

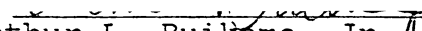
A COMPARISON OF TECHNIQUES FOR ESTIMATING THE HAZARD OF
CHEMICALS IN THE
AQUATIC ENVIRONMENT

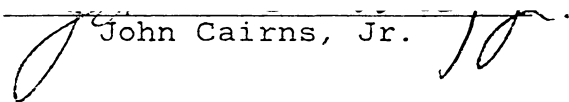
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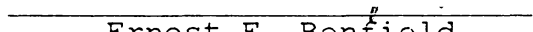
Barbara Ruth Niederlehner

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Zoology

APPROVED:


Arthur L. Buikema, Jr. Chairman


John Cairns, Jr.


Ernest F. Benfield

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A COMPARISON OF TECHNIQUES FOR ESTIMATING THE HAZARD OF CHEMICALS IN THE AQUATIC ENVIRONMENT

by

Barbara Ruth Niederlehner

(ABSTRACT)

Estimates of the concentration of cadmium constituting a threat to aquatic ecosystems were derived from laboratory tests conducted at two levels of the biological hierarchy. A population level estimate was derived from single species toxicity tests and a community level estimate was derived from laboratory tests on microbial communities. Estimates were compared to each other and to an ecosystem level estimate derived from reports of ecological health and ambient cadmium levels in rivers, lakes, and streams.

Estimates of permissible levels for short term exposures differed by an order of magnitude. Single species toxicity tests indicated that a level of 46.1 ug Cd/L would affect only 5% of taxa. The corresponding estimate from the community level test was 459.4 ug Cd/L. Similar estimates of permissible levels for chronic exposures were not significantly different (1.02 and 0.20 ug Cd/L, single species and community level tests, respectively). Both of the laboratory derived estimates of permissible levels for

chronic exposure fell within a rational range; the minimum level defined by median cadmium levels reported in healthy aquatic systems (0.05 ug Cd/L), and the maximum level defined by median cadmium levels reported in damaged aquatic systems (9.2 ug Cd/L). However, the community level estimate was obtained more efficiently, permitting an estimate of effects on diversity from a single test. Single species level tests, community level tests, and field studies each contributed unique information to hazard evaluation. Using information from all levels will strengthen predictions.

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Chapter I

INTRODUCTION

Biologists have been documenting pollution related damage to the aquatic environment with increasing frequency since the turn of the century. Concerns about the consequences of continued indiscriminate waste disposal were successfully communicated to the public, and, as a result, a body of legislation designed to protect human health and the environment from chemical insult was put into place in the 1970s (Tarlock 1979). However, legal challenges to the implementation of these programs have pointed out that effective pollution management techniques require a defensible, scientific basis (e. g. Cairns 1983). Although biologists can effectively document gross damage to aquatic ecosystems after the fact, proper management requires prediction of hazardous levels of pollution before damage has occurred. Management requires techniques that will enable the accurate and cost effective prediction of the concentration of a waste that will not harm an ecosystem.

The single species toxicity test as proposed in the 1940s (e. g. Hart et al. 1945; Doudoroff et al. 1951) has provided the great majority of data used in waste management attempts for aquatic systems. As a first approach, the

acute toxicity test with fish is exemplary because it demonstrates a clear effect on a response of obvious significance. Subsequent work has refined the single species toxicity test without major divergence (e. g. Buikema et al. 1982). The time scale and responses measured have been expanded to include less obvious but potentially important responses, and the variability of response across time, temperature, habitat, group or taxa, age or stage, and with changing water chemistry has been acknowledged and, in some cases, accommodated (e. g. USEPA 1980a).

However, a systematic bias may result from the sole use of single species toxicity tests in the prediction of ecosystem level effects (e. g. Cairns 1980; 1981; Odum et al. 1979; Giesey 1980). Conventional single species tests do not monitor behaviors and properties characteristic of the interactions between species or species and their environment yet these may be important in determining the response of ecosystems to stress. For example, single species toxicity tests do not address the functional redundancy in the ecosystem where the elimination or replacement of a single species is sometimes of little significance. Toxicants can impair the ability to capture prey or evade predation (e. g. Sullivan et al. 1978; Lawrence and Holka 1979), or effects on food and feeding

organisms can be so closely coordinated that there is no net effect (McAllister et al. 1972). Toxicants can impair the ability to resist disease (e. g. Couch and Courtney 1978; Knittel 1980). Harvesting pressure can interact with toxic stress (Woltering 1983). None of these interactive factors will be apparent in the course of a single species testing scheme yet they may be significant in the determination of effects on an ecosystem.

A hierarchical concept of biological systems provides a convenient theoretical framework for this problem (e. g. Odum et al. 1979; Cairns 1981; Buikema et al. 1982). Odum (1977), Webster (1978), and others, have described a hierarchy of the structure of biological systems, generally progressing from cell to organism to community and ecosystem. Each level is defined by the strength of its internal interactions. The levels of the hierarchy can be distinguished on the bases of size, speed, and complexity, with higher levels larger, slower, more complex, and composed of lower levels. At each level there are unique properties and behaviors that cannot be predicted apriori from properties and behaviors at lower levels. Organisms eat, reproduce, and are eaten, whereas ecosystems persist, cycle nutrients and energy, and progress through a successional path. The emergent properties of higher

hierarchical levels may be explained *a posteriori* by knowledge of the dynamics at the next lower level, but usually they cannot be predicted without an elaborate model. The consequences of this arrangement have been described (Webster 1978):

McFadyen (1975) suggested that the most obvious problems in ecology arise because ecology is concerned with at least two levels of organization. This should be turned into a strength rather than a weakness. All natural investigations should proceed at at least two levels... Behavior at any level is explained in terms of the level below, and its significance is found in the level above.

Given this, if the ultimate goal of pollution management strategies is the protection of ecosystems, exhibiting energy flow, diversity, etc., Population or single species level tests may be more successful in defining the mechanics of change and in explaining differences between toxicants, organisms, and conditions. For these reasons, it has been suggested that hazard evaluations can be significantly improved by the use of community or ecosystem level tests to complement the predominantly single species data base (e.g. Cairns 1980; Giesey and Odum 1980; Hammons 1981; NRC 1981; O'Neill and Waide 1981; USEPA 1979a).

Few studies have examined differences in toxic response corresponding to differences in hierarchical level. Hansen (1981) examined the effects of nalidixic acid on test

systems at 2 distinct levels of complexity. He determined the concentrations of nalidixic acid required to impair the growth rate of 2 strains of Escherichia coli in single species exposures and compared these values with the concentration necessary to affect the outcome of competition between the strains. The more complex, competitive exclusion test was chosen specifically because mechanistic models for this type of interaction existed. He found that the effect levels determined in the 2 test systems were virtually identical, and the single species tests were quicker and easier to conduct. However, he points out that "the range of the mechanistic approach is more limited; being applicable only to those interactions whose underlying mechanisms have been identified and experimentally proven."

The correspondence between levels may decrease as the lack of an adequate model for the interactions of components obscures the choice and translation of single species responses and as difference in complexity between the tests increases. In similar studies comparing the effects of PCBs on algal species tested alone and in competition, effect concentrations differed by 2 orders of magnitude (Mosser et al. 1972; Fisher et al. 1974). Hansen and Garton (1982) found that a battery of single species toxicity tests were somewhat successful in predicting the concentration of

diflubenzuron having an effect on laboratory stream communities, differing by an order of magnitude. However, the single species tests were less successful in predicting the nature and, thus, the significance, of community level effects.

Other researchers have evaluated the effects of chemicals on test systems at various levels of complexity, but have not bracketed effects levels in both systems (e. g. Davis and Woodling 1980; Eisele and Hartung 1976; Geckler et al. 1976; Hutchison and Czyrska 1975; Maki 1980; Weber and McFarlane 1981; Winner et al. 1975). And studies have evaluated effects using test systems at various level of complexity, but monitored only responses characteristic of populations in both system (e. g. Adams et al. 1983; Bailey 1982; Kettle et al. 1980; Marshall 1978). These data do not allow an evaluation of the magnitude of differences between effects levels as determined at various hierarchical levels.

The objective of this research was to independently estimate the deleterious effect level of a single toxicant, cadmium, at three levels of hierarchical complexity, and to compare these estimates in terms of their usefulness in pollution management. An estimate of an effect concentration was obtained from single species toxicity test

data by means of an abbreviated water quality criteria type protocol (Stephan et al. 1983, USEPA 1980a). Three aquatic invertebrates representing distinct structural or functional groups and spanning a range of sensitivities to toxic stress were tested in acute and chronic exposures. These determinations of the toxicity of cadmium to single species are presented in Chapters II through IV. A second type of estimate was derived from a laboratory toxicity test using an aquatic microbial community as the test system (e. g. Cairns et al. 1980; Buikema et al. 1983). The community level laboratory test was chosen to serve as an intermediate step between the simplicity of the single species toxicity test and the unreplicable complexity of the real world. Although similar in size and time scale to the single species tests, this test system was distinctly different in complexity. This work is presented in Chapter V. The final estimate of an effect level for cadmium was derived from literature describing experiences with cadmium in aquatic ecosystems. Levels of cadmium occurring in rivers, streams, and lakes strongly suspected of being damaged by cadmium were used to suggest a rational upper limit for laboratory derived estimates of permissible levels. Similarly, levels of cadmium in systems chosen for their ecological health or lack of anthropogenic inputs were used to suggest a rational

lower limit for laboratory derived estimates. This work is presented in Chapter VI. A comparison of the estimates and their specific applicability in hazard evaluation follows. The effective enforcement of existing legislation for the protection of human health and the environment from pollution damage is directly dependent on the availability of accurate and cost-effective hazard evaluation techniques. It is by continuous reevaluation of the merits of available testing techniques in reference to specified management goals that the hazard assessment process can be improved.

Chapter II

CADMIUM TOXICITY TO A CLADOCERAN

2.1 INTRODUCTION

The planktonic crustacean, Daphnia pulex, has become the most commonly tested animal in standard aquatic toxicity tests. This popularity stems primarily from the ease of culture, short lifecycle, and notable sensitivity to a variety of toxicants that characterize Daphnia. These characteristics permit an efficient and relatively inexpensive chronic test. The widespread use of daphnids in toxicity evaluations has also been justified by emphasizing their ecological importance. Daphnia is found worldwide in ponds, lakes, and slow moving rivers. They are size selective collector-filterers and feed primarily on algae, bacteria, and small particles of detritus suspended in the water column. Daphnids and other cladocerans are a major food source for young fish constituting 1 to 95% of the volume of stomach contents (Pennak 1978).

Daphnia pulex was chosen for use in this study over D. magna because it is more widely distributed and commonly found in softer waters. D. pulex generally reproduces parthenogenically. Eggs are extruded into and mature in a brood chamber. The fully developed young are released as

the female moults. Females are reproductively mature in 6 d and live for 35 to 40 d. During this period they produce from 1 up to 65 young every 2 d at 20° C.

2.2 MATERIALS AND METHODS

A culture of Daphnia pulex was started from organisms obtained from Carolina Biological Supply Company (Burlington, NC) and has been maintained in this laboratory for several years. The species was confirmed by keying females to D. pulex (Brooks 1957) and then inducing males in the population, preserving them in 0.4% buffered formalin, and examining the relative length of the dorsal abdominal process to distinguish D. pulex from D. pulicaria (Brandlova et al. 1972).

Cultures were maintained in carbon dechlorinated tap water in 5 L glass aquaria. The chemical characteristics of this water as determined by standard methods (APHA et al. 1981) are presented in Table 1. The temperature was maintained at 20±2° C. Light was provided by a combination of cool white fluorescent and Durotest Optima bulbs at an intensity of 50-100 ft-c at the air-water interface and a photoperiod of 16L:8D.

Daphnids were fed Chlamydomonas reinhardi (wild type, minus strain) ad libitum 3 times per wk. Algal cultures were started from material obtained from Carolina Biological

TABLE 1

Chemical characteristics of dechlorinated tap water.

Characteristic	Mean	Range
Hardness (mg/L)	65	55-80
pH (units)	7.75	7.7-7.9
Alkalinity (mg/L)	44	36-47
Conductivity (umhos)	168	115-189
Total cadmium (ug/L)	0.2	*-0.4

*Below detection limit (≤ 0.05 ug Cd/L).

Supply Company (Burlington, NC) and maintained in a vitamin enriched Bold's basal medium (Buikema 1970). Algae were centrifuged, washed, and resuspended in dechlorinated tap water before feeding. Artificial food, consisting of trout chow and dry yeast, was occasionally substituted (e. g. Comotto 1981).

An acute toxicity test was conducted exposing D. pulex to 5 concentrations of cadmium and a control for 48 h. Neonate daphnids were obtained by isolating ovigerous females 24 h before the test was begun. On the day of the test, 10 daphnids were randomly distributed to 60 mm glass petri dishes containing 5 ml diluent. Test media was prepared by diluting stock solutions of reagent grade $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$ with dechlorinated tap water. A 300 ml volume of test media was placed in each of 3 replicate 500 ml borosilicate glass beakers. The organisms were then transferred from petri dishes to the prepared test beakers. Beakers were covered with plastic film and randomly placed in a Sherer environmental chamber and maintained at $20 \pm 1^\circ \text{C}$. Light was provided by Durotest Optima bulbs at an intensity of 50 ft-c at the air-water interface on a 16L:8D photoperiod. Daphnia were not fed during acute exposures. Tests were static without renewal or aeration. The number of deaths in each beaker was recorded at 24 and 48 h. The criterion for death was no visible movement after gentle prodding.

Cadmium concentration of unfiltered, acidified samples were confirmed at the beginning and the end of the acute test by aspiration into the flame of a Perkin-Elmer (model 460) atomic absorption spectrophotometer (USEPA 1979b). The single operator estimates of precision and error based on USEPA quality control samples were 6.5% and 8.3%, respectively. Other chemical characteristics of the dilution water, pH, conductivity, hardness, alkalinity, were determined at the beginning of the test using standard methods (APHA et al. 1981). Wherever possible, LC50s and LC20s were calculated using probit analyses on log transformed cadmium concentrations. LC values for data characterized by few partial kills or irregular increases in mortality with dose were calculated using Spearman-Kärber analysis (Finney 1968; 1971; Daum 1970). Although acute data based on the lack of response to prodding are often expressed as an EC value based on immobilization, this severe response was called an LC value throughout this study in order to distinguish it from other, less drastic, chronic responses that were expressed as EC values. Results for the acute test are reported in terms of the mean measured acid labile cadmium. Values in parenthesis are 95% fiducial or confidence limits unless otherwise noted.

Daphnids were also exposed to 6 concentrations of cadmium and a control for 21 d. Methods were adapted from a draft ASTM method for static renewal tests with D. magna (Comotto 1981). Test solutions were prepared in the same manner as in acute tests. A 200 ml volume of test media was placed in 250 ml borosilicate glass Erlenmeyer flasks and covered with plastic film. Neonate D. pulex were obtained as described previously, and a single neonate daphnid was placed in 7 replicate vessels at each concentration. In addition, 3 replicate vessels containing 5 daphnids were set up at each concentration. Daphnia were fed Chlamydomonas reinhardtii at a rate of 2.5 mg/L on a dry weight basis 3 times per wk (Goulden et al. 1982). Test solutions were renewed 3 times per wk. At each renewal the survival and the number of young produced by daphnids tested individually were recorded. On days 7 and 21 the body lengths of Daphnia tested individually were determined to the nearest 0.04 mm. Individuals were measured from the most anterior part of the head to the base of the caudal spine. The vessels containing 5 daphnids were monitored for survival only.

During the chronic test, samples for chemical analyses were collected weekly. A sample of newly prepared test media and a composite sample of the replaced media were placed in polyethylene bottles and preserved with

redistilled nitric acid for cadmium analyses. These samples were digested in redistilled nitric acid and total cadmium was measured on a Perkin-Elmer (model 460) atomic absorption spectrophotometer equipped with a graphite furnace (USEPA 1979b). Based on analyses of USEPA quality control samples, the single operator precision for our cadmium determinations was 14% and the mean error was 16%. Despite precautions low level contamination was a problem in analysing samples with cadmium levels below 0.5 ug/L. The pH, alkalinity, hardness, and conductivity of the dilution water were determined weekly. Chronic test results are reported in terms of mean measured total cadmium concentrations.

Differences in long-term survival were examined by means of contingency table analysis. LC50s were calculated as previously described.

Toxicant effects on growth were evaluated by comparing mean body lengths for various exposure groups by means of one-way ANOVA. Least square means were computed and planned comparisons of exposed group means to the control were made with t-tests.

Effects of cadmium on the reproductive success of D. pulex were evaluated in 3 ways. First, the total numbers of young produced by each female over the 21 d test period were compared. This measure was affected by changes in both

survival and reproductive rate. Second, to separate effects on survival from those on reproduction, the number of young per reproductive day was calculated as the total number of young per female divided by the number of days of reproductive maturity. The means of both of these measures of reproductive success were compared as described above for body length. Third, the intrinsic rate of increase (r) was calculated for each exposure concentration as:

$$1 = L_x M_x e^{-rx}$$

where x is the age in d, L is the probability of survival to age x , and M is the number of female young per female produced during the surrounding time interval (Marshall 1962).

Several indices of chronic effect levels were calculated for each response monitored. Where significant differences between control and exposed group were found, a lowest observable effects concentration (LOEC) was defined as the lowest concentration tested statistically different from the control ($\alpha=0.05$). Similarly, a no observable effects concentration (NOEC) was defined as the highest concentration in which there was no difference from the control. The maximum acceptable toxic concentration (MATC) was calculated as the geometric mean of the LOEC and NOEC

(USEPA 1980a), or, when available information suggested that response changed with concentration on an arithmetic scale, an arithmetic mean was calculated. Dose-response relationships were evaluated using ordinary least squares regression analyses. A linear model using the natural log of the cadmium concentration was used. If there was significant deviation from this regression line other common models were tried sequentially. When a significant dose-response regression was established, an EC20, the cadmium concentration corresponding to a 20% impairment in response relative to the mean control value (e. g. Leffler 1981), was calculated from the dose-response regression by means of inverse prediction (Sokal and Rohlf 1969). The EC20 has an advantage over the MATC as calculated by the USEPA. If there is a linear dose-response curve, then the NOEC, LOEC, and MATC are artifacts of our skill in measurement, not empirical thresholds (e. g. Woodwell 1975), and the location of the estimate will vary with design factors such as sample size. An EC20 (or EC10, etc.) should show changes in measurement skill in the width of its confidence interval, not in its size.

2.3 RESULTS

The 48 h LC50 for D. pulex exposed to cadmium in dechlorinated tap water was 62 (50-73) ug Cd/L. The slope of the dose-response line was 5.23 (3.03-7.43).

During the chronic test, Daphnia were transferred to new media 3 times per wk. In the transfer process 3 of the 70 original test organisms were mechanically damaged. These animals were eliminated from the analyses and the sample sizes were reduced accordingly.

Table 2 summarizes the results of the chronic test. Mean total cadmium concentrations over all samples from the chronic test were comparable to nominal concentrations. Other chemical parameters remained within normal ranges for dechlorinated tap water throughout the chronic test (Table 1).

Contingency table analyses of the survival of D. pulex tested individually indicated no significant differences in survival after 7 d (LOEC and LC50 > 23.3; Table 2). After 14 d there was significant mortality in concentrations ≥ 10.2 ug Cd/L (LOEC = 10.2 ug Cd/L). The Spearman-Kärber estimate of the 14 d LC50 was 7.4 (5.5-10.0) ug Cd/L. After 21 d, there were no surviving daphnids in the 2 highest concentrations tested, 10.2 and 23.3 ug Cd/L. There were no significant differences from the control in survival at

TABLE 2

Effects of chronic cadmium exposure on *Daphnia pulex*.

Cadmium Concentration** (ug/L)		Survival Alive/Total (%)			Growth Mean Body Length** (mm)		Reproduction		Intrinsic Growth Rate
Nominal	Measured	Day 7	Day 14	Day 21	Day 7	Day 21	Mean Young per Female**	Mean Young per Day**	
Control	0.2 ± 0.2 6	7/7 (100)	6/6 (100)	6/6 (100)	1.93 ± 0.08 6	2.65 ± 0.12 6	200.3 ± 15.6 6	13.4 ± 1.0 6	0.62
0.6	0.8 ± 0.4 6	7/7 (100)	5/6 (83)	5/6 (83)	1.91 ± 0.20 6	2.68 ± 0.12 6	191.8 ± 56.9 6	14.0 ± 2.3 6	0.60
1.3	1.5 ± 0.8 6	7/7 (100)	6/6 (100)	6/6 (100)	1.80 ± 0.23 6	2.58 ± 0.13 6	180.8 ± 38.7 6	12.1 ± 2.6 6	0.48
2.5	3.2 ± 0.6 6	7/7 (100)	7/7 (100)	5/7 (71)	1.91 ± 0.07 7	2.67 ± 0.07 5	186.8 ± 39.0 7	13.1 ± 1.1 7	0.63
5.0	5.5 ± 1.3 6	7/7 (100)	7/7 (100)	6/7 (86)	1.89 ± 0.11 7	2.53 ± 0.10 6	182.9 ± 17.7 7	12.3 ± 1.1 7	0.70
10.0	10.2 ± 2.2 6	6/7 (86)	1/7 (14)*	0/7 (0)*	1.88 ± 0.04 6	---- 0	55.3* ± 42.3 7	9.8* ± 2.4 6	0.69
20.0	23.3 ± 3.1 4	5/7 (71)	0/7 (0)*	0/7 (0)*	1.67* ± 0.14 5	---- 0	25.6* ± 16.6 7	4.9* ± 2.9 6	0.65

*Significantly different from the control ($\alpha=0.05$).

**Standard deviations and samples size follow mean values.

lower concentrations (LOEC=10.2 ug Cd/L). The Spearman-Kärber estimate of the 21 d LC50 was 5.2 (3.7-7.3) ug Cd/L. Because survival did not decline regularly with increasing concentration LC20s could not be calculated.

Data on the survival of daphnids exposed 5 individuals per vessel were not used. Reproduction in these vessels resulted in densities of organisms sufficient to induce the production of males in at least 1 vessel. Because male production is generally regarded as a sign of stress which can interact with toxicant exposure and confound interpretation of results, these data were eliminated from analysis. This is likely to be a repetitive problem when testing 5 individuals in a single container, and, as such, the procedure may not be the best methods for obtaining data on longterm survival under toxic stress.

Exposure to 23.3 ug Cd/L for 7 d resulted in significantly smaller Daphnia than in the control (LOEC=23.3 ug Cd/L; Table 2). The relationship between body length on day 7 and cadmium concentration was significant ($p=0.0136$) but the coefficient of determination for this regression was very low ($r^2=0.16$), thus the predictive utility was poor. After 21 d there were no significant differences in the body lengths of surviving daphnids ($p=0.1243$).

There were significant declines in the total number of young produced per female in 10.2 and 23.3 ug Cd/L (LOEC=10.2 ug Cd/L; Table 2). The relationship between the total number of young per female and cadmium concentration was significant ($p=0.0001$), however, there was also a significant deviation from linearity ($p=0.0007$). A sigmoid response curve may be more appropriate.

Daphnids exposed to 10.2 and 23.3 ug Cd/L had fewer young per reproductive day (Table 2). Number of young per reproductive day decreased with increasing cadmium concentration (Figure 1). The EC20 estimated from this relationship was 8.4 (3.0-13.6) ug Cd/L.

There was no trend in intrinsic growth rate estimates across concentration. This measure emphasizes early reproduction and was not very different between groups.

2.4 DISCUSSION AND SUMMARY

The 48 h LC50 for D. pulex determined in this study was 62 ug Cd/L (hardness=65 ppm). This value is quite comparable to others determined for D. pulex; 71 ug Cd/L (Mount and Norberg 1983), 115 ug Cd/L in slightly harder water (Ingersoll and Winner 1982), and 62 ug Cd/L after 72 h (Bertram and Hart 1979). It is also similar to the 48 h LC50 of 65 ug Cd/L determined for D. magna, by Biesinger and

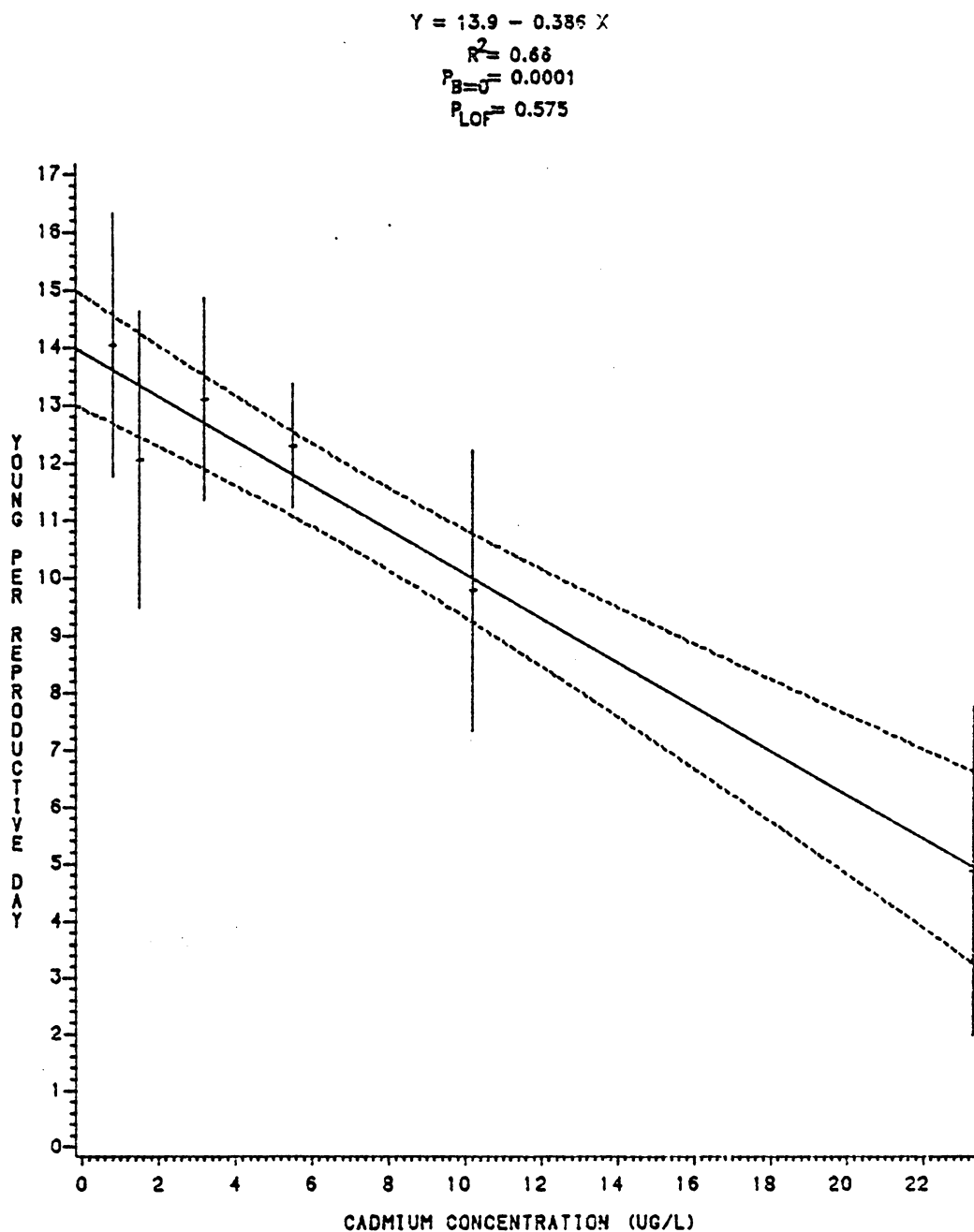


Figure 1: Relationship between cadmium dose and number of young per reproductive day for Daphnia pulex.
 Bars span 1 standard deviation.
 Dashed lines are 95% confidence limits for mean values.

Christensen (1972), but considerably higher than the 9.9 ug Cd/L determined by Chapman (USEPA 1980b).

There were significant effects of cadmium on the long-term survival and reproduction of D. pulex at 10.2 ug/L (Table 2). The MATCs for these parameters were 7.5 and 7.9 ug Cd/L, respectively. The EC20 for the number of young per reproductive day was 8.4 ug Cd/L. Growth as monitored by body length was not a sensitive indicator of toxic effects after 21 d.

These chronic effect levels are very similar to those determined by Ingersoll and Winner (1982) who found effects on survival and reproduction at 10 ug Cd/L and no effects on growth. Bertram and Hart (1979) found effects on survival and reproduction at 5 and 1 ug Cd/L, respectively. However, there were some problems with control survival after 21 d in that test.

Surprisingly, D. magna appears to be substantially more sensitive than D. pulex to chronic cadmium exposure. Chronic effect levels of 0.34 and 0.15 ug Cd/L have been reported for D. magna tested in soft waters (Biesinger and Christensen 1972; Chapman as cited by USEPA 1980, respectively).

Chapter III

CADMIUM TOXICITY TO AN OLIGOCHAETE

3.1 INTRODUCTION

Toxicity tests with oligochaetes can efficiently provide chronic effects data on an invertebrate with a distinctly different lifestyle than the most commonly tested invertebrate, Daphnia. These data are of particular interest because oligochaetes may be important mediators in the flow of energy and materials through some aquatic ecosystems. Oligochaetes are an important and high quality source of food for fish (see Chapman et al. 1982) and, possibly, later instars of aquatic insects (Anderson 1976). In addition to trophic importance, oligochaetes may affect the turnover of sediment associated materials both by physical disruption of the substrate and by food chain transfer of materials from the sediment to higher trophic levels (e. g. Whitten and Goodnight 1967). Although some oligochaetes, notably Tubifex and Limnodrilus, are quite tolerant of eutrophic conditions and have been suggested as indicators of organic pollution, others, such as Stylodrilus, require oligotrophic waters. It is difficult to generalize water quality requirements for the entire group, and Brinkhurst and Cook (1974) suggested that

toxicity tests with species other than those known to be tolerant to a specific pollutant may provide meaningful data. Available toxicity test data suggest that oligochaetes may be quite sensitive to some toxicants (Chapman et al. 1982; Bailey and Liu 1980; Giesey et al. 1979; Newman 1975). Toxicity tests with marine polychaetes have been successful (e. g. Reish et al. 1978), and similar utility may be expected from tests with a freshwater species.

Aeolosoma headleyi was chosen for this study because it is commercially available, is easily cultured, and has a very short lifecycle. The genus Aeolosoma is found world-wide in ponds, lakes, and rivers. These small oligochaetes (1 to 3 mm in length) are collector-gatherers and feed by ingesting sediment as they burrow. They probably derive most of their nutrition from microorganisms in the sediment (Brinkhurst and Jamieson 1971; Newman 1975). They reproduce parthenogenically by pygidial budding every 2 to 3 d at 25° C. Sexual reproduction has not been reported for this species.

3.2 MATERIALS AND METHODS

A stock culture of A. headleyi was obtained from Carolina Biological Supply Company (Burlington, NC). The species was confirmed by Dr. Robert Singer (Colgate University, Hamilton, NY). Cultures were maintained in dechlorinated tap water (Table 1) in 11.5 cm glass culture bowls at a temperature of $25 \pm 1^\circ \text{C}$, a light intensity of 80-100 ft-c from Durotest Optima bulbs, and a photoperiod of 16L:8D. A trout chow suspension provided both food and substrate. It was prepared by blending 3 g Trout Chow (Ralston-Purina Co., St. Louis, MO) in 200 ml dilution water for 1 min and filtering through 0.25 mm mesh netting to remove large particulates. The resulting suspension was diluted, 1 part to 19 parts diluent, to yield a culture medium containing approximately 450 mg/L trout chow on a dry weight basis. Cultures were inspected, and one-half the culture medium was replaced, weekly.

All tests were conducted in 60 mm diameter covered glass petri dishes. Media consisted of dechlorinated tap water, stock solutions of reagent reagent grade $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$, and trout chow suspension. Other conditions were identical to those used for culture. Young worms with no apparent pygidial budding were used to start all tests. All observations were made using a dissecting microscope at a total magnification of 120X.

An acute toxicity test was conducted exposing A. headleyi to 5 cadmium concentrations and a control. Duplicates of 10 worms were exposed in 10 ml media. Tests were static without renewal. Samples of test media were collected at the beginning of the test for chemical analysis. Deaths, defined as lack of movement in response to gentle prodding, were counted at 24 and 48 h. LC50s were calculated as previously described (Chapter II).

Chronic toxicity tests monitored the log phase population growth of A. headleyi in response to 5 cadmium concentrations and a control. Five vessels containing 5 worms were set up at each concentration. Worms were counted as they were pipetted into spot plates, then transferred with 1 ml media to 9 ml fresh media in the test vessel. Solutions were similarly renewed three times per wk and the worms were counted. Tests continued until densities of 300 worms per dish were exceeded.

On the basis of preliminary tests (Figure 2), log phase growth was defined as that occurring after the first doubling (10 worms per dish) up to an apparent inflection from linearity at densities of 300 worms per dish. The first significant deviation from linearity occurred after 14 d at a mean density of 311 worms per dish ($p=0.004$). Thereafter a logistic term was required to describe the

declines in growth rates. Slower growth continued for weeks and densities of 1,500 worms per dish were observed after 28 d. The carrying capacity of the system was extrapolated using nonlinear regression and a logistic model to be 3,000 worms.

Exponential growth curves were fitted to the natural log transformed count data for days on which average densities for all exposure groups were between 10 and 300 worms per dish. A Multivariate Analysis of Variance (MANOVA) procedure similar to that described by Capizzi and Burton (1978) and ordinary least squares regression analysis were used to fit and compare population growth curves. The slopes of the fitted lines were used as estimates of intrinsic growth rates. In addition, count data for the last day of observation within the log phase growth period were compared with one-way ANOVA and Duncan's multiple range test. The relationships between cadmium dose and numbers of worms and intrinsic growth rates were examined with ordinary least squares regression analysis. Indices of chronic effects, NOECs, LOECs, MATCs, and EC20s, were calculated as described previously (Chapter II).

Samples for chemical analyses were collected weekly. The total cadmium concentrations of the test media were determined by means of graphite furnace atomic absorption

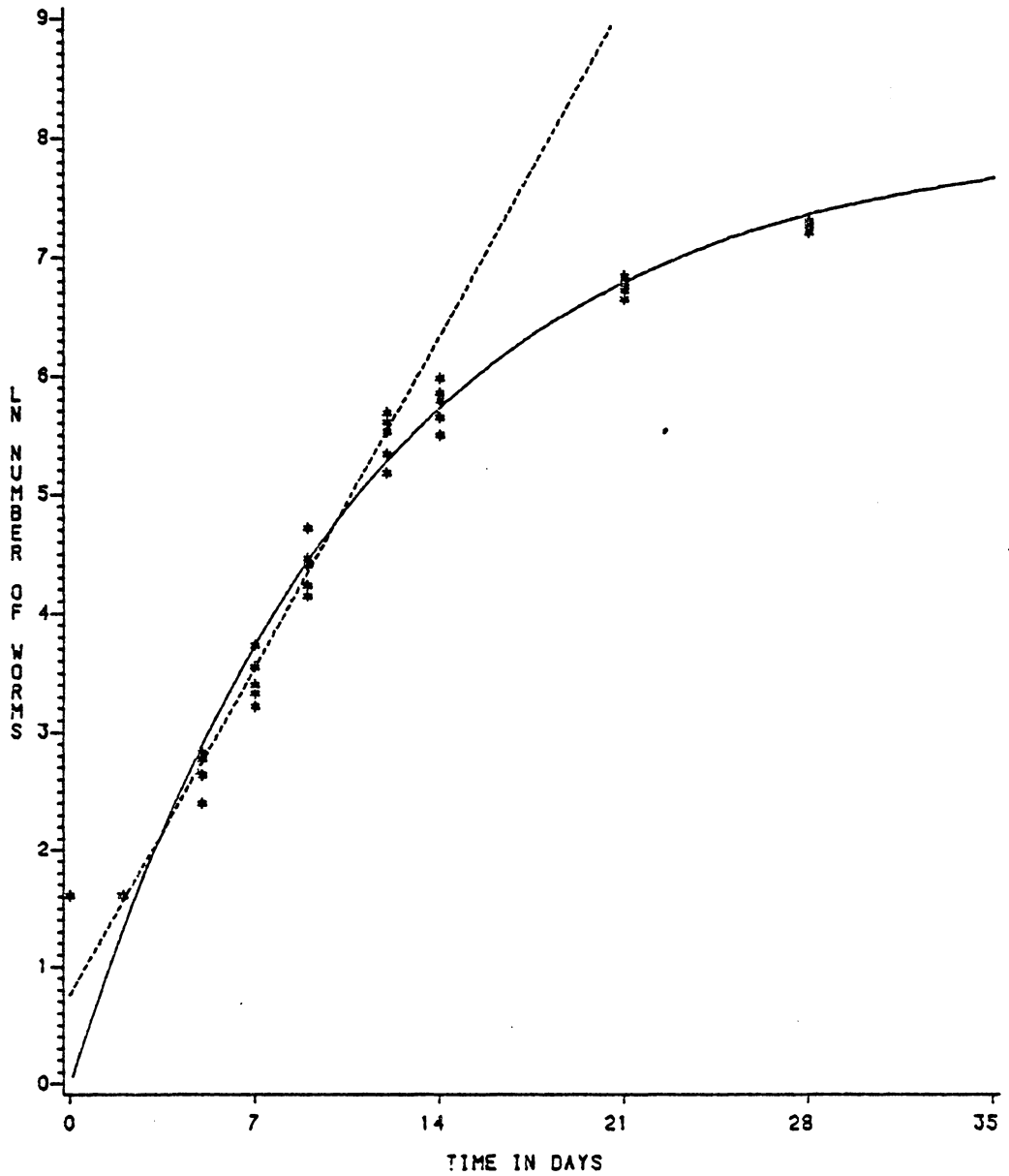


Figure 2: Log phase and logistic population growth of Aeolosoma headleyi.

spectrophotometry after acid digestion (USEPA 1979; Chapter II). Results are reported as mean measured total cadmium concentration.

3.3 RESULTS

The Spearman-Kärber estimate of the 48 h LC50 for A. headleyi was 1,200 (1,100-1,350) ug Cd/L. Results of cadmium exposure on population growth of A. headleyi are presented in Table 3 and Figure 3. There was no lag time before log phase growth and worms completed up to 6.7 generations in 12 d (up to 507 worms in the control). Other water quality parameters remained within normal ranges for dechlorinated tap water (Table 1). Variability in replicate counts resulted in significant deviations from the regression lines in lower concentrations (Control through 9.8 ug Cd/L), but these deviations were not systematic and all least squares coefficients of determination (r^2) were above 0.95. The LOEC based on growth rate was 36.9 ug Cd/L. There was no significant relationship between cadmium dose and intrinsic growth rate, however, such a relationship may exist at higher concentrations. The LOEC based on number of worms on day 10 was 36.9 ug Cd/L. The dose-response relationship was linear (Figure 4). The EC20 calculated from this relationship was 62.8 (53.2-81.6) ug Cd/L, but this value was outside the range of concentrations tested.

TABLE 3

Effects of chronic cadmium exposure on the population growth of Aeolosoma headleyi

Nominal Cadmium Concentration (ug/L)	Mean Measured Total Cadmium Concentration (ug/L) ±Standard Deviation	Intrinsic Growth Rate (r) ±Standard Error	Mean ln Number of Worms Day 10 ±Standard Deviation
Control	<0.5 ---	0.44 ±0.01	5.67 ±0.04
2.5	2.8 ±1.4	0.43 ±0.02	5.68 ±0.17
5.0	4.3 ±0.9	0.45 ±0.02	5.63 ±0.12
10.0	9.8 ±0.3	0.45 ±0.01	5.62 ±0.05
20.0	17.2 ±3.8	0.43 ±0.02	5.56 ±0.19
40.0	36.9 ±7.8	0.36* ±0.02	4.99* ±0.93

*Significantly different from the control ($\alpha=0.05$).

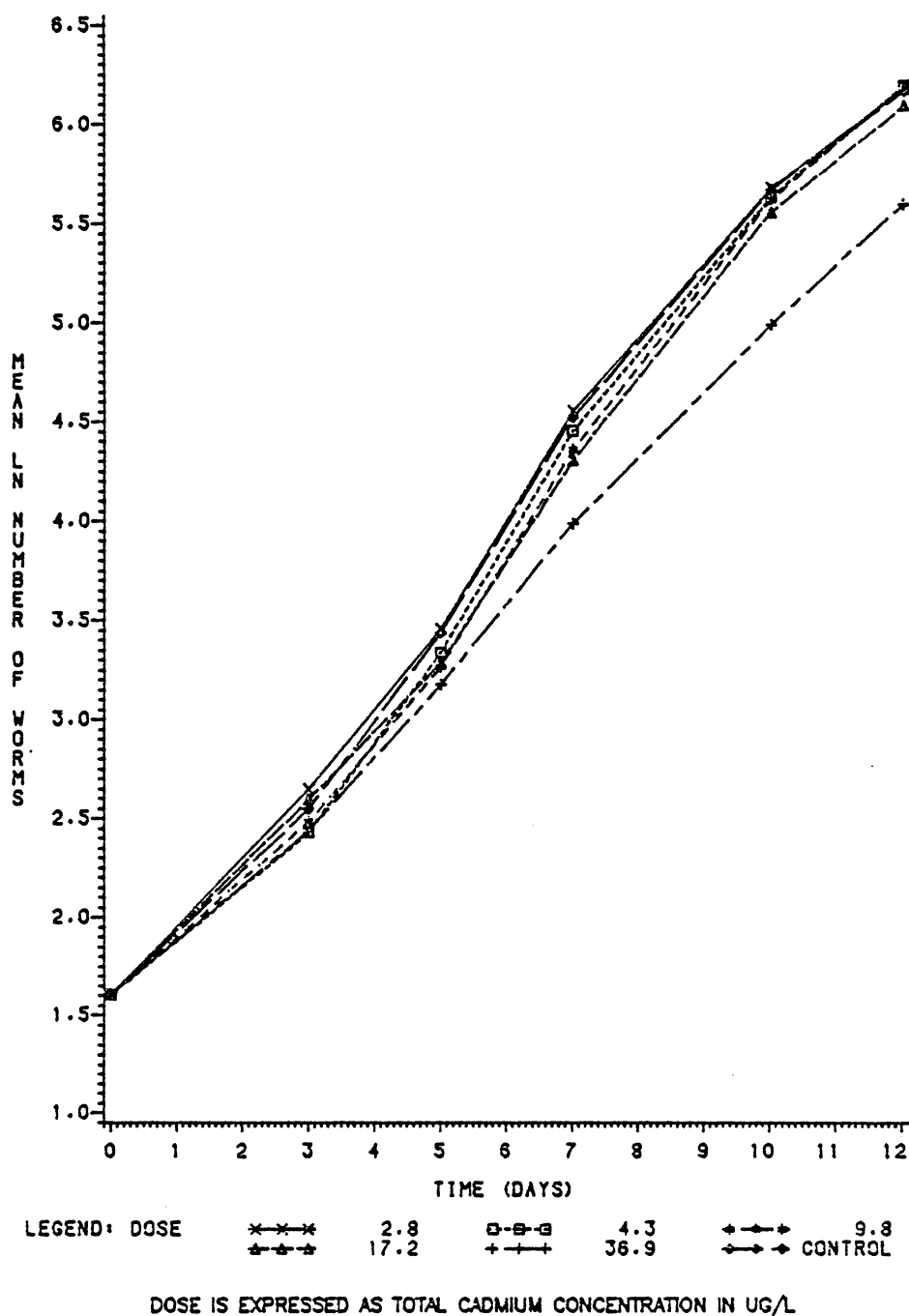


Figure 3: Population growth over time of cadmium exposed Aeolosoma headleyi.

$$Y = 5.77 - 0.020 X$$

$$R^2 = 0.68$$

$$P_{B=0} = 0.0001$$

$$P_{LOF} = 0.123$$

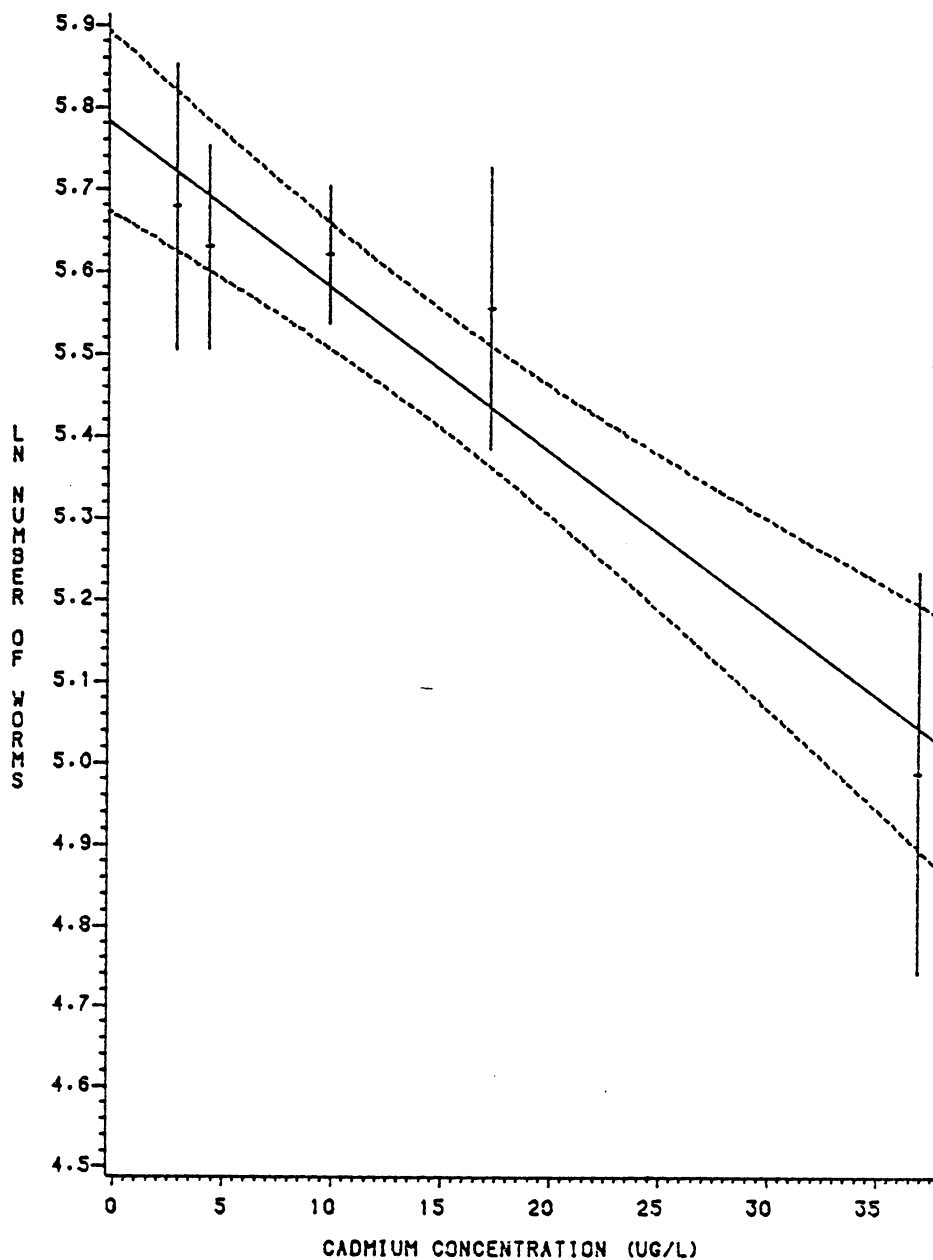


Figure 4: Relationship between cadmium dose and population increase of Aeolosoma headleyi.
 Bars span 1 standard deviation.
 Dashed lines are 95% confidence limits for mean values.

3.4 DISCUSSION AND SUMMARY

The 48 h LC50 of 1,200 is similar to others determined for freshwater oligochaetes. Rehwoldt et al. (1973) reported a 48 h LC50 of 1,700 ug Cd/L for Nais. Chapman et al. (1982) reported EC50s of 3,500 to 12,000 ug Cd/L for 6 species of oligochaetes tested with sediment and 170 to 630 ug Cd/L for animals tested without sediment. Bailey and Liu (1980) found Lubriculus variegatus to be somewhat more sensitive (48 h LC50=120 ug Cd/L), but, because they were tested without substrate greater sensitivity is expected. Brokovic-Popovic and Popovic (1977) evaluated the effects of acute cadmium exposure on Tubifex tubifex without substrate and found LC50s ranging from 310 to 720 ug Cd/L. The acute effect level determined by this study appears reasonable in comparison with other similar work.

A. headleyi appeared to be relatively insensitive to chronic cadmium exposure. Population growth was not impaired at concentrations up to 17.2 ug Cd/L. A 20% effect level would be higher than the concentrations tested and was extrapolated to 62.8 ug Cd/L. The MATC based on number of worms after a 10 d exposure was 27.1 ug Cd/L. No other single species chronic tests exposing freshwater oligochaetes to cadmium were found in the literature for comparison. However, Giesey et al. (1979) found that

populations of the oligochaete, Pristina, were adversely affected at cadmium concentrations of 5 ug/L in long term exposures in channel microcosms.

Chapter IV

CADMIUM TOXICITY TO A DIPTERAN

4.1 INTRODUCTION

The importance of insect larvae in the energy flow in aquatic ecosystems has become increasingly obvious in the past decade (e. g. Cummins 1974; Anderson and Sedell 1979). However, of the many important insect group in aquatic systems, relatively few are amenable to laboratory toxicity testing through an entire lifecycle. Dipterans of the family Chironomidae seem to be the most promising group for laboratory toxicity tests and Tanytarsus dissimilis, Chironomus tentans, and Chironomus riparius have been used for this purpose (e.g. Nebeker and Puglisi 1974; Macek et al. 1976; Lee et al. 1980; respectively). Chironomids can be an important structural component of aquatic ecosystems, sometimes constituting a majority of total community biomass and occurring in densities up to 50,000 individuals per m². They also can be important in energy and material flow in aquatic systems. Chironomids are an important food source for fish (e. g. Markle and Grant 1970). They also may influence the turnover and biological availability of sediment associated materials (e. g. Curry 1962).

Chironomus riparius was chosen for this study on the basis of availability and apparent ease of culture. C. riparius (Meigen) is a non-biting midge found in the sediment of ponds, lakes, streams, and rivers throughout North America and Europe (Curry 1962). It is a nonselective collector-gatherer and feeds on deposited detrital particles or algae (Edgar and Meadows 1969; Walshe 1951). C. riparius in natural systems is multivoltine. The number of generations in a year depend on temperature and food availability (Learner and Potter 1974). The life cycle consists of 7 distinct stages; egg, 4 larval instars, pupa, and adult. Reproduction is sexual and mating occurs in swarms (Downe and Caspary 1973). In the laboratory a lifecycle can be completed in 3 wk at 21° C.

4.2 MATERIALS AND METHODS

Several egg masses were obtained from stock cultures of C. riparius at the Savannah River Ecology Laboratory (Aiken, SC). The original culture was established from animals collected from Badfish Creek in Madison, WS, and identified by Dr. Richard Narf (Wisconsin Department of Natural Resources, Madison, WS). The species of our established culture was verified by Dr. Boris Kondratieff (Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA) as C. riparius.

Cultures were maintained in 5 L glass aquaria with 8 cm of ground paper towel substrate and 2 L of dechlorinated tap water (Table 1). Paper towel substrate was prepared by shredding brown industrial paper towels into water and boiling the mixture for 15 min. The substrate was aerated for >1 wk before use. Aquaria were covered with nylon netting to retain adults and disposable pipettes were used to aerate the containers without disturbing the substrate. Cultures were kept under a combination of Durotest Optima and cool-white fluorescent bulbs at an intensity of 25-50 ft-c at the air-water interface. The photoperiod was 16L:8D. Temperature was maintained at $22 \pm 1^\circ \text{C}$. Chironomids were fed a slurry of Tetramin fish food ad libitum 3 times per wk.

An acute test exposed 1st instar larvae to cadmium for 48 h. First instar larvae were obtained by isolating egg masses from stock cultures into containers without substrate and checking daily for hatching and departure from the egg mass. To begin the test 15 larvae were randomly distributed to triplicate glass baby food jars (50 mm diameter and 60 mm height) containing 25 ml dechlorinated tap water. Tetramin flake fish food was added to the test vessels as a slurry (2 g blended in 100 ml diluent) to yield a final concentration of 200 mg/L on a dry weight basis. Slurry provided both

food and substrate. After addition of food, the animals were allowed to acclimate (e.g. build cases) for 24 h before the addition of toxicant. Then a 25 ml volume of stock solution, consisting of reagent grade $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$ and dechlorinated tap water, was added to the 25 ml diluent in the test vessel to yield final concentrations. Vessels were covered with plastic film and randomly placed in a Sherer environmental chamber at $22 \pm 1^\circ \text{C}$. Light was provided on a 16L:8D photoperiod, at a light intensity of 50 ft-c at the air-water interface. The test was static without renewal or aeration. The number of deaths in each concentration was recorded at the end of 48 h. The lack of any response to prodding was the criterion for a death. Samples for chemical analyses were collected at the beginning and end of the test. Cadmium samples were digested in redistilled nitric acid and analyzed by flame atomic absorption spectrophotometry (USEPA 1979b; Chapter II). Other water quality characteristics, pH, conductivity, hardness, alkalinity, were measured at the beginning of the test. LC50s were determined as previously described (Chapter II). Results for the acute test are reported as mean measured total cadmium concentration.

A partial lifecycle chronic test was conducted exposing C. riparius to 6 concentrations of cadmium and a control in

a flowthrough system for 5 weeks. The test system consisted of a proportional dilutor delivering 125 ml of test media to duplicate 1.25 L chambers every 15 min. Test containers were rectangular glass chambers measuring 12.5 by 30.0 by 15.0 cm (WxLxH). Self starting siphons maintained water depth at approximately 6 cm but permitted some fluctuation in water level and mixing. Test media consisted of stock solutions of reagent grade $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$ diluted with dechlorinated tap water. Larvae were tested in egg cups consisting of a 60 mm diameter glass petri dish with a 6 cm collar of 200 um mesh Nitex screen. Nylon net screens were used to retain adults in the testing chamber. Water was adjusted to $22 \pm 1^\circ \text{C}$ before delivery to the dilution system with a stainless steel aquarium heater. Light was provided by Durotest Optima bulbs on a 16L:8D photoperiod. Light intensity was 10 ft-c at the air-water interface.

To begin the test, newly hatched larvae from 5 egg masses were combined, then 20 individuals were randomly distributed to each of 70 egg cups. Food was added as a slurry (12.5 mg dry weight per egg cup), and the cups stood overnight, allowing larvae to construct tubes. The next morning 5 egg cups were distributed to each test chamber. Larvae were fed 12.5 mg Tetramin per cup 3 times per wk, or less frequently if no clear, grazed areas were apparent around larval tubes.

After 7 d, surviving larvae in all cups were counted. Larval size measures were obtained by destructively sampling 1 egg cup from each exposure chamber. Organisms were killed by slow addition of hot water, and head capsule width and body length were measured to the nearest 0.02 and 0.08 mm, respectively. Animals were assigned to instars by comparing head capsule measurements to previously determined ranges.

After 14 d surviving larvae could not be accurately counted without removal from tubes, and so survival was monitored only for 2 egg cups destructively sampled from each concentration. Larval size and instar were determined as described above.

After the first pupae were observed, exuvia and live adults in each chamber were counted daily. Adults were retrieved from tanks after death and body length measured to the nearest 0.08 mm.

Samples for chemical analyses were collected weekly. Cadmium samples were digested in redistilled nitric acid and concentrations determined by means of graphite furnace atomic absorption spectrophotometry (USEPA 1979; Chapter II). Other water quality characteristics were monitored as described previously. Results are reported in terms of the mean measured total cadmium concentrations.

Survival after 7 and 14 d and survival to adult were compared to the control by means of contingency table analysis. LC50s and LC20s were calculated as previously described (Chapter II). A control death correction factor was used in calculating the LC values for survival to adult (Daum 1970). Body lengths were compared between concentrations with two-way ANOVA using replicates as a blocking factor. Least square means were computed and planned comparisons of exposed group means to the control were made with t-tests. The relative frequencies of the larval instars in exposed groups with replicates combined were compared to the control using contingency table analysis (Sokal and Rohlf 1969). Relationships between cadmium concentration and levels of responses were investigated using ordinary least squares regression. Indices of chronic effect levels, NOECs, LOECs, MATCs, and EC20s, were calculated as described previously (see Chapter II).

4.3 RESULTS

The 48 h LC50 for C. riparius was 7,900 (6,500-9,900) ug Cd/L. The slope of the dose-response line was 2.98 (2.08-3.88).

Although chronic exposures were initiated at 6 concentrations and a control, a mechanical failure resulted in excessive cadmium concentrations in the lowest exposure group during the first week of exposure. This group was eliminated from subsequent analyses. The results of cadmium determinations for the remaining exposure groups are summarized in Table 4. Mean measured concentrations were consistently higher than nominal concentrations and the differences increased with concentration. A combination of a hyper-concentrated primary stock solution and errors in delivery rates gave rise to the increases in concentration over nominal values. Although the intended 50% series of concentrations was not obtained, the measured concentrations were distributed over a reasonable range for a toxicity evaluation and they were consistent over the length of the test. Other water quality characteristics remained within normal ranges for dechlorinated tap water (Table 1).

The effects of cadmium exposure on the survival of C. riparius are summarized in Table 5. It was noted that survival measures in all exposure groups were slightly confounded by the occasional escape of an organism from the egg cup and exposure tank. As such, survival values represent both survival and retention in the testing system and are slightly lower than true values. After a 7 d

TABLE 4

Cadmium concentrations in the chronic test with Chironomus riparius.

Nominal Cadmium Exposure (ug/L)	Mean Measured Cadmium (ug/L)	Standard Deviation	Sample Size
Control	0.2	±0.1	7
1.5	1.8	±0.5	7
3.0	4.3	±2.4	8
6.0	10.2	±5.0	5
12.5	30.4	±8.6	8
25.0	41.5	±9.7	6

exposure there were significantly fewer surviving organisms in concentrations of 4.3 ug Cd/L and above (LOEC=4.3 ug Cd/L). The 7 d LC50 was 11.7 (7.3-19.4) ug Cd/L and the LC20 was 3.3 (1.2-5.5) ug Cd/L. The slope of the dose-reponse line was 1.51 (0.90-2.12). After 14 d the survival of organisms in all exposure groups was significantly less than in the control (LOEC $\leq$ 1.8 ug Cd/L). The 14 d LC50 was 5.4 (2.8-8.4) ug Cd/L and the LC20 was 0.7 (0.1-1.5) ug Cd/L. The slope of the dose-reponse line was 0.94 (0.58-1.30). Survival to adult was significantly impaired at cadmium concentrations of 10.2 ug/L and above (LOEC=10.2 ug Cd/L). The 2 highest concentrations, 30.4 and 41.5 ug Cd/L produced no adults. The LC50 for survival to adulthood was 7.2 (3.9-9.4) ug Cd/L and the LC20 was 4.6 (1.4-6.6) ug Cd/L. The slope of the dose-response line was 4.39 (1.70-7.08). Control survival rates were low but comparable to those obtained in other chronic tests with C. riparius (22% in this study vs 43-50%; Lee et al. 1980; Hax 1983). Much mortality after the second week was associated with pupation and emergence, a particularly physiologically demanding and vulnerable time in the lifecycle of aquatic insects. Test conditions, particularly inadequate food quality and lack of substrate, may have been inadequate for better survival.

TABLE 5

Effects of chronic cadmium exposure on the survival of
Chironomus riparius.

Treatment Group	Survival Week 1	Survival Week 2	Survival to adult
Control	174/200 (87)	37/40 (93)	26/120 (22)
1.8	175/200 (88)	25/40* (63)	23/120 (20)
4.3	159/200* (80)	24/40* (60)	21/120 (18)
10.2	98/200* (49)	17/40* (43)	7/120* (6)
30.4	67/200* (34)	12/40* (30)	0/120* (0)
41.5	69/200* (35)	5/40* (13)	0/120* (0)

*Significantly different from the control ($\alpha=0.05$).

The effects of cadmium exposure on the growth of C. riparius are summarized in Table 6. After 7 d, organisms exposed to ≥ 10.2 ug Cd/L were significantly smaller than controls (LOEC=10.2 ug Cd/L). Although there was considerable variability among individuals in body length, mean body length for each exposure group declined with increasing cadmium concentration (Figure 5). The EC20 calculated from this regression was 6.7 (2.7-8.8) ug Cd/L. After 14 d, organisms in cadmium concentrations of 10.2 ug/L and above remained significantly smaller than controls (LOEC=10.2 ug Cd/L). Again, there was a significant regression of mean body length on cadmium concentration (Figure 6). The EC20 estimated from this regression was 8.1 (3.2-10.0) ug Cd/L. There were no significant differences between exposure groups in mean adult body length ($p=0.453$).

Cadmium also affected the rate of development of the insects (Figure 7). The relative frequencies of larval instars were very similar in lower concentrations, but higher concentrations had more individuals in earlier instars. The LOECs based on larval instar distribution were all 10.2 ug Cd/L after 7, 14 and 35 d of exposure.

Adults produced eggs in cadmium concentrations ≤ 4.3 ug Cd/L. However, no egg masses developed, probably due to lack of fertilization. The emergence of adults was

TABLE 6

Effects of chronic cadmium exposure on the growth of
Chironomus riparius.

Treatment Group	Body Length Week 1	Body Length Week 2	Body Length Adult
Control	6.3 ±2.1 26	10.3 ±2.4 35	6.0 ±0.7 16
1.8	6.3 ±2.1 32	11.0 ±2.0 21	6.1 ±0.7 9
4.3	5.9 ±2.0 26	9.9 ±2.2 22	5.7 ±0.6 10
10.2	4.4* ±1.8 22	7.2* ±3.7 17	5.7 ±0.8 6
30.4	3.4* ±1.3 21	5.4* ±2.1 12	--- --- 0
41.5	3.4* ±1.4 16	5.5* ±2.4 5	--- --- 0

*Significantly different from the control ($\alpha=0.05$).

$$Y = 7.01 - 1.020 \ln X$$

$$R^2 = 0.97$$

$$P_{B=0} = 0.0001$$

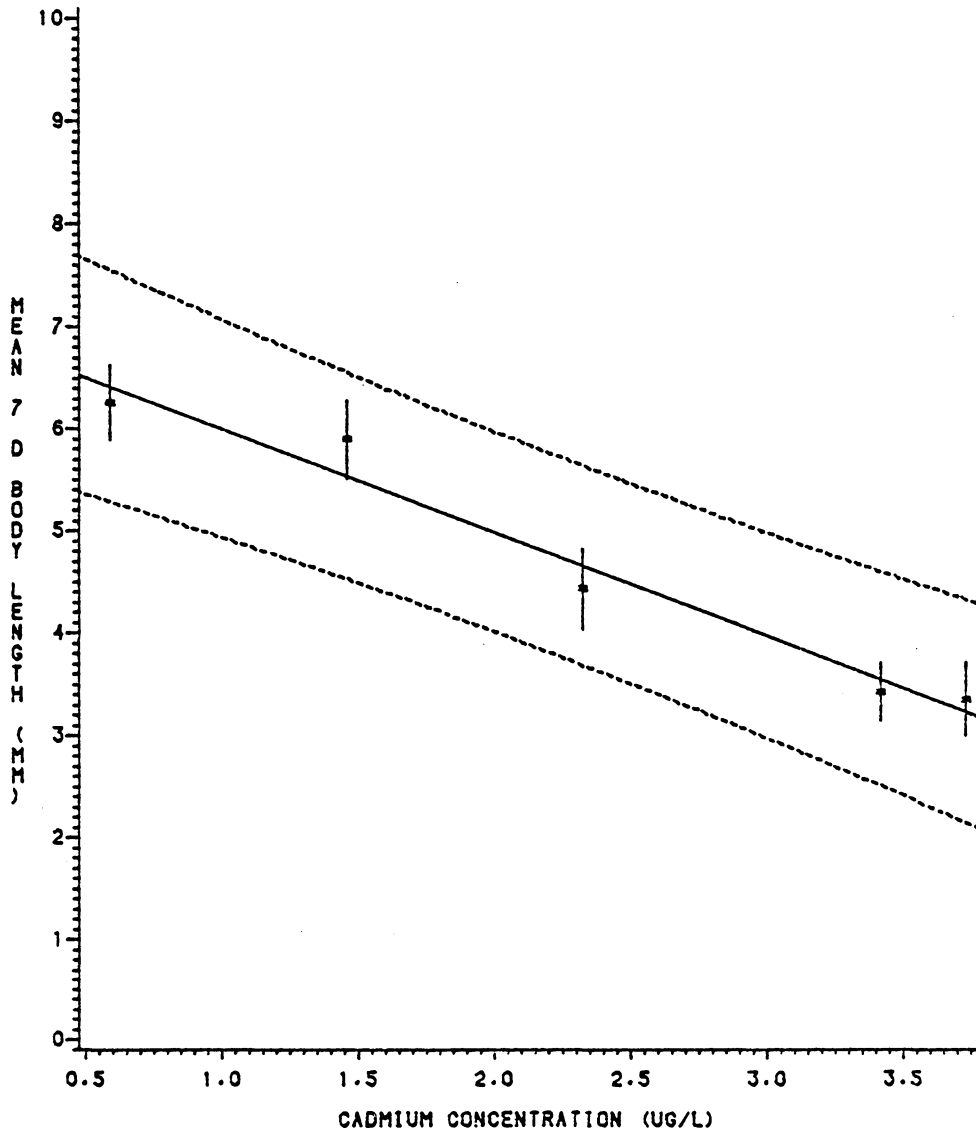


Figure 5: Relationship between cadmium dose and mean body length of Chironomus riparius after a 7 d exposure.

Bars span 1 standard error.

Dashed lines are 95% confidence limits for individual values.

$$Y = 12.30 - 1.900 \ln X$$

$$R^2 = 0.97$$

$$P_{B=0} = 0.0001$$

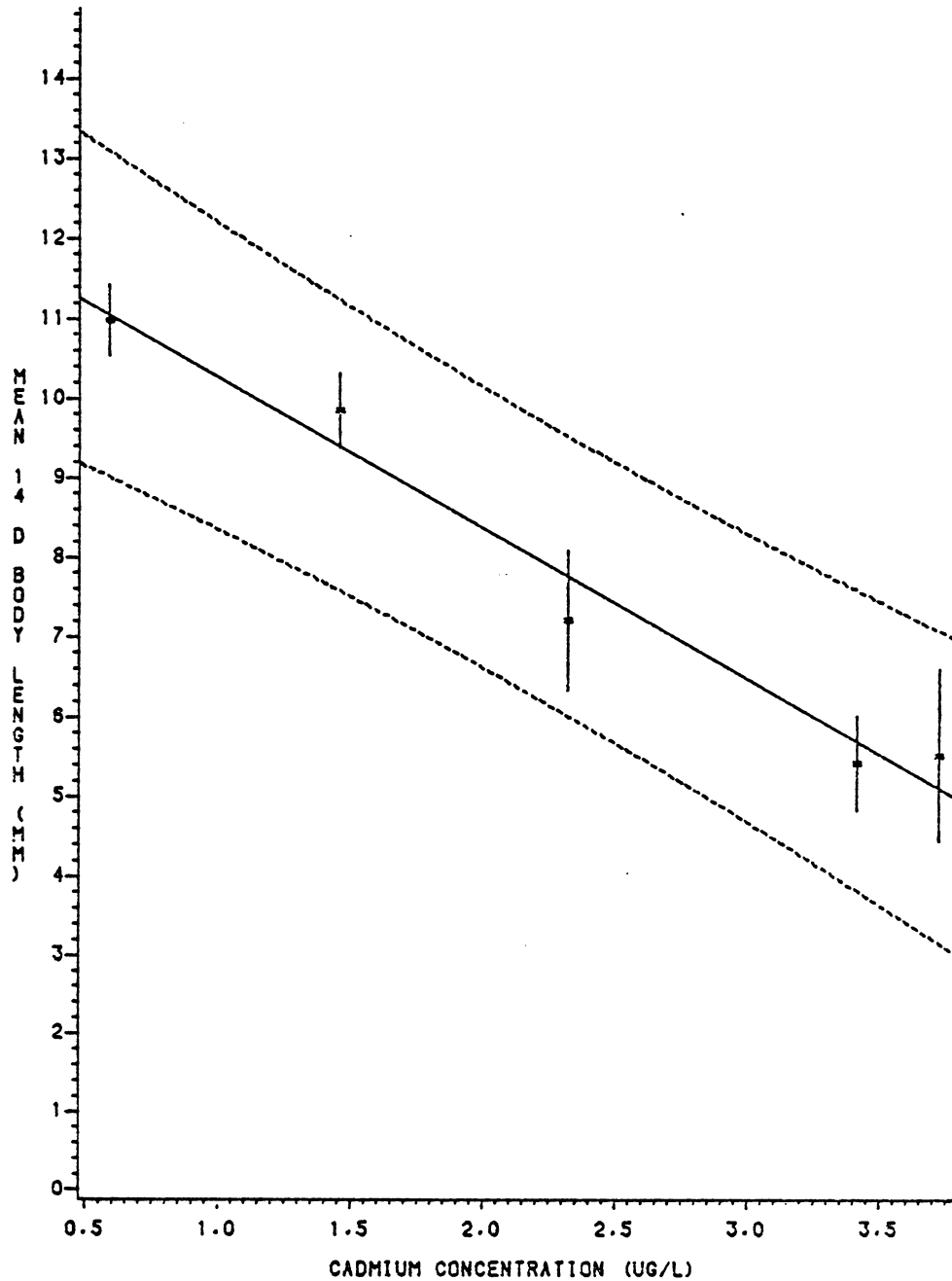


Figure 6: Relationship between cadmium dose and mean body length of Chironomus riparius after a 14 d exposure.

Bars span 1 standard error.

Dashed lines are 95% confidence limits for individual values.

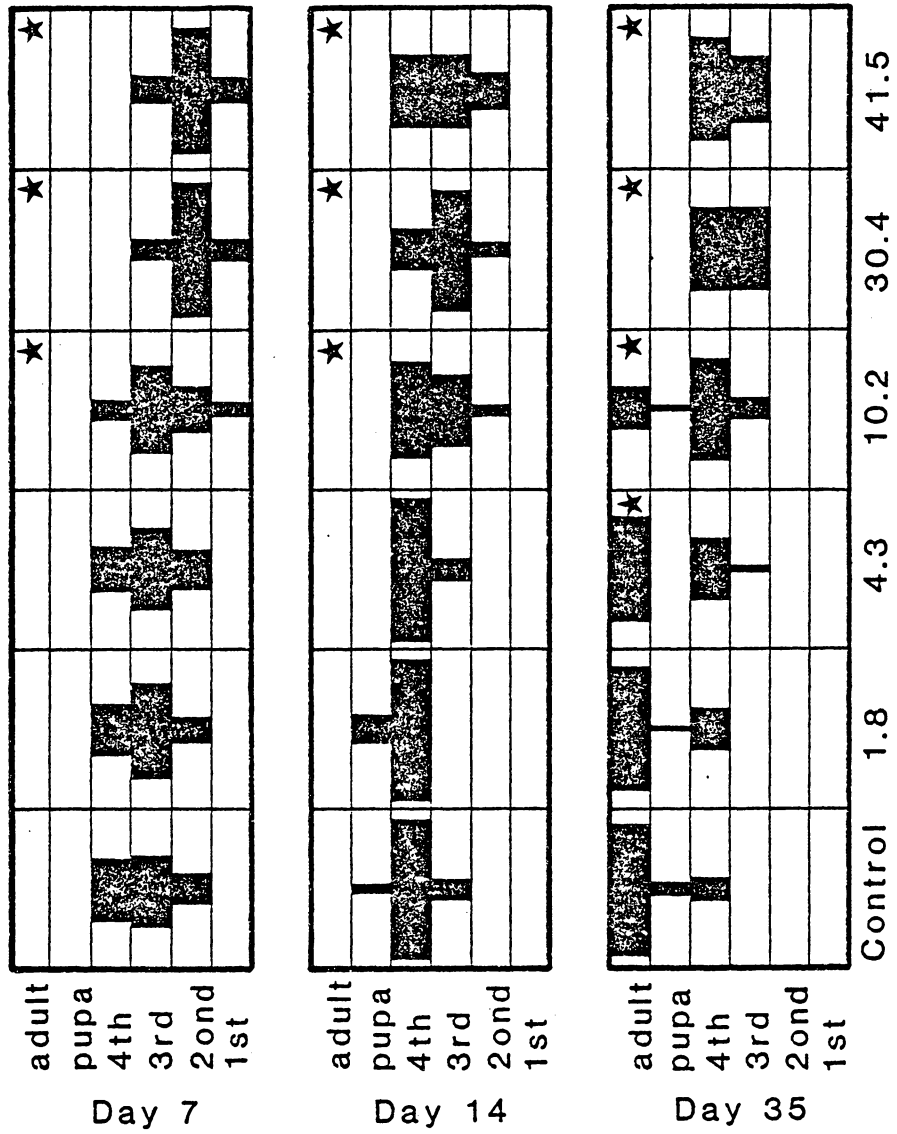


Figure 7: Effects of cadmium on the larval development of *Chironomus riparius*. Starred distributions were significantly different from the control (alpha=0.05).

staggered over a three week period. Consequently, there were never more than 6 live adults present in any tank at any time. This is below the critical swarming density of 7 individuals necessary for successful fertilization in C. riparius (Downe and Caspary 1973). As such, this test produced no data on the effects of cadmium on egg embryogenesis, hatchability, or second generation survival and growth. More data is needed for a definitive evaluation of the effects of cadmium on the on the reproductive success of C. riparius.

4.4 DISCUSSION AND SUMMARY

The 48 h LC50 for C. riparius exposed to cadmium in this study was 7,900 ug/L. As a class, aquatic insect larvae are resistant to heavy metals in acute exposure and this value is quite comparable to acute values obtained for other aquatic insect larvae (range=1,200-28,000 ug Cd/L; USEPA 1980b).

Chronic exposures to ≥ 10.2 ug Cd/L resulted in significant and persistent decreases in survival, growth, and development, and completely repressed the production of eggs in C. riparius. All responses monitored appeared to be equally sensitive measures of cadmium stress. The MATCs for these effects were 6.6 ug Cd/L. However, the LC20 for

survival to adult was 4.6 ug Cd/L suggesting that there may be a biologically significant effect below the MATC.

In one other long term study of the effects of cadmium on an aquatic insect Anderson et al. (1980) found the 10 d LC50 for Tanytarsus dissimilis to be 3.8 ug Cd/L. The 14 d LC50 of 5.4 ug Cd/L was quite comparable to this value.

Chapter V

CADMIUM TOXICITY TO MICROBIAL COMMUNITIES

5.1 INTRODUCTION

Several researchers have used Polyurethane Foam (PF) substrates to study the dynamics of freshwater protozoan communities (see Cairns 1982). A PF substrate which is colonized in a natural system typically contains the majority of protozoan species occurring in that system (Yongue and Cairns 1973) in addition to detritus, bacteria, fungi, diatoms and other algae, rotifers, and annelids. The inhabitants of the sponge constitute a complex species assemblage that has many of the characteristics of a community such as energy transfer, competition, succession, and internal homeostasis. The processes of colonization and maintenance of structural integrity on these substrates as monitored by observations of protozoan species abundance have proven to be reliable functions that reflect the quality of the surrounding water. Both processes have been studied in a variety of natural systems and in the laboratory with and without toxic stress (e.g. Buikema et al. 1983; Cairns et al. 1980; Cairns et al. 1979; Hart and Cairns 1984; Henebry and Cairns 1980a; 1980b; Kuhn and Plafkin 1977; Plafkin et al. 1980).

Several characteristics of PF substrate communities make them a promising system for the evaluation of toxic effects at a level of complexity greater than the single species. First, because they are naturally derived, both the individual species and the structure and organization of the test system are indigenous, e. g. presumably the successional process has already "designed" a system rather than an arbitrary assemblage of species, and this system can be transported and maintained in the laboratory. Second, the protozoan component of these microbial communities seems to be a practical yet effective focal point for analysis of community level toxic effects. Many individual species and interactions are exposed and tested, yet the small scale of the test minimizes the distortion inherent in laboratory work and makes this community level test comparable to more conventional single species tests in time and cost. The unusually broad range of life styles found in common Protozoa engenders a test that simultaneously monitors effects on multiple primary producers, detritivores, consumers, and predators. And the sensitivity of protozoans to toxic chemicals seems to be similar to that of more standard test organisms (Ruthven and Cairns 1973; Kneip et al. 1979). Finally, the responses measured, inertia (Cairns 1976) and colonization success, are functional

measures at the community level of organization. Colonization success reflects the ability of the community to replicate and organize itself and is somewhat analogous to reproduction in the single species. Because rapid and continual recolonization and succession are common in microbial communities, occurring with a frequency of days, not months or years, concentrations of substances affecting the colonization process may be expected to have effects that may be felt in other ecosystem compartments. For example, Matthews et al. (1980) found that disturbances in microbial communities on PF substrates were correlated to changes in the diversity and distribution of functional groups of macroinvertebrate communities in a small stream receiving two effluents.

5.2 MATERIALS AND METHODS

PF substrates (7.5 x 5.0 x 6.0 cm) were suspended 0.2 m below the surface in 1 to 1.5 m depth in Pandapas Pond (Montgomery County, VA). Pandapas Pond is a 3.24 ha soft water man-made impoundment. Studies in this system have indicated that an equilibrium number of 40 to 60 species is consistently reached within 10 to 14 d and then maintained with some fluctuations in numbers and changes in composition (J. R. Pratt, unpublished data). PF substrates

were suspended in the pond for 4 to 5 wk and were then retrieved and transported to the laboratory in water from Pandapas Pond. Two substrates were examined to verify that equilibrium species numbers had been reached and to establish the original number of species on these PF substrates. The replicability of species number on these substrates has been demonstrated by Cairns et al. (1976).

All laboratory tests were conducted in dechlorinated tap water (Table 1). Water was pasteurized before use by heating to 70° C for 10 min followed by cooling and aeration. Toxicant was introduced as stock solutions of reagent grade $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$. Tests were set up at temperatures near the ambient temperature in Pandapas Pond at the time the PF substrates were collected. Tests were static without renewal or aeration. Light was provided by cool-white fluorescent bulbs at an intensity of 150 ft-c at the air-water interface and a photoperiod of 16L:8D.

Species numbers were determined within 12 h after a PF substrate was harvested. Substrates were harvested by squeezing the contents into a 500 ml beaker and allowing them to settle for 20 min. Two to 4 subsamples of 2-3 drops of material from the bottom of the beaker were pipetted onto microscope slides, covered with a No. 1, 22 mm coverglass, and examined microscopically. The entire coverglass was

scanned systematically at 200 and/or 450x total magnification. Protozoans were identified to genus and to species whenever possible using standard taxonomic keys (e.g. Kahl 1930-1935; Kudo 1971; Page 1976; Pascher 1913-1927). Species occurrence was recorded on a master species list. Counts of number of species tend to be asymptotic with the majority of species appearing on the first slide (Cairns et al. 1979). Generally 2 slides from each sample were examined, but when the second slide increased the total species number by more than 30% a third slide was examined. Pearson's product-moment correlation (r) of species numbers determined for a single substrate by different investigators in this study was 0.98.

In an acute test, colonized PF substrates were exposed to 4 cadmium concentrations and a control in 2 L culture dishes containing 1.25 L test media and 1 PF substrate per triplicate vessel. The ambient temperature in Pandapas Pond at the time of collection was 9° C and the test was conducted at 10±1° C. Number of species remaining were determined after 48 h.

Samples for the confirmation of cadmium concentrations were collected from the stock solution at the beginning of the test and from test vessels at the end of the test. Samples from both the water column and the sampled PF

substrate were digested in redistilled nitric acid and total cadmium concentrations were determined by means of flame atomic absorption spectrophotometry (USEPA 1979; Chapter II). Hardness, alkalinity, conductivity, and pH of the diluent were monitored as previously described.

Mean numbers of species in each concentration were compared with one-way ANOVA and differences were located with Duncan's multiple range test. The relationship between cadmium dose and number of species was examined with ordinary least squares regression analysis. An EC50, representing the concentration required to reduce the species number to 50% of the mean number on control sponges, was estimated from this regression line by means of inverse prediction. An EC20 was determined similarly. Results are reported in terms of the mean measured total cadmium concentration of the substrate contents.

Chronic effects of cadmium stress were evaluated by monitoring species accrual on barren PF substrates over time. A colonized PF substrate epicenter from Pandapas Pond was anchored with nylon line just below the air-water interface in the center of a 30-L leached plastic tub (45 cm diameter and 22 cm height) containing 20 L of test media. Six barren PF islands were similarly suspended around the perimeter of the tub equidistant from the epicenter and from

each other. Duplicate tubs of five cadmium concentrations and a control were set up. The temperature in Pandapas Pond at the time of collection was 20° C and tests were conducted at room temperature, averaging 24° C and ranging from 21 to 27° C over the 28 d test. The tubs were randomly placed on lighted shelves surrounded by plastic sheeting. Test media were not renewed but distilled water was added to replace evaporative loss.

One PF island from each tank was destructively sampled 1, 3, 7, 14, 21, and 28 d after set up. Epicenters were sampled on day 29. PF substrates were harvested and the species numbers determined as described above.

Colonization curves describing species accrual on the islands over time according to the MacArthur-Wilson model of noninteractive island colonization (MacArthur and Wilson 1967) were fitted and compared using the SAS nonlinear regression program (Helwig and Council 1979). The model was:

$$Y = S_{eq} (1 - e^{-Gt})$$

where Y is the number of species on day t, S_{eq} is the estimate of asymptotic or equilibrium species number, and G is the estimate of growth or colonization rate. "Dummy"

variables were used to identify treatment groups and compare treatment group curves to the control (Kleinbaum and Kupper 1978). The relationship between the estimate of equilibrium species number and cadmium dose was examined using ordinary least squares regression analyses.

On day 28, chlorophyll a and ATP measurements were made on the contents of the PF islands. Epicenters were similarly analyzed on day 29. Triplicate chlorophyll a determinations were made on each sampled substrate using the phaeophytin corrected fluorometric method (APHA et al. 1981). A 1 ml sample was injected into 9 ml 100% spectrograde acetone. This 90% acetone mixture was extracted at 4° C for 24 h in the dark. Measurements were made immediately after extraction using a Turner Designs fluorometer. Similarly, three subsamples of the substrate contents were extracted for ATP analyses. Each 1 ml sample was injected into 9 ml boiling tris buffer (tris (hydroxymethyl) aminomethane, 0.02 M, pH 7.75) and heated in a boiling water bath for 5 min. The extractions were frozen at -20° C until analysis. ATP measures were made using the luciferin-luciferase bioluminescence technique (APHA et al. 1981). A Labline 9140 ATP photometer and FLE-50 firefly enzyme (Sigma Chemical Co.) were used. An Autotrophic Index (AI) was calculated (Matthews et al. 1980) for each

PF substrate from mean chlorophyll a and ATP values as follows:

$$AI = \frac{\text{ATP biomass (mg/L)}}{\text{chlorophyll a (mg/L)}}$$

where:

$$\text{ATP biomass} = \frac{\text{ATP (ng/L)}}{2,400 \text{ (mg biomass/ng ATP)}}$$

Differences between cadmium treatment groups in ATP biomass estimates and chlorophyll a content were examined with one-way nested ANOVA and differences were located by means of Least Significant Difference Tests. AI values were compared with one-way ANOVA and Duncan's multiple range test. Dose-response lines were fitted with ordinary least squares regression.

Samples for the determination of total cadmium were collected weekly from both the water column and the sampled substrate. Samples were digested in redistilled nitric acid and samples expected to have cadmium concentrations ≤ 2 ug/L were analyzed in concentrated form. Total cadmium concentrations were determined using graphite furnace atomic absorption spectrophotometry (USEPA 1979b; Chapter II).

Other water quality parameters, i. e. pH, hardness, alkalinity, dissolved oxygen, and conductivity were monitored weekly in the control and the highest cadmium concentration.

5.3 RESULTS

Table 7 summarizes the results of the acute test. The stock solution was 12% below its nominal concentration at the beginning of the test and nominal cadmium concentrations were adjusted accordingly. Water column concentrations at the end of 48 h were approximately 75% of the adjusted nominal concentration. Much of this drop may have been due to adsorption to the soft glass test vessels (Struempler 1973). The cadmium concentration of the substrate contents ranged from 35 to 54% of water column concentrations. Complete penetration of the substrate by the toxicant was not achieved within 48 h. Subsequent analyses were based on the mean measured total cadmium concentration of the substrate contents.

There were 46 and 47 species on PF substrates examined at the time of collection. After 48 h in the laboratory, control PF substrates had a mean of 50 species. There was no apparent effect due to transplanting the PF substrates. There were significant differences in the number of species

TABLE 7

Effects of 48 h cadmium exposures on structural inertia of protozoan communities.
Standard deviations follow mean values. Sample size=3.

Nominal Cadmium Concentration	Adjusted Cadmium Concentration	Mean Measured Cadmium Concentration		Mean Number of Species Remaining
		Water Column	PF Substrate	
Control	---	<25 ---	<25 ---	50.0 ±5.2
2,500	2,200	1,540 ±50	560 ±160	46.0 ±1.0
5,000	4,000	3,310 ±90	1,240 ±480	38.0* ±2.6
10,000	8,800	6,730 ±240	3,290 ±120	27.0* ±2.6
20,000	17,600	14,090 ±950	7,600 ±480	6.7* ±7.6

*Significantly different from the control ($\alpha=0.05$).

remaining after 48 h ($p \leq 0.0001$; Table 7). The LOEC was 1,240 ug Cd/L. The number of species remaining declined with increasing cadmium concentration (Figure 8). The EC50 estimated from this line was 4,000 (3,840-4,150) ug Cd/L. Similarly, the EC20 was 1188 ug Cd/L. However, the 95% confidence limits for the EC20 included zero (-15.74-2228.8 ug Cd/L) indicating no significant difference from the control.

Table 8 summarizes results of cadmium determinations for the chronic test. Despite precautions, problems occurred with cadmium determinations. Some samples were grossly contaminated, probably from glassware used in the digestion of samples. These samples were eliminated and sample sizes reduced accordingly. The total cadmium concentrations of the remaining samples were comparable to nominal concentrations with the exception of values for contents of control PF substrates. I suspect these values are artificially high due to low level contamination exacerbated by the concentration of the samples before analysis. Control values for the epicenter contents are more consistent with our previous experience. In contrast to the acute test, no discrepancy occurred between internal and external cadmium concentrations as early as day 1 because the islands were filled with test media as they were placed

$$Y = 46.33 - 0.0053 X$$

$$R^2 = 0.93$$

$$P_{B=0} = 0.0001$$

$$P_{LOF} = 0.367$$

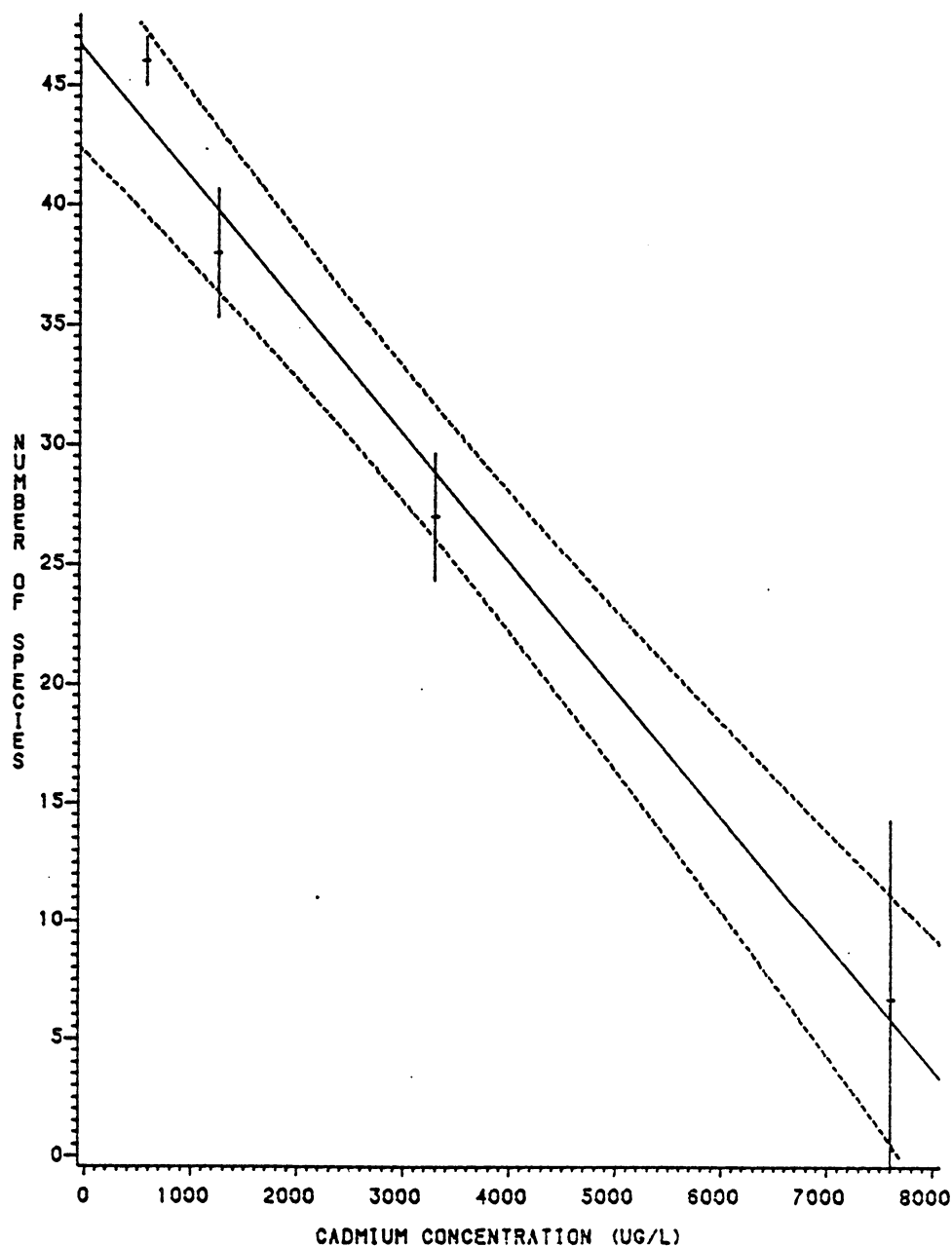


Figure 8: Relationship between cadmium dose and number of protozoan species after a 48 h exposure.

Bars span 1 standard deviation.

Dashed lines are 95% confidence limits for mean values.

in the tub. All results are reported in terms of mean measured total cadmium concentration of the PF island contents. Other chemical measures remained within normal ranges for dechlorinated tap water throughout the test period (Table 1). Dissolved oxygen remained above 80% saturation.

The numbers of species appearing on islands in each cadmium concentration over time are plotted in Figure 9, and results of nonlinear regressions using "dummy" variables to identify exposure groups are presented in Table 9. The regression was highly significant ($p \leq 0.0001$, $r^2 = 0.86$). No differences in colonization rate (G) occurred in comparisons of treatment groups to the control ($p = 0.414$). All groups approached their maximum number of species in a week. But there were significant differences between groups in estimates of equilibrium species number (Seq; $p \leq 0.0001$). All estimates of equilibrium species numbers except that for the lowest cadmium concentration tested (0.4 ug/L) were significantly different from the control (LOEC=1.4 ug Cd/L). The estimates of equilibrium species number were strongly related to cadmium concentrations (Figure 10). The EC20 calculated from this dose-response regression was 0.9 (0.2-3.3) ug Cd/L.

TABLE 8

Cadmium concentrations in the chronic test with microbial communities.

Mean Measured Cadmium Concentration (ug/L total cadmium)*			
Nominal	Water Column	PF Island Contents	PF Epicenter Contents
Control	0.5 ±0.2 9	1.2 ±0.5 8	0.3 --- 2
0.6	0.7 ±0.3 9	0.4 ±0.2 6	0.6 --- 2
1.3	1.0 ±0.7 8	1.4 ±1.3 10	1.7 --- 2
2.5	1.4 ±1.2 8	2.7 ±1.4 7	--- --- 0
5.0	3.4 ±1.7 9	5.6 ±2.1 9	4.3 --- 2
10.0	7.4 ±3.8 10	9.5 ±3.4 10	12.2 --- 1

*Standard deviations and samples sizes follow mean values.

TABLE 9

Effects of chronic cadmium exposure on the microbial
colonization of barren substrates.

Treatment Group	Seq Estimate $\pm$ ASE*	G Estimate $\pm$ ASE*
Control	33.9 $\pm$ 2.1	0.18 $\pm$ 0.04
0.4	30.1 $\pm$ 2.0	0.20 $\pm$ 0.05
1.4	26.6** $\pm$ 1.9	0.22 $\pm$ 0.06
2.7	22.6** $\pm$ 1.8	0.27 $\pm$ 0.09
5.6	19.4** $\pm$ 1.7	0.32 $\pm$ 0.12
9.5	13.1** $\pm$ 1.6	0.36 $\pm$ 0.20

*Asymptotic Standard Error

**Significantly different from the control ($\alpha=0.05$).

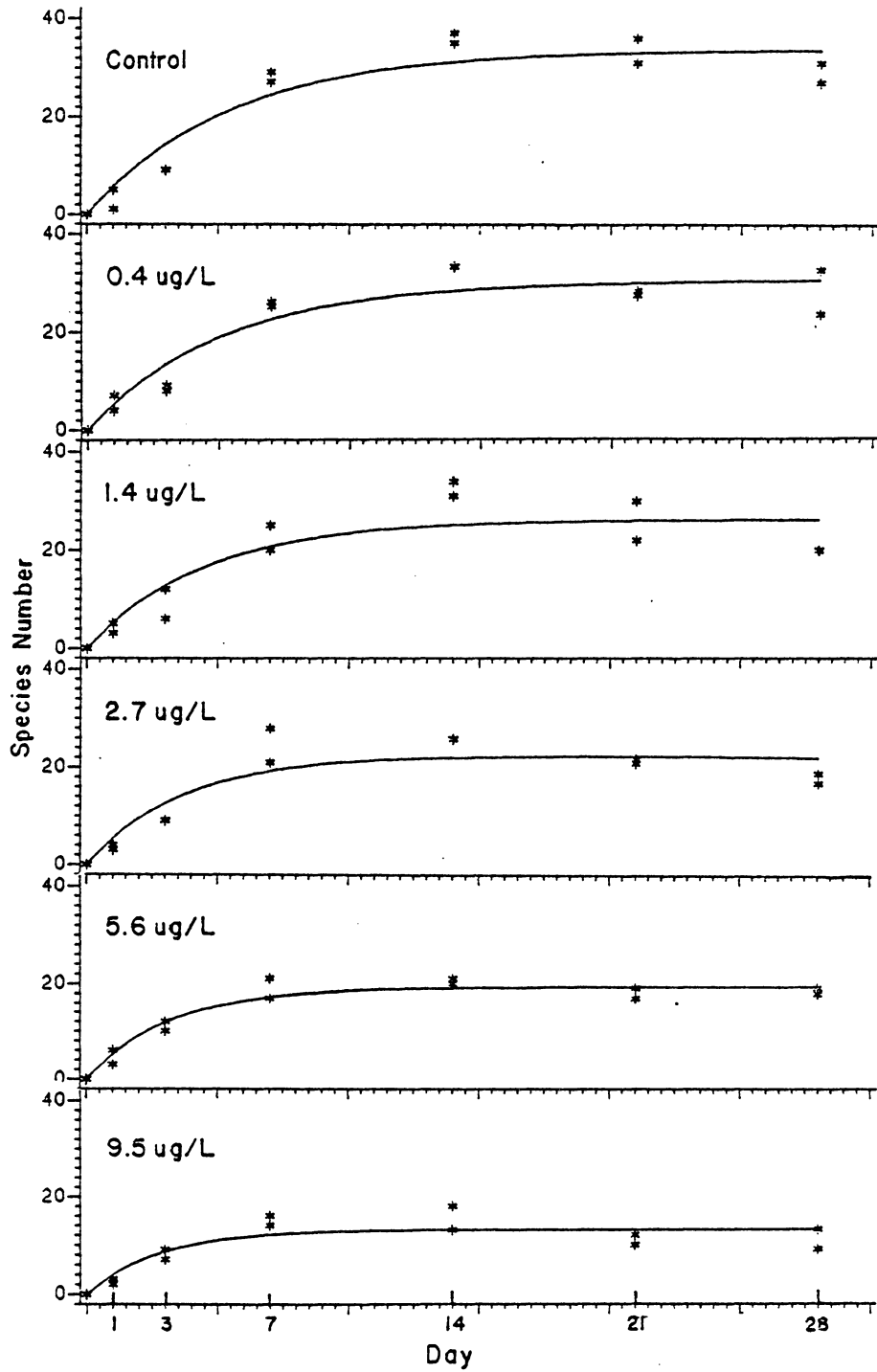


Figure 9: Number of protozoan species colonizing barren substrates over time.

$$Y = 26.8 - 5.10 \ln X$$

$$R^2 = 0.94$$

$$P_{B=0} = 0.007$$

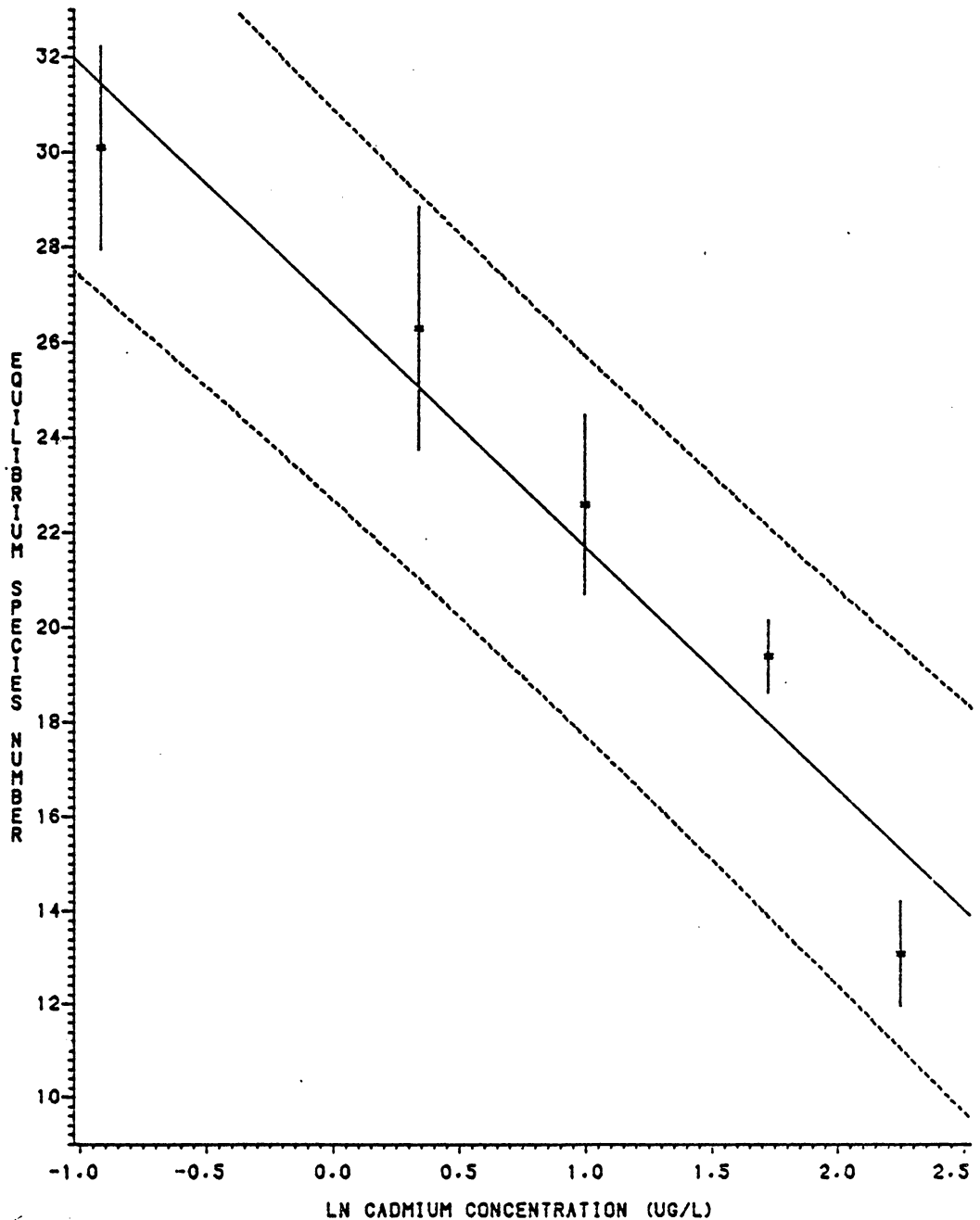


Figure 10: Relationship between cadmium dose and estimated number of protozoan species at equilibrium.

Bars span 1 standard error.

Dashed lines are 95% confidence limits for individual values.

Results of chlorophyll a, ATP biomass, and AI determinations for PF islands are presented in Table 10. Chlorophyll a content was significantly lower than the control at the highest cadmium concentration tested, 9.5 ug/L (LOEC=9.5 ug Cd/L). There was a significant regression of chlorophyll a on cadmium concentration ($p=0.050$, $r^2=0.70$, $Y = 0.013 - 0.003 \ln X$) and there was no significant lack of fit ($p=0.516$). The EC20 estimated from this line was 1.1 (0.2-3.3) ug Cd/L. ATP biomass was significantly greater than control values in cadmium concentrations ≥ 2.7 ug/L (LOEC=2.7 ug Cd/L). There was a significant regression of ATP biomass on cadmium ($p=0.003$, $r^2=0.69$, $Y = 1.671 + 0.783 \ln X$) and there was no significant lack of fit ($p=0.511$). The estimated EC20 was 0.7 (0.02-7.5) ug Cd/L. AI values increased at higher cadmium concentrations. An AI of 200 or above has been suggested as an indicator of pollution stress (APHA et al. 1981). All 3 higher cadmium concentrations exceeded this value. PF islands colonized in 2.7 and 9.5 ug/L cadmium were significantly different from the control but those in 5.6 ug Cd/L were not (LOEC=2.7 ug Cd/L). Although there was a significant trend toward higher AI values with higher cadmium levels ($p=0.010$, $r^2=0.59$, $Y = 166.2 + 225.7 \ln X$), there was also a significant lack of fit ($p=0.037$). The EC20 estimated from the regression was

0.7 ug/L cadmium but the variability of the response is reflected in a wide confidence interval, 0.003 to 20.3 ug Cd/L.

The cadmium concentrations tested had minimal effects on PF epicenters within 29 d (Table 11). The number of species did not differ significantly between treatment groups ($p=0.263$). In addition, the number of species in these mature communities did not change substantially over time. Mean species number on epicenter substrates was 49 at collection and 45 on control sponges after 29 d in the laboratory. There were no significant differences in chlorophyll a content, ATP biomass content, or AI for epicenters after a 29 d exposure to the cadmium concentrations tested ($p=0.500$, 0.250 , and 0.821 , respectively).

5.4 DISCUSSION AND SUMMARY

An acute exposure of 4,000 ug Cd/L was required to reduce the number of protozoan species on colonized PF substrates by 50%. No other acute exposures of microcosms to cadmium were found in the literature for comparison. The response level of the protozoan communities was within the range of response for single species (1 to 73,500 ug Cd/L; USEPA 1980b). It was noted that the acute EC50 derived in this

TABLE 10

Effects of chronic cadmium exposure on the Autotrophic Index
of PF islands after 21 d.

Treatment Group	Chlorophyll <u>a</u>	ATP Biomass	AI
Control	0.016	1.16	74.9
0.4	0.017	1.03	62.2
1.4	0.010	1.43	144.4
2.7	0.010	3.27*	400.2*
5.6	0.009	2.45*	269.2
9.5	0.004*	3.61*	945.2*

*Significantly different from the control ($\alpha=0.05$).

TABLE 11

Effects of chronic cadmium exposure on PF epicenters after
29 d.

Treatment Group	Species Number*	Chlorophyll <u>a</u> (mg/L)*	ATP Biomass (mg/l)*	AI*
Control	45.0	0.098	3.924	39.4
0.4	46.5	0.163	5.406	35.1
1.4	42.5	0.125	5.184	45.7
2.7	48.0	0.148	4.130	27.7
5.6	42.0	0.089	3.076	34.3
9.5	, 40.5	0.143	5.681	43.0

*No significant differences from the control ($\alpha=0.05$).

test did not represent an incipient effect level. In longer exposures, much lower toxicant concentrations were sufficient to produce a reduction in species number on colonized substrates. Although there was no reduction in species number after a 2 d exposure to 560 ug Cd/L in this test, a previous test found a 76% reduction in species number after a 21 d exposure to 442 ug Cd/L (Pratt et al. 1983). Both delayed effects and a time-dose dependency are likely. Another state variable, e.g. adenylate energy charge ratio, DO, pH, pE, may be a more sensitive indicator of acute effects than species elimination.

Measures of the chronic effects of cadmium on protozoan communities suggest 2 distinct effect levels. The colonization of barren substrates was consistently affected at concentrations around 1 ug Cd/L. The equilibrium species number was the most regular and sensitive response. The MATC for this parameter was 0.7 ug Cd/L and the EC20 was 0.9 ug Cd/L. In contrast, PF epicenters were not affected at the highest cadmium concentration tested, 9.5 ug/L. Results of a previous test suggest that concentrations 2 orders of magnitude greater may be required to disrupt the structural integrity of colonized PF substrates even in long term exposures (Pratt et al. 1983). This discrepancy in effect levels is consistent with other work suggesting increased

stress tolerance in more mature communities (e.g. Cairns et al. 1980; Mellinger and McNaughton 1975; Odum 1981), and supports the underlying assumption that these microbial communities are, indeed, "microcosms", small systems behaving in the same way larger systems do.

Similarly, Odum (1981) hypothesized that communities completing ecological succession under stress will be less complex than those developed under more favorable conditions. The continuously declining equilibrium species numbers of the communities developed under increasing cadmium stress in this study may provide an illustration of this relationship.

However, biomass, another measure often cited as an indication of organizational complexity, increased with cadmium stress in this study. Samples in higher concentrations appeared quite different from controls with markedly less detritus, more fungal material, and great abundances of a few heterotrophic protozoans including Entosiphon sulcatum, Bodo celer, and Microthorax costatus. Duddridge and Wainwright (1980) found cadmium stimulated fungal growth at some concentrations and Hendrix et al. (1981) also found increases in fungal abundance and biomass with increasing cadmium concentration in 7 L microcosms.

The chronic effect levels determined in this test were similar to other available data on cadmium toxicity to complex test systems. Microcosm studies have found effects of cadmium at concentrations of 0.2, 3.8, 5, 10, and 10 ug Cd/L (Marshall and Mellinger 1980; Taub 1976; Giesey et al. 1979; Heath 1979; and Hendrix et al. 1981, respectively).

Chapter VI

CADMIUM TOXICITY IN THE AQUATIC ENVIRONMENT

6.1 INTRODUCTION

The particular concern about heavy metals in the aquatic environment stems from unfortunate experience. Losses of natural resources and human health effects have been linked to cadmium in the aquatic environment (e. g. Friberg et al. 1974; Forstner and Wittmann 1981). These incidents not only focus our attention on the necessity of waste management, they also can serve to validate our current hazard evaluation strategies. By comparing our laboratory based hazard estimates to experience, a rough indication of the adequacy of our estimation techniques can be made. If the estimated effect levels fall above levels associated with environmental damage or human health effects in the past, regulation on the basis of those laboratory estimates are likely to allow damage and the costs associated with damage. Similarly, if the effect levels estimated by laboratory tests fall below naturally occurring levels of the trace element in areas uninfluenced by man, regulation on the basis of those laboratory estimates are likely to engender treatment costs not justified by ecological benefit.

6.2 MATERIALS AND METHODS

A review of the literature and several national water quality monitoring data bases was undertaken. Chemical Abstracts and the Government Report Announcement Index were searched from 1978-1983. Bibliographies on the effects of heavy metals were also searched (e. g. Fulkerson and Goeller 1973; Hammons et al. 1978; Eisler 1979). The United States Geological Survey provided raw data for chemical observations on Benchmark Stations from 1964-1981. These stations were established in 1958 to provide a data base on relatively pristine systems "in order to provide a comparative base for studying the effects of man on the hydrological environment" (Cobb and Biesecker 1971). Additional data on ambient cadmium levels were obtained from individuals involved in similar research. Several Canadian documents were provided by Dr. Donald MacGregor, Freshwater Institute (Manitoba, Canada). An analysis of data for USEPA region 10 was provided by Dr. Charles Delos, USEPA, Office of Water Regulations and Standards (Washington, DC).

There are a number of problems inherent in this type of approach. First, caution is always appropriate when using data for purposes other than that for which it was originally collected. Second, cadmium does not occur alone in the environment. It is always associated with other

substances, particularly other heavy metals, and deleterious effects can not be separated. Third, fate studies indicate that cadmium inputs will rapidly concentrate in the sediment (e. g. Huckabee and Blaylock 1974). So, although sediment levels will be the most relevant environmental measure of ambient levels, water column measures are more common and more comparable to data generated from prescribed single species toxicity tests. Also, there are a variety of methods for measuring cadmium in water. Most measures will be similar but not necessarily identical. Fourth, the ability to measure cadmium in background levels is a relatively new development. Many papers report values of questionable validity because they overstep the bounds of the analytical technique used or there is inadequate attention to quality control during chemical analysis. Because ambient levels of cadmium are so close to detection limits for the more available analytical techniques, both contamination (e. g. Salmela and Vuari 1979; Lutz 1980) and extrapolation error (MagGregor, personal communication; USEPA 1981) may be significant problems. Both errors are consistently positive, suggesting higher levels of cadmium than true values. Fifth, field studies often suffer from wide variability in measurements and the lack of an adequate control. Both these factors make the determination of the significance of an effect more difficult.

With these problems in mind, the following stipulations were developed to select reliable reports for cadmium levels in the environment.

1. Selected reports included an adequate description of the analytical methods used and the concentrations of cadmium reported fell within a generally acceptable range for the analytical method used (Table 12) or well above the reported detection limits for the individual analysis.
2. Unhealthy sites were chosen on the basis of an observed adverse biological effect, not merely the presence of elevated cadmium. An adverse effect included decreased density or diversity of organisms, elimination of specific taxa, poisoned food resources, or degradation of a fishery resource. An effect was determined on the basis of a statistical comparison to a control, or in some cases, professional judgement or consensus. Bioaccumulation was not considered an ill effect in and of itself.
3. Reports of damage in systems where another pollutant, such as pH, was obviously the predominant problem were eliminated. However, reports where problems stemmed from mixtures of heavy metals were retained because they are typical of situations in which

TABLE 12

Common methods for the analysis of cadmium in water.

Method	Abbreviation	Example of Reported Optimal Range*	Example of Reported Detection Limit*
Flame Atomic Absorption Spectrophotometry	FAAS	25-2000	5
Solvent Extraction- Flame Atomic Absorption Spectrophotometry	SE-FAAS	variable	0.05-1.1
Concentration- Flame Atomic Absorption Spectrophotometry	C-FAAS	>0.5	0.1
Graphite Furnace Atomic Absorption Spectrophotometry	GFAAS	0.5-10.0	0.01
Inductively Coupled Plasma Spectrometry	ICAP	>5.0	2.7
Isotope Dilution-Mass Spectrometry	ID-MS	>1.0	---
Anodic Stripping Voltammetry	ASV	>0.01	0.005
Differential Pulse Polarography	DPP	>1.0	---

*All values are in ug Cd/L.

cadmium is encountered. Although cadmium may be present in very small concentrations relative to other metals, its greater toxicity may make it the most significant portion of these mixtures. In addition to using cadmium levels alone, a crude attempt was made to acknowledge the contributions of other metals to toxic effects in the field. A total metal index (TMI) was calculated for sites receiving mixtures of heavy metals by converting observed levels of all heavy metals in the water column to cadmium equivalent toxic units and summing these units. Cadmium equivalent toxic units were calculated by dividing observed metal concentrations by their relative chronic toxicity to Daphnia magna as determined by Biesinger and Christensen (1972). The ratios used are presented in Table 13.

4. Healthy systems were chosen on the basis of either observed ecological health, e.g. diversity, healthy sport fishery, or on the basis of an absence of major anthropogenic inputs including industrial effluents or nearby sources of air pollution. Some healthy systems may have been subjected to some agricultural runoff and deposition of aerially transported pollutants, thus, measured cadmium concentrations may be above true natural levels.

TABLE 13

Ratios for the calculation of cadmium equivalent toxic units. Data are from Biesinger and Christensen (1972).

Metal	Ratio of effect levels (Metal/Cd)*			
	Survival (3 wk LC50)	Growth (weight, GOT, total protein)	Reproduction (16% impairment)	Geometric Mean of Ratios
Zn	158/5	175/1	70.0/0.17	132
Pb	300/5	62/1	30.0/0.17	87
Cu	44/5	40/1	22.0/0.17	36
Cr	2,000/5	619/1	330.0/0.17	783
Ni	130/5	125/1	30.0/0.17	83
Fe	5,900/5	7,480/1	4,380.0/0.17	6,104
Mn	5,700/5	5,000/1	4,100.0/0.17	5,161

*All values are in ug/L.

To facilitate the comparison of resulting data, reported cadmium values for water were converted to ug/L total cadmium wherever possible. If both dissolved and suspended cadmium concentrations in the water column were reported, the figures were summed for an estimation of total cadmium. Sediment values were converted to ug/g dry weight. If authors did not summarize chemical data for a site into a single index, a geometric median was calculated from all the data presented. Resulting table entries were summarized by calculating geometric means and medians. Values reported as < or > were not included in these calculations.

6.3 RESULTS

The results of the literature review are presented in Tables 14 and 15. Reported levels of cadmium in the water of healthy systems ranged from 0.019 to 0.7 ug Cd/L. The geometric mean and median for these values were 0.060 and 0.05 ug Cd/L, respectively. Sediment contained 0.43 to 10.0 ug Cd/g (mean=1.14, median=0.78). Reports for systems damaged by anthropogenic inputs were substantially above these levels. Cadmium levels in the water of damaged ecosystems ranged from 0.6 to 47.6 ug/L. The geometric mean and median for these values were 5.25 and 3.80 ug Cd/L, respectively. Values for the total metal index were higher

(mean=11.23, median=9.2 cadmium equivalent toxic units). Reported sediment levels of cadmium in damaged systems were quite variable. The geometric mean and median were 478.4 and 439.4 ug Cd/g, respectively.

6.4 DISCUSSION AND SUMMARY

These field data indicate that natural levels of cadmium fall in the range of 0.01 to 0.10 ug/L. This small amount of cadmium is probably not of anthropogenic origin. Weathering of bedrock may be the primary source of cadmium in these these systems. Cadmium content of common rock types of 0.02 to 0.43 mg/kg have been reported (Hem 1970). These values are in the same range as sediment levels in healthy systems. Management goals falling below the values in Table 14 would not be rational or obtainable.

Levels of cadmium in the water column of systems damaged by anthropogenic wastes were around 3.80 ug/L. When expressed as total metals in cadmium equivalent toxic units this value was 9.2 ug/L. Because these values are based on observed damage they are most comparable to a lowest observable effect concentration (LOEC) as determined in single species chronic toxicity tests. Management goals substantially higher than these values may not provide an adequate basis for the protection of ecosystems.

TABLE 14

Cadmium levels in healthy aquatic ecosystems.

Site	Reference	Cadmium in Water (ug/L)	Type of Measure	Cadmium in Sediment (ug/g)	Type of Measure	Method of Analysis*	Criteria for Inclusion
Lake Ontario	Nriagu et al. 1981	0.05	Mean Labile	10.0	Mean Dry wt	SE-FAAS	Healthy fishery
Lake Michigan	Muhlbaier et al. 1982	0.026	Mean Total	---	---	ID-MS	Healthy fishery
Helena River, Australia	Rosman & DeLaeter 1976	0.02	Mean Total	---	---	ID-MS	Absence of inputs
Lake Superior and Superior National Forest Streams, MN	Poldoski & Glass 1978, Poldoski & Glass 1975	0.019 0.026	Mean Acid- exchangeable, Mean Total	---	---	ASV	Healthy fishery, absence of inputs
Lake Erie	Nriagu et al. 1979	---	---	1.0	Mean Dry wt	FAAS	Precolonial levels
Jubilee Creek, IL	Enk & Mathis 1977 Mathis & Cummins 1973	0.1	Mean ---	---	---	SE-FAAS	Diversity, Absence of inputs
Swedish Lakes	Borg 1983	0.040	Mean ---	---	---	ASV	Absence of inputs
Norwegian Lakes	Henriksen & Wright 1978	0.22	Median Acidified	---	---	GFAAS	Absence of inputs

CONTINUED ON NEXT PAGE

*See Table 12 for explanation of abbreviations.

TABLE 14 (Continued)

Site	Reference	Cadmium in Water (ug/L)	Type of Measure	Cadmium in Sediment (ug/g)	Type of Measure	Method of Analysis*	Criteria for Inclusion
Wildlife Area Lake, IN	Mayes <i>et al.</i> 1977	---	---	0.60	Median Dry wt	FAAS	Absence of inputs
Rural Streams, Ontario	Inhat 1981, Inhat <i>et al.</i> 1980	0.08	Median Total	---	---	5 methods compared	Absence of industrial inputs
Rivers, USEPA Region 10	USEPA 1981	0.107	Mean ---	---	---	GFAAS	No point discharge <20 mi upstream
USGS Benchmark Stations	WATSTORE Data Base 1978-1981	0.7	Mean Total Recoverable	<10.0	Mean Dry wt	GFAAS	Absence of inputs
Skinface Pond, SC	Giesey <i>et al.</i> 1977	0.020	Mean Total	---	---	GFAAS	Absence of inputs
Upper St. Francis River, MO	Proctor & Sinha 1978	0.09	Mean Dissolved	0.43	Mean Dry wt	GFAAS	Absence of inputs
Wisconsin Lakes	Iskandar & Keeney 1974	---	---	1.6	Median Dry wt	FAAS	Precolonial levels
Algonquin Lake, IL	Anderson <i>et al.</i> 1978	---	---	0.54	Mean Dry wt	SE-FAAS	Absence of inputs

*See Table 12 for explanation of abbreviations.

TABLE 15

Cadmium levels in damaged aquatic ecosystems.

Site	Reference	Cadmium in Water (ug/L)	Type of Measure	Cadmium in Sediment (ug/g)	Type of Measure	Method of Analysis*	Criteria for Inclusion	Other Toxicants (ug/L)	Total Metal Index	Source of Metals
Palostine Lake, IL	Wentzel et al. 1977, Yost 1979	47.6	Mean Total	490	Mean Dry wt	SE-FAAS	Decreased chironomid abundance	Zn 571 Cr 95	52.0	Electro- plating effluent
	Nugent et al. 1980	---	---	585	Mean Dry wt	D-FAAS	Decreased microbial biomass	Zn 17,840 Cr 4,380	---	
Little Center Lake, IL	McIntosh & Bishop 1976	3.2	Mean Total	394	Mean Dry wt	C-FAAS	Elimination of cladocera and benthos	Zn 138 Pb 19	4.4	Electro- plating effluent
Foundry Cove, Hudson River, NY	Knelp et al. 1979	18.0	Median Total	26,969	Median Dry wt	GFAAS	Decreased benthos abundance	Ni 18	18.2	Battery Plant effluent
		3.8	Median Total	127	Median Dry wt	GFAAS	Potential human health effects-- Contaminated crab	---	---	
Homell Lake, Canada	McFarlane & Franzin 1978	0.6	Mean Total	---	---	GFAAS	Decreased fish abundance	Zn 245 Cu 13 Fe 59 Mn 30	2.9	Smelter
Jintsu River, Japan	Yagamata & Shigematsu 1970	1.7	Median Dissolved	31.2	Median, ---	C-FAAS	Human Health Effects-- Itai-itai Disease	Zn 54 Pb 12	2.2	Metal mine
River Conwy, England	Johnson et al. 1980	22.3	Mean Total	---	---	GFAAS	"Severely depleted flora and fauna"	Zn 3,256 Cu 7 Pb 57 Fe 248	47.8	Metal mine

CONTINUED ON NEXT PAGE

*See Table 12 for explanation of abbreviations.

TABLE 15 (Continued)

Site	Reference	Cadmium in Water (ug/L)	Type of Measure	Cadmium in Sediment (ug/g)	Type of Measure	Method of Analysis*	Criteria for Inclusion	Other Toxicants (ug/L)	Total Metal Index	Source of Metals
Molongo River, Australia	Weatherly & Dawson 1973, Doolan & Smythe 1973	3.5	---	---	---	GFAAS SE-FAAS	"Wasteland"	Zn-- level not reported	---	Metal mine
Coal Creek, State River, CO	Davies & Woodling 1980	1.3	Mean labile	---	---	SE-FAAS	Decreased fish abundance	Zn 990 Cu 13 Pb <2 Mn 350	9.2	Metal mine
	Janik et al. 1982	15.5	Mean Total	---	---	ICAP	Decreased benthic diversity	Zn 958 Cu 27 Cr 14 Pb 148 Ni 116	26.6	
Coner d'Alene River, ID	Bartlett et al. 1974	5.0	Mean Dissolved	---	---	SE-FAAS	Algal growth inhibition	Zn 550 Cu 2	9.2	Metal mine
Prickly Pear Creek, MO	Miller et al. 1982	<7.5	Mean Total	---	---	ICAP	Decreased benthic abundance	Zn 465 Cu 19 Pb <120	<12.9	Metal mine

*See Table 12 for explanation of abbreviations.

Chapter VII

COMPARISONS AND CONCLUSIONS

7.1 SUMMARY OF TEST RESULTS

The toxicities of cadmium to test systems at 3 levels of the structural hierarchy are summarized in Table 16. In acute tests, Daphnia was most sensitive followed by Aeolosoma, microbial communities, and Chironomus. In chronic tests, microbial communities were most sensitive followed by Chironomus, Daphnia, and Aeolosoma. The acute-chronic ratio was not a stable quantity and did not provide a reasonable method for predicting chronic effects from acute tests.

7.2 DERIVING PREDICTIONS OF PERMISSIBLE CONCENTRATIONS

7.2.1 Single Species Level Tests

Predictions of the level of toxicant that would not cause significant harm to aquatic ecosystems were derived from single species toxicity tests using 2 data bases. Data from the 3 single species tests conducted in this study were summarized, but such a small data base is unlikely to accurately reflect the range of sensitivities of the thousands of organisms likely to be affected by any discharge. A larger data base was compiled from test

TABLE 16

Toxicities of cadmium to test systems at 3 levels of complexity.

Test Level	Test System Response	Acute Effect	Chronic Effect				Acute-Chronic Ratio
			NOEC	LOEC	MATC	EC20	
Population							
A. <u>headleyi</u>							
	Survival	1,200					
	Growth	---	---	---	---	---	
	Reproduction	---	17.2	36.9	27.1*	62.8**	44.3
C. <u>riparius</u>							
	Survival	7,900	4.3	10.2	6.6	4.6	
	Growth	---	4.3	10.2	6.6	6.7	1,197.0
	Reproduction	---	4.3	10.2	6.6	---	
D. <u>pulex</u>							
	Survival	62	5.5	10.2	7.5	---	8.3
	Growth	---	10.2	23.3	16.8*	---	
	Reproduction	---	5.5	10.2	7.9*	8.4	
Community							
Microbial Community							
	Inertia	4,000	≥9.5	>9.5	---	---	
	Colonization	---	0.4	1.4	0.7	0.9	5,714.3
Ecosystem							
Field Observation							
	General Health	---	≥0.05	≤9.2	---	---	---

*Arithmetic mean of NOEC, LOEC.

**Extrapolated beyond tested range.

results cited by the USEPA (1980b; 1983) supplemented by data from this study, more recently published reports, and data from other researchers in this laboratory (Appendix A). This data base is more inclusive than that which the USEPA specifies for use in the derivation of water quality criteria. Specifically, it includes data on nonresident species, acute data for tests in which organisms were fed, and data for static toxicity tests and tests in which the concentration of toxicant was not confirmed by chemical analysis. The assumption underlying these inclusions was that the increase in the numbers of observations would more than offset any possible loss of precision in individual observations. There were other differences from the USEPA data base. If there were sensitivity differences between lifestages of an organism, the lower value was used in calculations, and invertebrate EC50s were preferentially expressed as 48 h observations.

Predictions of permissible levels were derived from the single species level data bases in 2 ways. The first method for summarizing single species data was that suggested by the USEPA (Stephan et al. 1983) for calculating Final Acute Values (FAVs) and Final Chronic Values (FCVs) in deriving water quality criteria. These values are not criteria, but are used by the USEPA in the determination of criteria along

with toxicity determinations for important animal species and data on toxicity to plants and bioaccumulation. FAVs are calculated from mean EC50s for organisms within a taxonomic family (Family Mean Acute Values; FMAVs). The FMAVs are ranked and the cumulative proportion of taxa responding at that concentration is calculated. The FAV, a concentration corresponding to an effect on 5% of taxa, is extrapolated or interpolated from the 4 lowest FMAVs. FCVs can be calculated similarly from all available MATCs. Thus, FAVs and FCVs are intended to protect 95% of taxa. The USEPA suggests that a minimum data base of 8 values from specified groups are necessary for these methods to yield a representative estimate. Values here were calculated using all available data with a relative assessment of adequacy: larger data bases will yield better estimates.

Because there was a substantial data base on the toxicity of cadmium to aquatic organisms, another method of deriving FAVs and FCVs, suggested by Buikema and Cherry (1982), was also applied. The plot of the cumulative % of taxa responding vs cadmium concentration (FMAV) is an empirical distribution function (EDF; Figure 11; e. g. Sokal and Rohlf 1969). The sigmoid shape of the EDF suggests a cumulative normal distribution of the tolerance of aquatic organisms to cadmium. A few taxa are affected at very low cadmium

concentrations, most taxa respond at intermediate concentrations, and a few taxa are not affected until cadmium concentrations are very high. The general impression of normality was confirmed. The Shapiro-Wilk test failed to reject the null ($p=0.439$), suggesting that the distribution did not deviate significantly from normality. In this case, a probit transformation of the Y axis describes the entire distribution with a straight line (Figure 11). The median tolerance of aquatic organisms can be inversely predicted from this regression line at a cumulative probability of 50% (probit=5.0) in the same way an LC50 is calculated. Similarly, the concentration inversely predicted at a cumulative probability of 5% (probit= 3.36) is analogous to the USEPA's FAV. Probit analysis has the advantage of making full use of all the data available rather than just the 4 lowest data points. It will be less affected by possible outliers. Decreased sensitivity to outliers is particularly important because the confidence in the cumulative proportions assigned at the extreme ends of the distribution is low. In addition, in cases where data are normally distributed, this approach is more consistent with standard statistical methods (Buikema and Cherry 1982). Acute data were analysed both as reported and after transformation to a common hardness value of 65

ppm (USEPA 1983). However, there were no significant differences between the 2 distributions ($p=0.830$ and 0.867 , variance and mean, respectively) and only results on untransformed data are reported. The 9 family mean chronic values (Appendix A) were similarly analyzed. The Shapiro-Wilk test of normality failed to reject the null ($p=0.479$) indicating no significant deviation from a normal distribution. Because neither of the methods for deriving FAVs or FCVs provide for the assessment or inclusion of indirect or interactive effects, there is an implicit assumption that protecting individual species will also protect interactions between species.

7.2.2 Community Level Tests

Data from community level toxicity tests required less manipulation to obtain estimates of permissible levels because the "effects of pollutants on biological community diversity" (USEPA 1980a) were directly observed. The acute MATC of 890 ug Cd/L and the chronic MATC of 0.7 ug Cd/L are directly applicable to estimates of permissible concentrations. It is also a simple matter to express results as a percentage of species affected or protected and to calculate corresponding confidence limits. The EC20s calculated from the dose-response regressions are examples

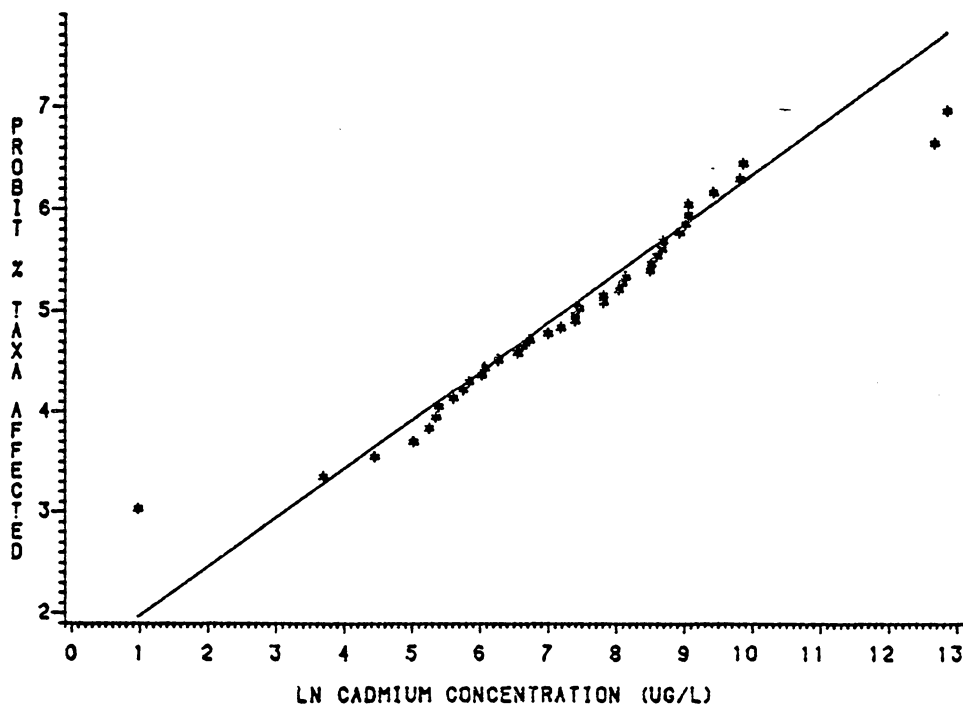
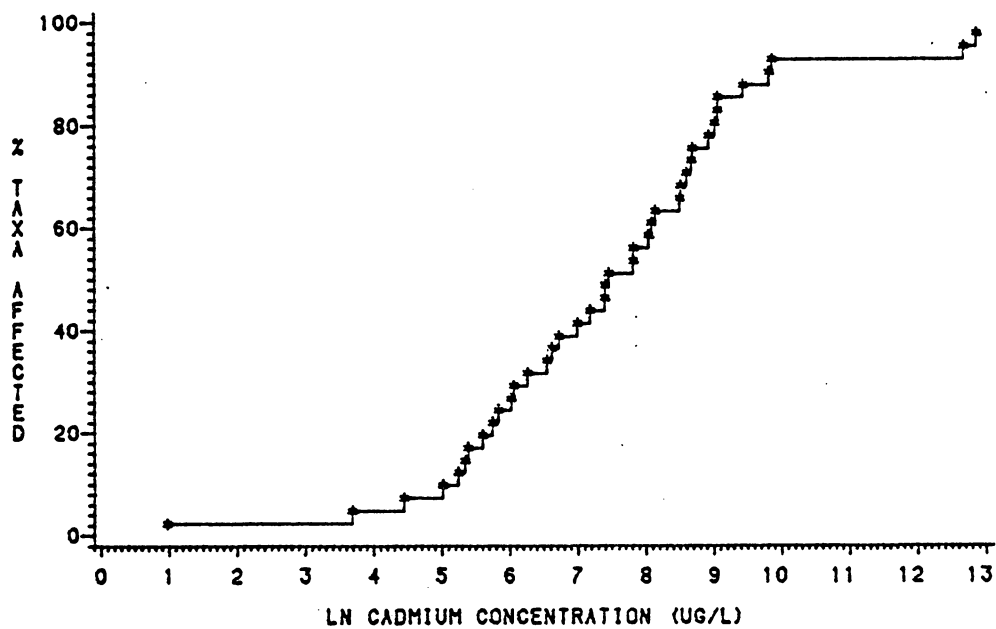


Figure 11: Empirical distribution function for family mean acute cadmium values.

(Chapter V). However, to facilitate comparison to the single species data base, the community level test data were also expressed as cumulative tolerance distributions. For both acute and chronic tests, the cumulative percentages of taxa affected at each tested concentration were estimated as:

$$\% \text{ taxa affected} = 1 - \frac{\text{mean species number on exposed}}{\text{mean species number on control}} \times 100$$

Probit analysis was used to estimate concentrations affecting 50 and 5% of taxa.

It was noted that standard errors for the mean number of species on control substrates in the community level tests exceeded 5% of the mean. This suggests that background fluctuations in species numbers, unattributable to any chemical insult, can exceed the effect level defined by the USEPA.

7.2.3 Ecosystem Level Observations

Obviously, field data required no translation before application as ecosystem effect levels. However, permissible levels were not readily defined by these data. Instead, ecosystem level observations were used to delimit a rational range of concentrations for laboratory based

estimates of permissible levels. These limits seem more relevant to chronic than acute exposure levels.

7.3 COMPARISON OF PREDICTIONS

Predictions of permissible levels resulting from observations at 3 levels of the structural hierarchy are presented in Table 17. The acute estimates were quite variable, ranging from 1.66 to 890.00 ug Cd/L. Chronic estimates were more uniform and ranged from 0.20 to 5.51 ug Cd/L.

Predictions of permissible concentrations from single species data are relatively similar. However, use of all of the data in the the larger data base should result in a more stable estimate, less influenced by possible outlying value.. For this reason, the single species estimates derived using the larger data base and the second method (46.1 and 1.02 ug Cd/L, acute and chronic, respectively) may be the most reliable estimates of this kind.

In comparisons of acute response between hierarchical levels (Table 17), the median tolerance derived from single species and community level tests were somewhat similar, 1,366 (1,153-1,588) and 1,119 (713-13,767) ug Cd/L, repectively. However, the permissible level estimates at the 5th percentile were less similar, 46.1 (28.2-66.0) and

TABLE 17

Predictions of permissible cadmium concentrations.

Hierarchical Level	Source of Data, Method	Acute Effects		Chronic Effects	
		Permissible Level	Median Tolerance	Permissible Level	Median Tolerance
Single Species	Table 17, USEPA 1983	1.7	---	1.55	---
	Table 17, Probit Analysis	5.4	261	2.10	2.33
	Appendix A, USEPA 1983	22.0	---	4.46	---
	Appendix A, Probit Analysis	46.1 (28.2-66.0)	1,366 (1,153-1,588)	1.02 (0.21-1.99)	6.96 (4.57-10.28)
Community	Table 17, MATC	890.0	---	0.7	---
	Chapter V, Probit Analysis	459.4 (54.0-1,032.0)	1,119 (713-13,767)	0.20 (0.04-0.44)	6.49 (2.86-19.49)
Ecosystem	Chapter VI, Median Field Levels	---	---	--- (0.05-9.2)	---

459.4 (54.0-1,032.0) ug Cd/L, single species and community levels, respectively. In addition to this qualitative comparison of point estimates, the cumulative tolerance distributions estimated from single species and community level tests were compared using relative potency analysis (Figure 12; Daum 1970). The slope of the community level tolerance distribution was significantly greater than that from single species data (0.896 and 0.486, respectively, $p < 0.005$).

There are several factors which may contribute to differences in single species and community level acute response. First, there could be a taxonomic bias introduced by monitoring only protozoans. Although protozoans as a group are neither more sensitive nor more resistant than aquatic organisms in general, they may be less variable in sensitivity. Decreased variability would result in a steeper slope as observed in Figure 12. Second, differences in the responses measured in the 2 test systems may contribute to overall response differences. The response monitored in the community level test was the complete elimination of a taxon. This is a more severe effect than a 50% reduction in numbers of individuals (LC50) and would occur at higher toxicant concentrations. Third, the community level test was conducted at 10° C while the

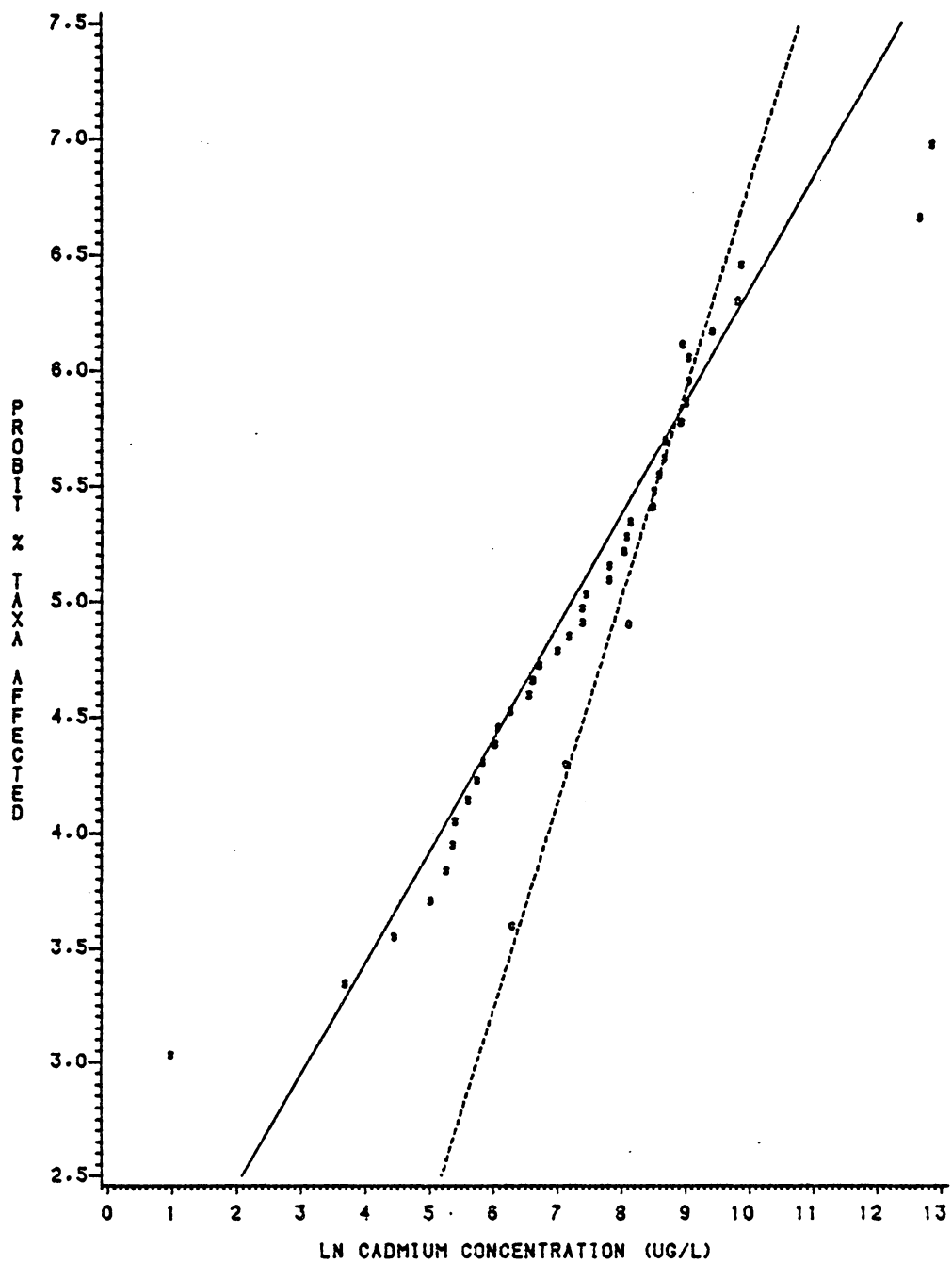


Figure 12; Comparison of acute tolerance distributions derived from single species and community level tests.

majority of single species determinations are conducted at temperatures around 20° C. Isolated organisms tend to be more sensitive or quicker to respond to toxicant stress with increasing temperature (Cairns et al. 1975). It is possible that communities would also be more sensitive at higher test temperatures. Fourth, the chemical form of cadmium may differ in the 2 types of tests. Colonized substrates have an abundance of detritus, and cadmium readily binds to humic materials. Fifth, the community level EDF may differ because the microbial community may be a cybernetic system, with intact interactions, feedback loops, homeostatic mechanisms (e. g. Patten and Odum 1981), and may be able to adjust to the effects of stress until some point at which the system collapses. At low doses there would be less effect than expected, but severe stress would destroy feedback mechanisms and accelerate the decline of the system. A steeper slope, as observed in Figure 12 would, again, be expected. It may be that communities display inertia (Cairns 1976), while single species do not.

The difference between acute estimates presents some cause for concern. Clearly, there is the potential for significant error. No field reports of acute or episodic cadmium exposure were found for comparison or validation.

In a somewhat redundant comparison back to the single species toxicity test data, the single species level final acute value (46.1 ug Cd/L) would appear to protect all but 2 of the 40 families for which data are available (95%). The susceptible families are Salmonidae and Daphnidae. The salmonids are generally considered important and irreplaceable, and the final water quality criteria derived by the USEPA (1983) would be adjusted in reference to this fact. In contrast, if organisms in communities respond to toxic stress identically to organisms tested in isolation, the community level final acute value (459.4 ug Cd/L) would appear to protect 28 of the 40 families tested (70%). Those with substantially lower mean acute values include salmonid fishes, fathead minnow fry, daphnids, amphipods, isopods, and mayflies. However, differences in response of 2 orders of magnitude have been reported for species tested alone and in interacting combinations (Chapter I). Appropriate field validation studies of acute estimates are necessary before the usefulness of either estimate can be confirmed. It is likely that both laboratory determinations are equally disadvantaged by the simplicity of their environments, lacking refuge and recolonists, and by the short duration of observation after an acute exposure.

There are other characteristics of these test systems that may contribute to their acceptance, although not necessarily to their predictive utility. Single species acute toxicity tests are convincing, unambiguous displays of a potential for harm. The cost of such tests is not unreasonable, and experience increases replicability and confidence in results. The microbial community test provided effects data on 50 species simultaneously in a more natural test situation.

The chronic tolerance distributions from single species and community level tests were more similar (Figure 13). The slopes of the 2 lines were not significantly different (0.854 and 0.473, single species and community, respectively, $0.10 > p > 0.05$). And estimates of median tolerance did not differ significantly (6.96 and 6.49 ug Cd/L, single species and community, respectively, $p = 0.09$). There was also less difference in estimates of permissible levels for chronic exposures than in acute tests (1.02 and 0.20 ug Cd/L, single species and community, respectively, Table 17).

The community level chronic test was more comparable to single species tests than the community level acute test in 2 respects. The test was conducted at room temperature, and the complexities of the test systems at the beginning of

exposure were more similar. In measures of colonization success, where there were no preexisting communities with intact homeostatic mechanisms at the start of exposure, the effect level was somewhat similar to that determined for single species. In contrast, there were no significant effects on epicenters (established communities with considerable complexity) after 29 d exposure to 9.5 ug Cd/L. As in acute exposures, established microbial communities appear less sensitive at low exposure levels. The difference between effect levels suggests that successional maturity, or complexity, or information content of a system may be important determinants of the response of an ecosystem to toxic stress, especially in the short term. Single species tests cannot account for this factor.

In comparisons of the laboratory based predictions of permissible level for chronic cadmium exposure to field data, both of the estimates fell within the the rational range of concentrations as delimited by field observations (Figure 14). This is an essential first step in the validation of such estimates. The 1980 water quality criterion for the protection of aquatic life from effects of chronic cadmium exposure was criticized because it often fell below naturally occurring levels of cadmium, making it unattainable and an unsuitable basis for regulation (Buikema

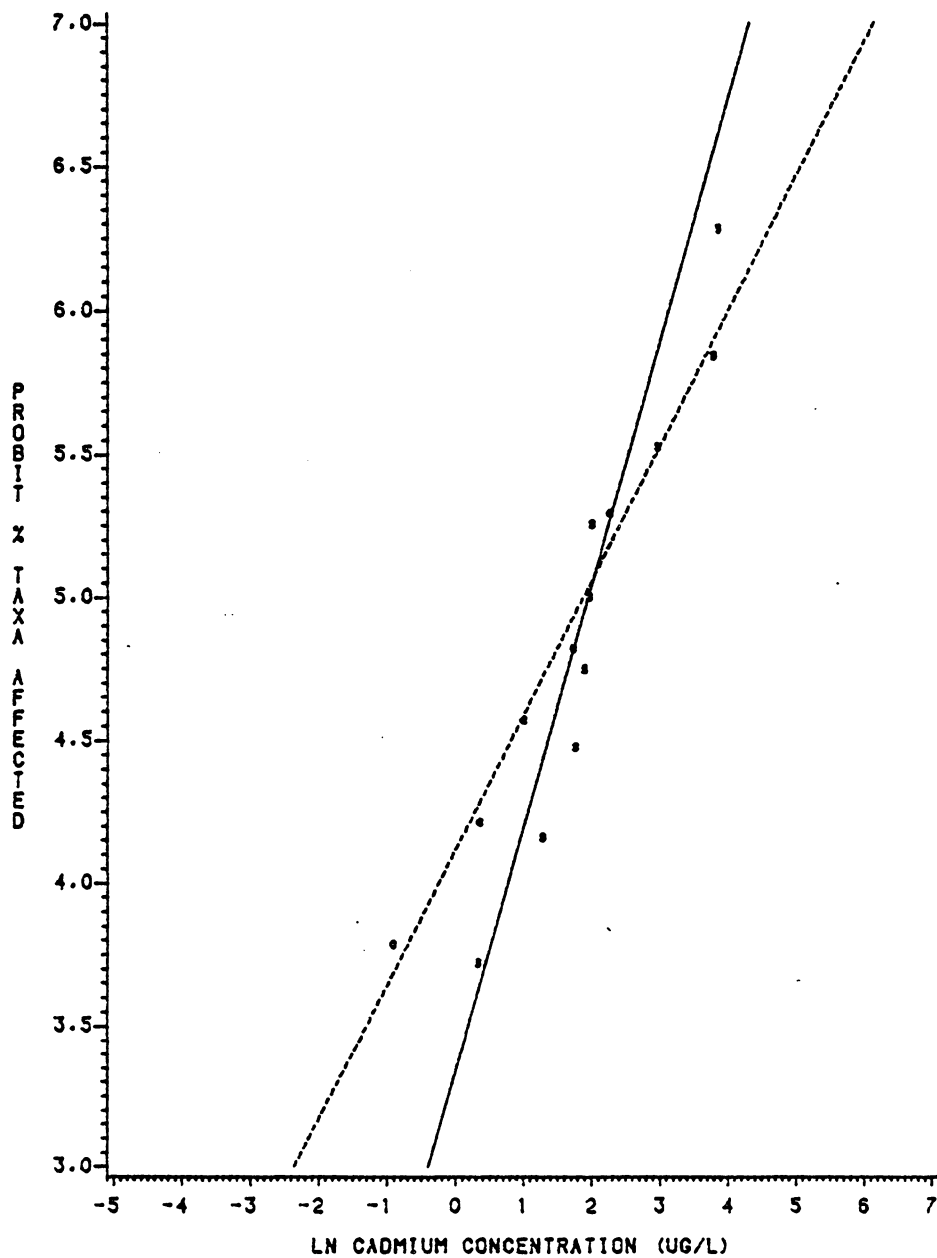


Figure 13: Comparison of chronic tolerance distributions derived from single species and community level tests.

and Cherry 1982). Both of the chronic estimates from this study and the proposed revised criterion avoid this problem and fall in the rational range (USEPA 1983). Specific field validation studies would permit a more definitive evaluation of the adequacy of these estimates.

Because single species and community level predictions were similar and reasonable on the basis of field comparison, other characteristics must be the basis of any practical decision to use one type of test over the other. Single species chronic toxicity tests are a natural extension of the widespread use of single species acute toxicity tests. They share the same unambiguous conceptual basis and widespread acceptance. However, they have unique problems. The cost of multiple chronic determinations is a significant problem when trying to define the probable range of response of aquatic organisms. In addition, most species that are tested chronically are from a select group amenable to laboratory testing (Buikema and Benfield 1979), and this may bias conclusions about the range of response of aquatic organisms in general to toxic stress. Also, there is more ambiguity about the significance of responses in chronic tests than in acute tests. For example, a 10% decrease in longevity of a daphnid can be a statistically significant response to a toxicant, but in the environment, where the

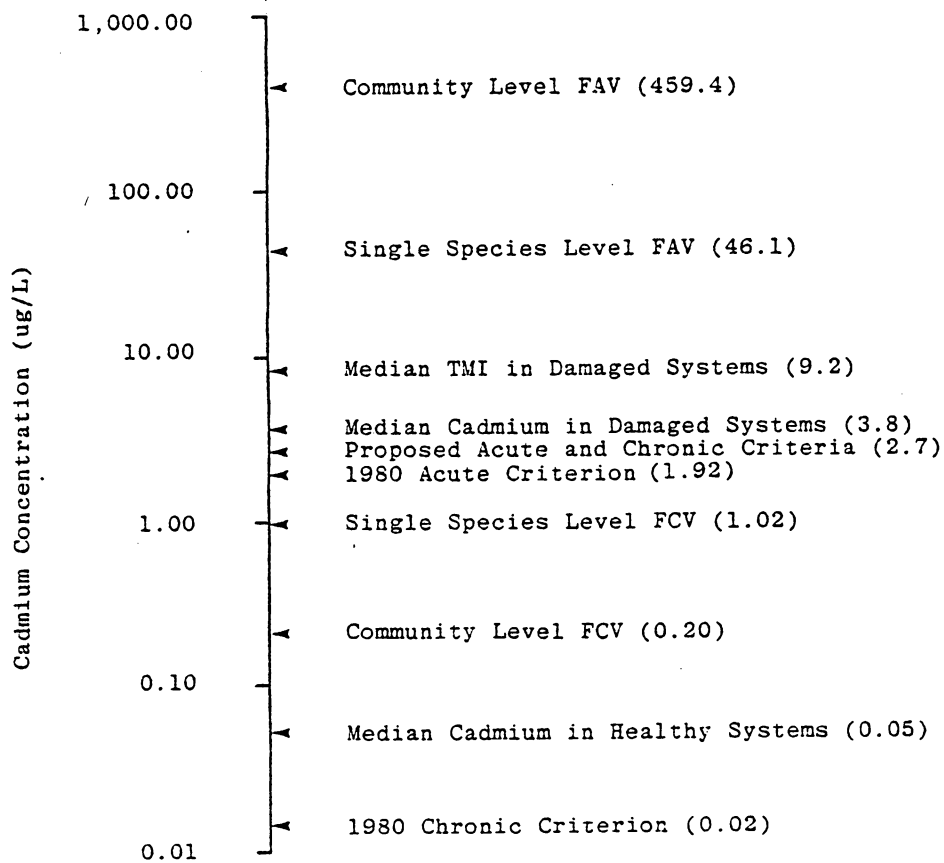


Figure 14: Estimates of permissible concentrations. Water Quality Criteria were calculated for a hardness of 65 ppm.

organisms would rarely escape predation for a prolonged period, this response may have less biological significance.

The community level toxicity test provided a reasonable chronic effect level based on many interacting species in 4 weeks with a single test. The corresponding single species data based used for comparison represents years of effort and the minimum single species data base as defined by the USEPA (Stephan et al. 1983) would require a minimum of 18 weeks of testing. This potential savings of time and cost becomes particularly significant when the number of discharges for which hazard evaluations are desirable is considered. It has been reported that up to 2 million man-made chemicals are in the environment, and up to 250,000 are added each year (Hansen 1981). There are additional advantages. The microbial community toxicity test is more complex than the single species test, yet the results are easy to interpret. The effects of a toxicant on community diversity are directly observed. These tests may be more realistic because the species tested and the system of interactions are indigenous, not an arbitrary selection or assemblage. Direct and indirect toxic effects are simultaneously tested. The test system itself "weighs" the importance of individual species or response in the persistence of the system. However, the usefulness of the

microbial community toxicity test in hazard evaluation depends upon the assumption that the microbial community on the PF substrate is, indeed, a "microcosm", i.e. that it behaves in the same manner as larger systems, the only salient differences being size and time frame, not response to toxic materials (Heath 1980). Field validation studies are necessary and are feasible. Tests with different classes of toxicants are also needed.

The laboratory tests at both levels of the structural hierarchy share some problems that affect their predictive utility. For both there is likely to be variability in toxic effects with changing chemical and physical parameters, and distortions due to miniaturization and limited time frame.

There are many applications of toxicity tests that are not directly related to the prediction of ecosystem level effects and so have different design requirements. When one of the stated management goals is protection of a single species rather than ecosystem function or diversity (e. g. Coutant 1973), single species determinations of pollutant toxicity are essential, although not sufficient. Single species tests also explain, a posteriori, specific field observations about single species. For example, in Palestine Lake, IL, and the Hudson River, NY, the last

remaining organisms in the face of gross cadmium pollution were oligochaetes. Data from the present study suggest that a representative of that group may not be seriously impaired by concentrations up to 63 ug Cd/L (EC20; Chapter III). The simplicity and unambiguous nature of single species acute toxicity tests makes them useful in the examination of "third factors". The relationship between hardness and the acute toxicity of various metals elucidated through single species tests is convincing. Similar relationships may be derived for many other contributing factors, facilitating the construction of defensible mechanistic models which will allow the transfer of information between systems or hierarchical levels. Because the behavior at one hierarchical level is generally explained by mechanics of interactions at the next lower level, this approach is sound, although ambitious.

7.4 CONCLUSIONS

Based on experience with cadmium, hazard evaluations will be most accurate if they are conducted at several hierarchical levels. Single species level tests on important species are irreplaceable. These tests are widely accepted and have effectively focused attention on the potential for harm stemming from indiscriminant waste

disposal. However, formulations of single species response information may not reflect the nature and significance of environmental changes with sufficient accuracy. Community level toxicity tests provide unique information about the response of interactive, cybernetic systems to toxic stress. These responses may prove to be different in kind from those of single species. Community level tests may eventually provide a more efficient, empirical basis for prediction of permissible levels for chronic exposure based on a concern about reductions in biological diversity. This efficiency makes site specific and effluent specific determinations more practical, and so the difficult task of construction of models for the transfer of information from one chemical situation or hierarchical level to another is less pressing. Field studies are essential to evaluate the significance of toxicant induced changes in the system of true interest, and validation studies comparing laboratory estimates to field effects are necessary to determine if the accuracy of our predictions is sufficient to our management needs. At this point each test level contributes unique information to hazard assessment.

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Appendix A

TOXICITY OF CADMIUM TO SINGLE SPECIES

Part I: Acute Values

Family: Organism	Hardness	Effect Level	Reference
Philodinidae:			
<u>Philodina acuticornis</u>	25	500	USEPA 1980b
<u>Philodina acuticornis</u>	25	200	USEPA 1980b
<u>Philodina acuticornis</u>	81	300	USEPA 1980b
Naididae:			
<u>Nais sp.</u>	50	1,700	USEPA 1980b
Aeolosomatidae:			
<u>Aeolosoma headleyi</u>	62	1,200	This study
<u>Aeolosoma headleyi</u>	168	4,980	Niederlehner <u>et al.</u> 1984
Tubificidae:			
<u>Limnodrilus hoffmeiseri</u>	5	3,500	Chapman <u>et al.</u> 1982
<u>Brachiura sowerbyi</u>	5	5,700	Chapman <u>et al.</u> 1982
<u>Tubifex tubifex</u>	5	3,800	Chapman <u>et al.</u> 1982
<u>Quistadrilus multisetosus</u>	5	7,400	Chapman <u>et al.</u> 1982
<u>Spirosperma nikolskyi</u>	5	12,000	Chapman <u>et al.</u> 1982
Lubriculidae:			
<u>Lubriculus variegatus</u>	30	74	Bailey & Liu 1982
<u>Stilodrilus heringianus</u>	5	7,500	Chapman <u>et al.</u> 1982
Planariidae:			
<u>Dugesia sp.</u>	20	4,900	Kneip <u>et al.</u> 1979
Hydridae:			
<u>Hydra oligactis</u>	100	1,600	Slooff <u>et al.</u> 1983
Bithyniidae:			
<u>Amnicola sp.</u>	50	8,400	USEPA 1980b

Appendix A (continued)

Part I: Acute Values (continued)

Family:

Organism	Hardness	Effect Level	Reference
Physidae:			
<u>Physa gyrina</u>	200	1,370*	USEPA 1980b
<u>Physa gyrina</u> (immature)	200	410	USEPA 1980b
Planorbidae:			
<u>Helisoma trivolvis trivolvis</u> (juvenile)	55	201	Buikema <u>et al.</u> 1981
Lymnaeidae:			
<u>Lymnaea stagnalis</u>	100	1,600	Slooff <u>et al.</u> 1982
Daphnidae:			
<u>Daphnia magna</u>	51	9.9	USEPA 1980b
<u>Daphnia magna</u>	104	33	USEPA 1980b
<u>Daphnia magna</u>	105	34	USEPA 1980b
<u>Daphnia magna</u>	197	63	USEPA 1980b
<u>Daphnia magna</u>	209	49	USEPA 1980b
<u>Daphnia magna</u>	---	47	USEPA 1980b
<u>Daphnia magna</u>	45	65	Mount & Norberg 1982
<u>Daphnia magna</u>	--	30.2	USEPA 1983
<u>Daphnia magna</u>	100	30	Canton & Slooff 1982
<u>Daphnia magna</u>	200	30	Canton & Slooff 1982
<u>Daphnia pulex</u>	--	93.5	USEPA 1983
<u>Daphnia pulex</u>	45	62	Mount & Norberg 1982
<u>Daphnia pulex</u>	106	115	Ingersoll & Winner 1982
<u>Daphnia pulex</u>	65	62	This study
<u>Daphnia cucullatus</u>	100	200	Canton & Slooff 1982
<u>Simocephalus serrulatus</u>	10	35.0	USEPA 1980b
<u>Simocephalus serrulatus</u>	11	7.0	USEPA 1980b
<u>Simocephalus serrulatus</u>	11	3.5	USEPA 1980b

*Not included in calculations. Value for more sensitive
lifestage used.

Appendix A (continued)

Part I: Acute Values (continued)

Family:

Organism	Hardness	Effect Level	Reference
Daphnidae (continued):			
<u>Simocephalus serrulatus</u>	11	12.0	USEPA 1980b
<u>Simocephalus serrulatus</u>	11	16.5	USEPA 1980b
<u>Simocephalus serrulatus</u>	11	8.6	USEPA 1980b
<u>Simocephalus vetulus</u>	45	24	Mount & Norberg 1982
<u>Ceriodaphnia reticulata</u>	45	24	Mount & Norberg 1982
Cyclopidae:			
<u>Cyclops sp.</u>	20	340	Kneip et al. 1979
Cypridopsidae:			
<u>Cypridopsis sp.</u>	20	190	Kneip et al. 1979
Talitridae:			
<u>Hyalella azteca</u>	20	85	Kneip et al. 1979
Gammaridae:			
<u>Gammarus sp.</u>	50	70	USEPA 1980b
<u>Gammarus pulex</u>	71	680	Wright & Frain 1981
Ephemerellidae:			
<u>Ephemerella grandis grandis</u>	--	28,000	USEPA 1980b
<u>Ephemerella grandis grandis</u>	44	2,000	USEPA 1980b
Heptageniidae:			
<u>Stenonema pudicum</u>	65	270	Specht 1984
Pteronarcyidae:			
<u>Pteronarcella badia</u>	--	18,000	USEPA 1980b
<u>Pteronarcys dorsata</u>	65	>18,880**	Lechleitner 1982
Unidentified odonate:			
Damselfly, unidentified	50	8,100	USEPA 1980b

**Not included in calculations unless <1 or >10,000 ug Cd/L, then included with sign dropped.

Appendix A (continued)

Part I: Acute Values (continued)

Family:

Organism	Hardness	Effect Level	Reference
Chironomidae:			
<u>Chironomus</u> sp.	50	1,200	USEPA 1980b
<u>Chironomus riparius</u>	65	7,900	This study
Culicidae:			
<u>Aedes aegypti</u>	100	11,000	Slooff et al. 1983
<u>Culex pipiens</u>	100	2,100	Slooff et al. 1983
Unidentified tricopteran:			
Caddisfly, unidentified	50	3,400	USEPA 1980b
Glossomatidae:			
<u>Glossosoma nigrior</u>	65	>308,750**	Trapp 1984
Hydropsychidae:			
<u>Hydropsyche slossonae</u>	65	5,750	Specht 1984
Psephenidae:			
<u>Psephenus herricki</u>	65	>372,120**	Specht 1984
Pectinatellidae:			
<u>Pectinatella magnifica</u>	205	700	- USEPA 1983
Lophopodidae:			
<u>Lophopodella carteri</u>	205	150	USEPA 1983
Plumatellidae:			
<u>Plumatella emarginata</u>	205	1,090	USEPA 1983
Anguillidae:			
<u>Anguilla rostrata</u>	55	820	USEPA 1980b
Salmonidae:			
<u>Oncorhynchus kisutch</u> (parr)	23	2.7	USEPA 1983
<u>Oncorhynchus kisutch</u>	23	17.5*	USEPA 1983
<u>Oncorhynchus tshawytscha</u> (swim-up)	23	1.8	USEPA 1980b
<u>Oncorhynchus tshawytscha</u> (parr)	23	3.5	USEPA 1980b
<u>Oncorhynchus tshawytscha</u> (alevin)	23	>26**	USEPA 1983

*Not included in calculations. Value for more sensitive lifestage used.

**Not included in calculations unless <1 or >10,000 ug Cd/L, then included with sign dropped.

Appendix A (continued)

Part I: Acute Values (continued)

Family:

Organism	Hardness	Effect Level	Reference
Salmonidae (continued):			
<u>Oncorhynchus tshawytscha</u> (smolt)	23	>2.9**	USEPA 1983
<u>Oncorhynchus tshawytscha</u> (juvenile)	25	1.41	USEPA 1983
<u>Salmo gairdneri</u>	--	6.0	USEPA 1980b
<u>Salmo gairdneri</u> (swim-up)	23	1.3	USEPA 1980b
<u>Salmo gairdneri</u> (parr)	23	1.0	USEPA 1980b
<u>Salmo gairdneri</u> (2-mo)	--	6.6	USEPA 1980b
<u>Salmo gairdneri</u>	31	1.75	USEPA 1980b
<u>Salmo gairdneri</u>	--	6	USEPA 1980b
<u>Salmo gairdneri</u>	--	7	USEPA 1980b
<u>Salmo gairdneri</u> (alevin)	23	>27**	USEPA 1983
<u>Salmo gairdneri</u> (smolt)	23	4.1	
		>2.9**	USEPA 1983
<u>Salvelinus fontinalis</u>	340	26***	USEPA 1980b
<u>Salvelinus fontinalis</u>	340	29***	USEPA 1980b
<u>Salvelinus fontinalis</u>	350	3.8***	USEPA 1980b
<u>Salvelinus fontinalis</u>	330	4.4***	USEPA 1980b
<u>Salvelinus fontinalis</u>	44	2.4***	USEPA 1980b
<u>Salvelinus fontinalis</u>	47	5,080***	USEPA 1983
<u>Salvelinus fontinalis</u>	42	<1.5***	USEPA 1983
Cyprinidae:			
<u>Carassius auratus</u>	20	2,340	USEPA 1980b
<u>Carassius auratus</u>	20	2,130	USEPA 1980b
<u>Carassius auratus</u>	140	46,800	USEPA 1980b
<u>Carassius auratus</u>	22	2,500	Kneip et al. 1979
<u>Pimephales promelas</u>	20	1,050*	USEPA 1980b
<u>Pimephales promelas</u>	20	630*	USEPA 1980b
<u>Pimephales promelas</u>	360	72,600*	USEPA 1980b
<u>Pimephales promelas</u>	360	73,500*	USEPA 1980b
<u>Pimephales promelas</u>	201	11,000*	USEPA 1980b

*Not included in calculations. Value for more sensitive lifestage used.

**Not included in calculations unless <1 or >10,000 ug Cd/L, then included with sign dropped.

***Not included in calculations due to discrepancies in test results for this species.

Appendix A (continued)

Part I: Acute Values (continued)

Family:

Organism	Hardness	Effect Level	Reference
Cyprinidae (continued):			
<u>Pimephales promelas</u>	201	12,000*	USEPA 1980b
<u>Pimephales promelas</u>	201	6,400*	USEPA 1980b
<u>Pimephales promelas</u>	201	2,000*	USEPA 1980b
<u>Pimephales promelas</u>	201	4,500*	USEPA 1980b
<u>Pimephales promelas</u> (fry)	40	21.5	USEPA 1983
<u>Pimephales promelas</u> (fry)	48	11.7	USEPA 1983
<u>Pimephales promelas</u> (fry)	39	19.3	USEPA 1983
<u>Pimephales promelas</u> (fry)	45	42.4	USEPA 1983
<u>Pimephales promelas</u> (fry)	47	54.2	USEPA 1983
<u>Pimephales promelas</u> (fry)	44	29.0	USEPA 1983
<u>Ptychocheilus oregonensis</u>	25	1,092	USEPA 1980b
<u>Ptychocheilus oregonensis</u>	25	1,104	USEPA 1980b
<u>Cyprinus carpio</u>	55	240	USEPA 1980b
Cyprinodontidae:			
<u>Fundulus diaphanus</u>	55	110	USEPA 1980b
<u>Jordaneila floridae</u>	44	2,500	USEPA 1980b
Poeciliidae:			
<u>Gambusia affinis</u>	10	1,300	USEPA 1980b
<u>Gambusia affinis</u>	10	1,500	USEPA 1980b
<u>Gambusia affinis</u>	10	2,600	USEPA 1980b
<u>Gambusia affinis</u>	11	900	USEPA 1980b
<u>Gambusia affinis</u>	11	2,200	USEPA 1980b
<u>Poecilia reticulata</u>	20	1,270	USEPA 1980b
<u>Poecilia reticulata</u>	200	11,100	Canton & Slooff 1982
<u>Poecilia reticulata</u>	100	3,800	Canton & Slooff 1982
Oryziatidae:			
<u>Oryzias latipes</u>	200	130	Canton & Slooff 1982
<u>Oryzias latipes</u>	100	350	Canton & Slooff 1982
Gasterosteidae:			
<u>Gasterosteus aculeatus</u>	115	6,500	USEPA 1980b
<u>Gasterosteus aculeatus</u>	107	23,000	USEPA 1980b

*Not included in calculations. Values for more sensitive lifestage used.

Appendix A (continued)

Part I: Acute Values (continued)

Family:

Organism	Hardness	Effect Level	Reference
Percichthyidae:			
<u>Morone americanus</u>	55	8,400	USEPA 1980b
<u>Morone saxatilis</u>	55	1,100***	USEPA 1980b
<u>Morone saxatilis</u> (larvae)	70	1***	USEPA 1980b
<u>Morone saxatilis</u> (fingerling)	70	2***	USEPA 1980b
Centrarchidae:			
<u>Lepomis cyanellus</u>	20	2,840	USEPA 1980b
<u>Lepomis cyanellus</u>	360	66,000	USEPA 1980b
<u>Lepomis cyanellus</u>	335	20,500	USEPA 1980b
<u>Lepomis gibbosus</u>	55	1,500	USEPA 1980b
<u>Lepomis macrochirus</u>	20	1,940	USEPA 1980b
<u>Lepomis macrochirus</u>	207	21,100	USEPA 1980b
Ambystomidae:			
<u>Ambystoma mexicanum</u>	170	1,300	Canton & Slooff 1982
Pipidae:			
<u>Xenopus laevis</u>	170	3,200	Canton & Slooff 1982

***Not included in calculations due to discrepancies in test results for this species.

Appendix A (continued)

Part II: Chronic Values

Family:

Organism	Hardness	Effect Level	Reference
Daphnidae:			
<u>Daphnia magna</u>	45	0.34	USEPA 1980b
<u>Daphnia magna</u>	53	0.15	USEPA 1980b
<u>Daphnia magna</u>	103	0.21	USEPA 1980b
<u>Daphnia magna</u>	209	0.44	USEPA 1980b
<u>Daphnia pulex</u>	106	7.1	Ingersoll & Winner 1983
<u>Daphnia pulex</u>	65	7.5	This study
Aeolosomatidae:			
<u>Aeolosoma headleyi</u>	65	27.1	This study
<u>Aeolosoma headleyi</u>	168	41.1	Niederlehner et al. 1984
<u>Aeolosoma headleyi</u>	189	72.8	Niederlehner et al. 1984
Chironomidae:			
<u>Chironomus riparius</u>	65	6.6	This study
Salmonidae:			
<u>Oncorhynchus kisutch</u>	44	2.1	USEPA 1980b
<u>Oncorhynchus kisutch</u>	44	7.2	USEPA 1980b
<u>Oncorhynchus tshawytscha</u>	25	1.56	USEPA 1983
<u>Salvelinus fontinalis</u>	44	2.0	USEPA 1980b
<u>Salvelinus fontinalis</u>	44	2.4	USEPA 1980b
<u>Salvelinus fontinalis</u>	36	1.7	USEPA 1980b
<u>Salvelinus namaycush</u>	44	7.4	USEPA 1980b
<u>Salmo trutta</u>	44	6.7	USEPA 1980b
Esocidae:			
<u>Esox lucius</u>	44	7.4	USEPA 1980b
Cyprinidae:			
<u>Pimephales promelas</u>	201	46	USEPA 1980b
Catostomidae:			
<u>Catostomus commersoni</u>	44	7.1	USEPA 1980b
Cyprinodontidae:			
<u>Jordanella floridae</u>	44	5.8	USEPA 1980b
Centrarchidae:			
<u>Micropterus dolomieu</u>	44	7.4	USEPA 1980b
<u>Lepomis macrochirus</u>	207	50	USEPA 1980b

Appendix A (continued)

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