0.012 mg/liter cadmium sulfate). The mean development time ($\bar{X}$), the standard deviation (S) and the number of animals (N) is shown in Table IV. The development times for test animals were less than those for the controls but Student-Newman-Keuls Multiple Range Test (Table V) revealed no significant differences at ($P<0.10$). There were however significant differences in development times between 15.5 C and 19.5 C (Table V). Robertson (1971) reported mean development times for adult *D. pulex* of 49.57 hours at 20 C and 82.25 hours at 15 C.

Mean instar times and their standard deviations for unknown age adult *D. pulex* are shown in Table VI. At 15.5 C the mean instar time was longer for the test animals (76.31 hours) than for the controls (74.53 hours). The reverse was true at 19.5 C with the control mean instar time (49.20 hours) longer than the mean instar time for test animals (47.82 hours). Student-Newman-Keuls Multiple Range Test (Table VII) applied to the data revealed a significant difference ($P<0.10$) between instar time for temperature, but not for dose. Data from Robertson (1971) indicated mean adult instar times of 53.25 hours and 87.22 hours for 20 C and 15 C, respectively. The differences between Robertsons' times and the times reported here were probably due to the slightly different temperatures at which the animals were tested.

TABLE IV

Mean Development Times in Hours and Standard Deviations for Unknown Age *Daphnia pulex*

		15.5 C	19.5 C
Control	$\bar{X}$	70.34	46.80
	S	17.22	14.13
	N	65	40
0.012 dose	$\bar{X}$	65.42	45.63
	S	17.41	11.12
	N	31	38

TABLE V

Results of Student-Newman-Keuls Multiple Range Test[a] Applied to Mean Development Times for Unknown Age *Daphnia pulex*

15.5 C		19.5 C	
Control	0.012 dose	Control	0.012 dose
70.34	65.42	46.80	45.63

[a]Any two means not underlined by the same line are significantly different at $P<0.10$.

TABLE VI

Mean Instar Times in Hours and Standard Deviations for Adult Unknown Age *Daphnia pulex*

		15.5 C	19.5 C
Control	$\bar{X}$	74.53	49.20
	S	14.92	12.18
	N	64	45
0.012 dose	$\bar{X}$	76.31	47.82
	S	20.00	9.18
	N	32	33

TABLE VII

Results of Student-Newman-Keuls Multiple Range Test[a] Applied to the Mean Instar Times for Adult Unknown Age *Daphnia pulex*

15.5 C		19.5 C	
0.012 dose	control	control	0.012 dose
76.31	74.53	49.20	47.82

[a]Any two means not underlined by the same line are significantly different at $P<0.10$.

A comparison of the 15.5 C instar time with the 15.5 C development time (Tables IV and VI) suggested that development time was shorter in the cadmium sulfate dosed animals than in the control, while the instar time was longer in those dosed with cadmium sulfate than in the controls. It appeared that the young were being hatched prematurely in the cadmium sulfate dosed specimens. The poorly developed young hatched from the dosed animals tended to confirm this hypothesis.

The mean number of young produced by unknown age adult female Daphnia pulex is presented in Table VIII and ranged from a high of 11.11 at 19.5 C in the control to a low of 8.75 at 15.5 C in the dose. These data were tested by Student-Newman-Keuls Multiple Range Test (Table IX) and were not statistically different ($P<0.10$). Robertson (1971) reported the mean brood number in her experiments to be 21.2 for 20 C and 16.4 for 15 C. Green (1956) found an average brood size for D. pulex of 10-12. Hutchinson (1967) reported that the number of eggs per clutch is dependent primarily on age, nutrition and genetic constitution of the mother. The small number of young produced by the unknown age Daphnia pulex may indicate that they were in poor condition or were fed below optimium levels. Since the age of the animals used in this experiment was not known, age could be the reason for the small average number of young produced as compared with Robertsons' data.

TABLE VIII

Mean Number of Young and Standard Deviations for Unknown Age *Daphnia pulex*

		15.5 C	19.5 C
Control	$\bar{X}$	10.98	11.11
	S	5.30	5.01
	N	186	155
0.012 dose	$\bar{X}$	8.75	10.17
	S	5.96	7.62
	N	32	51

TABLE IX

Results of Student-Newman-Keuls Multiple Range Test Applied to the Mean Number of Young Produced by Unknown Age *Daphnia pulex* [a]

Control		0.012 dose	
19.5 C	15.5 C	19.5 C	15.5 C
11.11	10.98	10.17	8.75

[a]Any two means not underlined by the same line are significantly different at $P<0.10$.

Table X shows the mean length of life for the unknown age adult female Daphnia pulex tested. The 15.5 C control animals lived more than twice as long as did the 19.5 C animals, 500 and 232 hours, respectively. The 15.5 C test animals lived slightly longer than the 19.5 C test animals. The mean lengths of life under the experimental conditions were tested for significance using Student-Newman-Keuls Multiple Range Test (Table XI). The 15.5 C control mean was significantly higher ($P<0.10$) than the other means.

Long-term Studies using Specimens of Known Age

Because age was an unknown factor in the previous study, known age Daphnia pulex were tested at two temperatures (15.5 C and 19.5 C) and two doses (0.012 and 0.025 mg/liter) of cadmium sulfate.

The mean development time for Daphnia pulex in the known age experiments is given in Table XII. The longest development time was 76.51 hours at 15.5 C in the controls while the shortest time was 52.22 hours at 19.5 C in the 0.025 dose of cadmium sulfate. The development times for the known age of D. pulex (Table XII) were longer than those of the unknown age D. pulex (Table IV, p.28). A possible explanation for the differences was that the development of the first or second brood of young takes longer due to the immaturity of the mother. It was noted by Robertson (1971) that development time at 10 C and 15 C was significantly longer for broods of adult instars one and two than for broods of adult instars three through seven.

TABLE X

Mean Length of Life in Hours and Standard Deviations for Unknown Age *Daphnia pulex*

		15.5 C	19.5 C
Control	$\bar{X}$	500.04	232.50
	S	104.12	44.00
	N	10	10
0.012 dose	$\bar{X}$	271.80	204.60
	S	123.94	54.22
	N	10	10

TABLE XI

Results of Student-Newman-Keuls Multiple Range Test[a] Applied to Length of Life for Unknown Age *Daphnia pulex*

15.5 C		19.5 C	
Control	0.012 dose	Control	0.025 dose
500.04	271.80	232.50	204.60

[a]Any two means not underlined by the same line are significantly different at P<0.10.

TABLE XII

Mean Development Times in Hours and Standard Deviations For Known Age *Daphnia pulex*

		15.5 C	19.5 C
Control	$\bar{X}$	76.51	54.37
	S	4.54	3.72
	N	123	119
0.012 dose	$\bar{X}$	73.33	53.47
	S	4.16	7.65
	N	3	15
0.025 dose	$\bar{X}$	72.00	52.22
	S	3.89	4.44
	N	20	18

Testing mean development times with Student-Newman-Keuls Multiple Range Test revealed no significant differences between doses ($P<0.10$). It was observed, however, that development time was of shorter duration in the dosed animals. These results agreed with those from the experiments on unknown age test animals.

Mean instar time for adults in the experiments with known aged Daphnia pulex ranged from 77.74 hours in the 15.5 C control group to 55.63 hours in the 19.5 C control group (Table XIII). Application of Student-Newman-Keuls Multiple Range Test showed a significant difference between the control and the 0.025 dose at 15.5 C (Table XIV). At 19.5 C however, the mean instar time in the 0.012 dose was of significantly longer duration than either the 0.025 dose or the control (Table XIV). Instar times were longer than development times in both doses at 19.5 C while they were only slightly longer in the controls. As observed in the experiments with unknown age D. pulex, the young may be hatched prematurely when exposed to these concentrations of cadmium sulfate.

The mean number of young produced by Daphnia pulex of known age under the various experimental conditions are shown in Table XV. The control animals produced the largest mean brood size, 9.60 and 11.58 young per brood at 15.5 C and 19.5 C, respectively. Testing of these means with Student-Newman-Keuls Multiple Range Test (Table XVI) showed that the mean number of young produced by the controls was significantly higher ($P<0.10$) than either dose at both temperatures. A larger number of young were produced by the D. pulex cultured

TABLE XIII

Mean Instar Times and Standard Deviations for Adult Known Age *Daphnia pulex*

		15.5 C	19.5 C
Control	$\bar{x}$	77.74	55.63
	S	7.33	5.29
	N	127	126
0.012 dose	$\bar{x}$	75.00	64.62
	S	10.13	7.89
	N	4	16
0.025 dose	$\bar{x}$	71.00	57.26
	S	16.38	8.69
	N	28	31

TABLE XIV

Results of Student-Newman-Keuls Multiple Range Test[a]
Applied to Mean Instar Time for Adult
Known Age *Daphnia pulex*

	Control	0.012 dose	0.025 dose
15.5 C	77.74	75.00	71.00
	0.012 dose	0.025 dose	Control
19.5 C	64.62	57.26	55.63

[a]Any two means not underlined by the same line are significantly different at $P<0.10$.

TABLE XV

Mean Number of Young and Standard Deviations for Known Age *Daphnia pulex*

		15.5 C	19.5 C
Control	$\bar{X}$	9.60	11.58
	S	4.27	5.98
	N	123	118
0.012 dose	$\bar{X}$	4.33	6.00
	S	2.52	2.80
	N	3	15
0.025 dose	$\bar{X}$	7.22	8.39
	S	2.73	5.34
	N	18	18

TABLE XVI

Results of Student-Newman-Keuls Multiple Range Test[a] Applied to the Mean Number of Young for Known Age *Daphnia pulex*

	Control	0.025 dose	0.012 dose
15.5 C	9.60	7.22	4.33
19.5 C	11.58	8.39	6.00

[a]Any two means not underlined by the same line are significantly different at P<0.10.

at 19.5 C than by those cultured at 15.5 C. The 0.025 mg/liter cadmium sulfate dosed animals also produced more young per clutch than did the 0.012 mg/liter cadmium sulfate dosed animals. Biesinger and Christensen (1972) reported that reproductive impairment was a more sensitive measure of toxicity than survival. The reduction in numbers of young produced between the controls and the 0.012 mg/liter cadmium sulfate dosed *Daphnia* was about 50 percent at both 15.5 C and 19.5 C. However, the reduction in numbers of young between the controls and 0.025 mg/liter cadmium dosed *Daphnia* was only 25 percent.

Table XVII presents the mean length of life for known age *Daphnia pulex*. Controls lived more than 100 hours longer than the cadmium sulfate dosed animals and the 0.025 mg/liter of cadmium sulfate dosed animals lived approximately 100 hours longer than the 0.012 mg/liter of cadmium sulfate dosed *D. pulex*. At 15.5 C the mean length of life for the controls and both doses were significantly different ($P<0.10$) from each other as revealed by Student-Neuman-Keuls Multiple Range Test (Table XVIII). However, at 19.5 C the control and the 0.025 dose were not significantly different, although they were both significantly different from the 0.012 dosed animals. *Daphnia pulex* lived longer at 15.5 C than at 19.5 C in all doses.

TABLE XVII

Mean Length of Life and Standard Deviations for Known Age *Daphnia pulex*

		15.5 C	19.5 C
Control	$\bar{X}$	428.7	358.2
	S	143.1	105.3
	N	39	32
0.012 dose	$\bar{X}$	213.5	173.1
	S	80.1	119.8
	N	22	30
0.025 dose	$\bar{X}$	320.0	283.0
	S	144.5	78.48
	N	16	14

TABLE XVIII

Results of Student-Newman-Keuls Multiple Range Test [a]
Applied to Length of Life for Known Age
Daphnia pulex

	Control	0.025 dose	0.012 dose
15.5 C	428.7	320.0	213.5
19.5 C	358.2	283.0	173.1

[a]Any two means not underlined by the same line are significantly different at $P<0.10$.

CHAPTER V

SUMMARY AND CONCLUSIONS

The LD50's determined during the acute toxicity experiments varied between species, temperatures, and experiments. Most of the LD50 concentrations were intermediate in value when compared to the literature. For example Baudouin and Scoppa (1974), Bringmann and Kuhn (1959), and Sporn et. al. (1970) reported LD50's higher than those found during these studies. However, Anderson (1950) and Biesinger and Christensen (1972) found 64-hour LD50's which were much lower than the LD50's determined during the acute toxicity experiments presented here.

The toxicity of cadmium to *Daphnia* was not significantly different at the two temperatures (15.5 C and 19.5 C) used during the short-term studies.

The LD50 concentrations decreased with time of exposure in all experiments. However the incipient LD50 could not be estimated at all times by the 96-hour LD50 as suggested by Sprague (1969). Therefore in acute toxicity bioassay involving species of *Daphnia*, longer exposure times of 120 to 168 hours should be used in order to estimate the incipient LD50 concentrations of cadmium.

Long-term studies of individual *Daphnia pulex* in small vials indicated that concentrations of cadmium sulfate of 0.012 and 0.025 mg/liter were sufficiently high to cause an increase in mortality and a reduction in the number of young per individual.

Test *Daphnia* exposed to 0.012 mg per liter of cadmium sulfate lived approximately 50 percent fewer hours than the controls. The 0.025 mg per liter dosed animals lived 25 percent fewer hours than the controls. Although the *Daphnia* tested at 15.5 C lived longer than those tested at 19.5 C there did not appear to be any difference in the toxic effect of cadmium sulfate between temperatures.

A critical time for *Daphnia* exposed to cadmium may be during molting. The cadmium sulfate dosed animals were often observed having difficulty during molting and were often found dead right after or in the process of molting. Anderson (1950) observed marked inflections in the concentration-immobilization time curves at the time when molting was expected for many of the chloride compounds that he tested.

The test animals during the long-term experiments appeared to hatch young prematurely and even ejected eggs out of the brood chamber. Evidence for the premature hatching of young was a shorter development time for dosed animals as compared to the controls and poorly developed young as revealed by microscopic examination.

The test animals produced 50 percent fewer young per clutch in the 0.012 mg per liter dose than in the controls. Although the number of young produced was not significantly different between the two doses of cadmium sulfate tested, the 0.012 dose appeared to be more toxic than the 0.025 dose.

The results presented in this paper indicate the need for further research on the effects of cadmium on aquatic invertebrates, especially members of the plankton community. Future work should concentrate on the long-term (chronic) effects of cadmium on reproduction and fecundity. Present water quality standards were based on bioassay work with fish and may not be realistic for *Daphnia* and other members of the plankton community.

LITERATURE CITED

LITERATURE CITED

Anderson, B.C. 1950. The apparent thresholds of toxicity to *Daphnia magna* for chlorides of various metals when added to Lake Erie water. Trans. Amer. Fish. Soc. 78:96-113.

Baudouin, M.F., and P. Scoppa. 1974. Acute toxicity of various metals to freshwater zooplankton. Bull. Env. Cont. Tox. 12(6): 745-751.

Becker, C.P. 1973. Some effects of temperature, calcium concentration, and age of animal on the toxicity of zinc sulfate to *Daphnia pulex* Leydig. The University of Tennessee M.S. Thesis. 101 p.

Biesinger, K.E., and G.H. Christensen. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. J. Fish. Res. Bd. Canada. 29: 1691-1700.

Bringmann, G., and R. Kuhn. 1959. The toxic effects of waste water on aquatic bacteria, algae, and small crustaceans. Gesundheits-Ing. 80:115-120.

Cairns, J. and Scheier, A. 1957. The effects of temperature and hardness of water upon the toxicity of zinc to the common bluegill (*Lepomis macrochirus* Raf.) Notul. Nat. No. 299. 12 p.

Clubb, R.W. 1974. Some aspects of cadmium toxicity upon several species of aquatic insects. University of Utah. Ph.D. dissertation. 71 p.

Davis, P., and G.W. Ozburn. 1969. The pH tolerance of *Daphnia pulex* (Leydig, Emend., Richard). Can. J. Zool. 47:1173-1175.

Doudoroff, P., 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 Industr. Wastes. 25:802-839.

Finney, D.J. 1962. Probit Analysis. Cambridge University Press, London. 318 p.

Freeman, L., and I. Fowler. 1953. Toxicity of combinations of certain inorganic compounds to *Daphnia magna* (Straus). Sewage Ind. Wastes. 25:1191-1195.

Fry, F.E.J. 1947. Effects of the environment on animal activity. Univ. Toronto. Stud. Biol. Ser. No. 55. Pub. Ontario Fish Res. Lab. No. 68. 62 p.

Green, J. 1956. Growth, size and reproduction in Daphnia (Crustacea: Cladocera). Proc. Zool. Soc. Lond. 126:173-204.

Hutchinson, G.E. 1967. A treatise on limnology. Volume II. Introduction to lake biology and the limnoplankton. John Wiley and Sons, Inc. 1115 p.

Kopp, J.F., and R.C. Kroner. 1968. A five year summary of trace metals in rivers and lakes of the United States (October 1, 1962 - September 30, 1967). Federal Water Pollution Control Admin., Cincinnati, Ohio.

Lloyd, R. 1960. The toxicity of zinc sulphate to rainbow trout. Ann. Appl. Biol. 48:84-94.

McKee, J.E., and H.W. Wolf, eds. 1963. Water quality criteria. Calif. State Water Quality Control Bd. Sacramento Pub. No. 3A. 548 p.

Pickering, Q.H., and C. Henderson. 1965. The acute toxicity of some heavy metals to different species of warm water fishes. Proc. 19th Indust. Waste Conf. Purdue Univ. 578-591.

Powers, E.B. 1920. Influence of temperature and concentration on the toxicity of salts to fishes. Ecology. 1:95-111.

Rehwoldt, R., L. Lasko, C. Shaw, and E. Wirhowski. 1973. The acute toxicity of some heavy metal ions toward benthic organisms. Bull. Env. Cont. Tox. 10(5): 291-294.

Robertson, S.D. 1971. The effects of temperature on instar duration and egg development of Daphnia pulex Leydig. University of Tennessee. M.S. Thesis.

Rudolfs, W., G.E. Barnes, G.P. Edwards, H. Heukelekian, E. Hurwitz, C.E. Renn, S. Steinberg, and W.F. Vaughan. 1950. Review of literature on toxic materials affecting sewage treatment processes, streams, and B.O.D. determinations. Sewage Industr. Wastes. 22:1157-1191.

Sporn, A., I. Dinv, L. Stoenescu, A. Cristea, and L.G. Ghizelea. 1970. The acute and subacute toxicity of cadmium. Igiena. 19(4):201-208.

Sprague, J.B. 1969. Review paper: Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3:793-821.

Sprague, J.B. 1970. Review paper: Measurement of pollutant toxicity to fish. II. Utilizing and applying results. Water Res. 4:3-32.

Sprague, J.B. 1971. Review paper: Measurement of pollutant toxicity to fish. III. Sublethal effects and "safe" concentrations. Water Res. 5:245-266.

Thorp, V.J., and P.S. Lake. 1974. Toxicity bioassays of cadmium on selected freshwater invertebrates and the interaction of cadmium and zinc on the freshwater shrimp, *Paratya tasmaniensis* Riek. Aust. J. Mar. Freshwater Res. 25(1): 97-104.

Warnick, S.L., and H.L. Bell. 1969. The acute toxicity of some heavy metals to different species of aquatic insects. J. Water Pollut. Contr. Fed. 41:280-284.

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