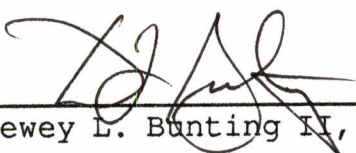
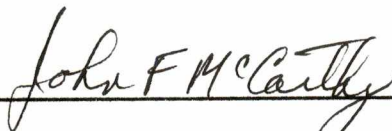


To the Graduate Council:

I am submitting herein a dissertation written by Willodean Delores Burton entitled "An Evaluation of Aquatic Toxicity Data with a Population Growth Model for Application to Environmental Hazard Assessment." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology.

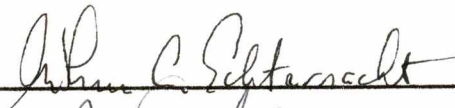

Dewey L. Bunting II, Major Professor

We have read this dissertation
and recommend its acceptance:











Accepted for the Council:


Associate Vice Chancellor
and Dean of the Graduate School

AN EVALUATION OF AQUATIC TOXICITY DATA WITH A POPULATION
GROWTH MODEL FOR APPLICATION TO ENVIRONMENTAL
HAZARD ASSESSMENT

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Willodean Delores Burton

May 1991

DEDICATION

To the memory of Messrs. Robert S. Stevenson, my father; Breman G. Fort, my grandfather; and John N. Burton, my father-in-law. Three men whose lives inspired me to be persistent in achieving my goals.

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Gratitude is also extended to numerous friends of the University of Tennessee and otherwise who provided emotional support and other contributions throughout my tenure in graduate school. My work on this project has been blessed with love, support, and inspiration from David E. Burton, my husband, whose belief in me--and in my work--has been steadfast; Freda and Phillip, my children, whose wisdom and integrity have enhanced the meaning of everything for me; Wilhelmenia F. Stevenson, my mother, and other family members whose love and encouragement sustained me.

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ABSTRACT

Acute and chronic bioassays with the cladocerans Ceriodaphnia dubia and Daphnia magna were conducted using four chemicals, each having a different mode of action. The chemicals were: cadmium (chloride), pentachlorophenol (PCP), 1-octanol, and 1-naphthyl-N-methylcarbamate (carbaryl). The data obtained from the tests were used to compare species sensitivities, endpoint sensitivities, and the relative toxicities of the chemicals.

In the acute bioassays, estimates of the median lethal concentrations (48-h LC_{50} s) were used to compare the species sensitivities. Ceriodaphnia dubia was more sensitive than D. magna to all four chemicals, and carbaryl was the most toxic chemical to both species.

The sensitivity of three endpoints (survival, reproduction and the intrinsic rate of natural increase, r) were used to evaluate the chronic toxicity of the four chemicals to C. dubia and D. magna with tests of two different durations. Survival and reproduction were measured during the chronic tests, then integrated using the Lotka equation to calculate r . Estimates of the variance in r was calculated using a bootstrap statistical procedure.

Survival, reproduction and r all declined with greater concentrations of the chemicals. These effects were evident in both the 7- or 14-d exposures. The lowest observed effect concentration (LOEC) determined from studies with the four chemicals showed that neither survival nor reproduction was consistently the more sensitive endpoint for either C. dubia or D. magna. The LOECs for r were the same for both exposure durations for each species.

The LOEC comparisons among endpoints showed that r was at least as sensitive, and often more sensitive than either of the other chronic endpoints. Thus, the data from this study demonstrate that the calculations of r from chronic toxicity data can provide equally sensitive bioassay results for protecting the environment, while eliminating conflicting interpretations of toxicant effects on separate endpoints. Furthermore, statistical decisions drawn from comparisons of the toxicity of exposure concentrations with the controls may not provide the most ecologically meaningful criteria for environmental protection.

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CHAPTER I

GENERAL INTRODUCTION

The primary goal of this study was to develop a conceptual framework and data to investigate the use of the intrinsic rate of natural increase (r) as an index for environmental hazard assessment of aquatic ecosystems. The data development process involved conducting acute and chronic bioassays using two common aquatic microcrustacean species (Ceriodaphnia dubia and Daphnia magna). Preliminary acute tests provided the data needed to set the concentration levels for the chronic bioassays. Then chronic bioassays were conducted following EPA aquatic toxicity protocols. Comparisons made between species, test durations, and integrated endpoints of chronic toxicity provided the data necessary to investigate the use of r as an environmental hazard assessment index.

Hazard assessment involves the comparison of the magnitude and length of duration of exposure to chemical concentrations occurring in various segments of the environment (Kenaga, 1982). Therefore, chronic bioassays are necessary tools for detecting effects of low-level toxicant exposures to organisms. During chronic bioassays, the endpoints usually measured are survival and

reproductive impairments. These endpoints provide meaningful biological data for the protection of biota within an aquatic system; however, the endpoints are usually considered separately, and this can lead to conflicting interpretations of the data. Chronic toxicity endpoints measured for test organisms capable of producing several broods can be integrated to improve the interpretations of chronic data.

Many aquatic invertebrates (e.g., zooplankton, benthos) are capable of producing several broods within short time periods, and they can be exposed to numerous pollutants within their natural habitats. Therefore, some species are used in bioassays as a part of the hazard assessment process. Invertebrates of the genera Daphnia and Ceriodaphnia are often used to estimate acute and chronic toxicity of effluents and receiving waters. The genus Daphnia, especially Daphnia magna, has a long history of use as a bioassay organism in tests lasting for 21-days (21-d). However, this organism is not a common species in North America, thus test results may not be representative of a specific site. Furthermore, testing can be relatively laborious and costly to perform (Mount and Norberg, 1984). To replace the 21-d Daphnia test Geiger, et al.(1980) and Adams and Heidolph (1985) proposed a 14-d Daphnia test duration. This is now an

accepted standard toxicity protocol (OECD, 1981). Even with the 14-d Daphnia test duration, the search continued for a shorter test duration without loss of information. This motivated the development of a seven-day test with C. dubia (Mount and Norberg, 1984).

The use of C. dubia for chronic toxicity testing reduces the labor, time and expense of chronic bioassays, but if short-term tests are used for regulatory purposes they should be representative of the data obtained from longer term tests (Winner, 1988). Because the toxicity data base for C. dubia is small compared to that of D. magna, studies are needed to examine the response of C. dubia to a broad array of pollutants, and to determine whether the responses at short-test durations can reliably predict the results of longer test durations.

Chapter II describes the acute and chronic bioassays that used C. dubia and D. magna. Tests with these two species were conducted with four different chemicals, each which has a different mode of action: cadmium chloride (cadmium), pentachlorophenol (PCP), 1-octanol and 1-naphthyl-N-methylcarbamate (carbaryl). The objectives of the study were: (1) to determine the relative sensitivities of the two species and the relative toxicities of the chemicals, and (2) to determine the sensitivity of the chronic toxicity endpoints (survival

and reproduction) for the two species at short-term and long-term test durations. It was hypothesized that the sensitivities of the two species are the same for similar exposures to the same chemical, and that endpoint sensitivities do not differ for short- and long-term exposure periods.

Few toxicity studies have compared the sensitivity of C. dubia and D. magna. In acute toxicity studies, (Mount and Norberg, 1984; Cowgill, et al., 1985; Elnabarawy, et al., 1986; Hickey, 1989) researchers have concluded that the two species were about equally sensitive to the chemicals tested. There are fewer studies utilizing chronic comparisons (Elnabarawy, et al., 1986; Winner, 1988; Hickey, 1989), but the conclusions are the same as for the acute studies.

To accomplish the objectives and test the hypotheses proposed in this study, chronic tests were performed for two exposure durations, subchronic (7-d and 14-d for C. dubia and D. magna, respectively) and chronic (18-d).

To understand the effects of toxic chemicals on ecosystems it is necessary to consider effects not only to individuals, but also on species, populations, and the interactive effects on the community of organisms in an ecosystem (Gentile, et al., 1982; Westman, 1985). Integrative parameters which account for these aspects

would have obvious advantages for assessing environmental hazards of toxic chemicals. In this study, I propose r as a more ecologically relevant endpoint than either of the separate measures of survival and reproduction obtained from chronic bioassays. Calculations of r using the Lotka (1925) equation (Daniels and Allan, 1981; Gentile, et al., 1982; Van Leeuwen, et al., 1986) integrate data concurrently gathered to measure the responses of those two chronic endpoints. However, because r is a calculated summation product for all individuals within a chronic bioassay and results in a single value for a cohort, its application has been hampered by the difficulties in determining its variance (Lenski and Service, 1982 and Meyer, et al., 1986).

Two recently developed statistical procedures (the jackknife and bootstrap) offer solutions to this problem. The variance about a statistic can be estimated from a single sample (Efron, 1979 and Diaconis and Efron, 1983). Both statistical procedures have been used to estimate the variance in r for data from long-term studies, and hypothetical data (Lenski and Service, 1982; Rago and Dorazio, 1984; Meyer, et al., 1986).

Chapter III describes the integration of the survival and reproductive data obtained through the use of chronic bioassays outlined in Chapter II. Mean r values and

estimates of the variance in r were calculated for two exposure durations using a bootstrap statistical procedure developed to be user-friendly and flexible; it requires a simple data input. The objectives were: (1) to quantify the variance in r for use in hypothesis testing; (2) to statistically determine the lowest observed effect concentration (LOEC) for r for comparison with survival and reproduction; and (3) to evaluate r based on statistical decisions drawn from comparisons of the exposure concentrations with controls, and the ecological criteria for adverse effects on r .

The null hypotheses tested were: (1) there is no difference in r measured in short-term and long-term exposure durations of C. dubia and D. magna to each of the four chemicals, and (2) there is no difference in the sensitivity of r and the separate chronic endpoints (survival and reproduction).

Although r may not be the most sensitive endpoint measured during chronic bioassays, its application in hazard assessment can increase the ability to predict toxic effects of chemicals above the individual level of organization for laboratory populations. Effluent discharge and water quality regulators are among the sources that rely upon chronic bioassay data for decision-making. Of the chronic endpoints, survival and

reproduction, the more sensitive endpoint is usually chosen during the decision-making process. An integration of the two endpoints into a single endpoint would eliminate the dichotomous chronic endpoint interpretation.

Essential to hazard assessment is the modelling of ecosystem processes. The integrated endpoint, r , can be used in models that require the input of both survival and reproductive data; thus, reducing the number of parameters required for certain models.

CHAPTER II

ACUTE AND CHRONIC TOXICITY OF FOUR CHEMICALS WITH DIFFERENT MODES OF ACTION TO Ceriodaphnia dubia AND Daphnia magna

Introduction

Laboratory bioassays with aquatic species provide the important function of predicting the effects of chemicals and effluents on the organisms inhabiting an aquatic ecosystems. Thus, bioassay data for aquatic invertebrates are necessary to the development of aquatic safety assessments for new or expanded use of chemicals, and for the establishment of water quality criteria (Maki and Bishop, 1979). Acute and chronic bioassays using cladocerans are now accepted parts of the toxicological data base used to evaluate hazardous environmental contaminants (Adams and Heidolph, 1985).

Acute bioassays are used to screen new chemicals, establish water quality and monitor effluents. They are rapid, inexpensive and provide a starting point in hazard assessment where acute lethality is the endpoint measured (Parrish, 1985). Acute lethality results from short-term exposure to high concentrations of a toxicant, but usually

does not represent an environmental condition, except in the case of an accidental spill.

Since the biological effect of a toxicant on a species is a function of both the concentration of the toxicant in the environment and the duration of exposure of the organism to the chemical, an assessment of chronic effects provide a measurement to protect aquatic biota. A chronic response resulting from an organism's exposure to low concentrations of a toxicant over long periods of time is probably more representative of many environmental conditions.

While the cladoceran, Daphnia magna, has been used frequently in aquatic toxicity testing, an alternative genus, Ceriodaphnia, has been proposed for more rapid chronic bioassays (Mount and Norberg, 1984). Since its development, emphasis has been placed on the C. dubia (7-d) subchronic test because, when compared to D. magna, C. dubia: (a) produces three broods in 7-d (half the time required for D. magna) for measurements of the survival and reproductive endpoints, (b) is considered to be more sensitive to toxicants, although its response to numerous chemicals has not been ascertained (Cowgill, et al., 1985), (c) can be easily maintained in laboratory cultures, and (d) is globally distributed in ponds and lakes.

Species Sensitivities. Despite the prevalent use of cladoceran in bioassays, few studies have compared the sensitivity of C. dubia and D. magna in either acute or chronic toxicity tests. To describe the 7-d Ceriodaphnia test, Mount and Norberg (1984) initially performed acute toxicity tests with four cladoceran species, D. pulex, D. magna, Simocephalus vetulus, and C. reticulata exposed to 13 different chemicals. Based on the median lethal concentration (LC_{50}) values, C. reticulata was more sensitive than D. magna to seven of the 13 chemicals tested, but they did not differ in their response by more than a factor of two. The authors concluded that neither species was distinctly more sensitive than the other.

The acute toxicities of four chemicals (phenol, chlorobenzene, diethanolamine, and ethylene glycol) to D. magna and C. dubia were estimated at two temperatures (20 and 24 °C) (Cowgill, et al., 1985). A comparison of the response of the two organisms showed that C. dubia was more sensitive than D. magna to all four chemicals at 24°C. At 20°C, C. dubia was more sensitive than D. magna to chlorobenzene and ethylene glycol; the response to phenol and diethanolamine was similar for both organisms.

In a study of the relative sensitivities of D. magna, D. pulex, and C. reticulata to inorganic and organic chemicals, Elnabarawy, et al. (1986) performed acute

toxicity tests with eight organic compounds and seven metals, and chronic toxicity tests with two organic compounds and three metals. The acute toxicities of the chemicals to the Daphnia and the Ceriodaphnia species were similar. The concentration of chemical needed to reduce survival by 50 percent (EC_{50} value) varied by a factor of <2 times. The chronic values for reproduction were within one order of magnitude for all the chemicals tested. The authors concluded that these two cladocerans were about equally sensitive to the test chemicals.

Hickey (1989) compared the sensitivity of five cladocerans in acute and chronic exposures to boron, chromium, fluoride, and pentachlorophenol (PCP). In the acute studies, C. dubia was about 4 times more sensitive than D. magna. In chronic studies, differences in sensitivity were <10 times.

Winner (1988) used cadmium and PCP to compare the responses of D. magna and C. dubia in 7-d tests. The endpoints measured were survival and reproduction. The results indicated that the two species were about equally sensitive to PCP. However, C. dubia was more sensitive than D. magna to cadmium. In this study, D. magna survival and reproduction data were for animals exposed to the test chemicals for 7-d beginning when they were 4-d old. The results were then compared to the 7-d C. dubia

tests for both chemicals. This experimental design eliminated exposure of first-instar D. magna to the toxicants, and so did not provide an equivalent comparison between organisms of the same age.

Such studies indicate that C. dubia is about as sensitive or slightly more sensitive than D. magna in acute toxicity tests. However, results from these chronic studies are less conclusive than the acute studies due to differences among test designs.

Test Durations. The use of C. dubia reduces the exposure duration of bioassays; the expenses of testing are reduced accordingly. For cladocerans, 48-h tests appear to provide sufficient time to obtain reliable estimates of acute toxicity (Parrish, 1985). However, the appropriate duration for chronic toxicity testing depends upon the species and the test endpoints, as well as upon specific environmental conditions and nutritional status, both of which need to remain constant for a relatively long time period. Among the advantages of shorter chronic bioassays are that data in support of regulations can be more rapidly obtained, and that the variability associated with the health of an organism during chronic testing can be reduced.

Winner (1988), points out that the use of 7- or 14-d tests as toxicological tools for regulatory purposes

requires the consideration of three important questions:

(1) are the tests ecologically relevant, (2) are the commonly-used species as sensitive as the natural biota to specific chemicals, and (3) are the relative sensitivities of the toxicity endpoints (survival and reproduction) about the same for any species in subchronic tests?

The answer to the first of these questions has been addressed by comparing the sensitivities of laboratory toxicity tests with the sensitivities of natural communities (Mount, et al., 1984; Moore and Winner, 1989; Nimmo, et al., 1990; and Eagleson, et al., 1990). In each of these studies, when toxicity test results indicated negative effects of the test chemicals or effluents there was decreased zooplankton and benthic species abundance in the natural ecosystem.

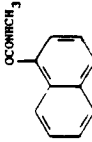
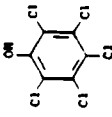
Species Comparisons. My study was conducted to address the last two questions posed by Winner (1988). How well the results of the 7-d C. dubia test compare with the results of the 14-d D. magna test, and with longer test durations has not been determined to the extent needed for adequate risk assessment. Because C. dubia is rapidly outpacing D. magna with respect to use in toxicity estimates, in spite of the fact that its response to numerous chemicals has not been determined, the present study compares the relative sensitivities of these two

species to four chemicals, each of which has a different mode of action (Table II-1). Two hypotheses were tested: (1) there is no difference in the sensitivity of C. dubia and D. magna exposed to chemicals that have different modes of toxic action, and (2) there is no difference in the sensitivities of the toxicity endpoints (survival and reproduction) for the two species in short-term and long-term test durations. The underlying question here is whether survival (or reproduction) is the more sensitive endpoint for both C. dubia and D. magna exposed to a certain chemical. The chemicals tested were:

(1) Cadmium (as cadmium chloride), a metal that affects multiple systems through inhibition or modification of specific enzyme systems (e.g., carboxypeptidase). The toxic effect of cadmium involves interactions between the free metal ion and the target site on functional proteins (Goyer, 1986).

(2) Pentachlorophenol (PCP), used as a wood preservative and pesticide. PCP is an uncoupler of oxidative phosphorylation; it interferes with the production of high-energy phosphate compounds essential for cell respiration (Eisler, 1989). The uncoupling of oxidative phosphorylation stimulates or inhibits many enzyme systems, including the mitochondrial electron transport system (Ahlborg and Thunberg, 1980). Holmberg,

Table II-1. The four different modes of action chemicals used for acute and chronic exposures of *Ceriodaphnia dubia* and *Daphnia magna*.

Chemical	Formula/ Structure	Partition Coefficient (log P) ^a	Aqueous Solubility (mg/L)	Molecular Weight	Priority Pollutant ^b	Modes of Toxic Action
Cadmium	CdCl ₂	----	1.4 X 10 ⁸	183.32	L	protein/enz metabolism
Carbaryl		2.36	40	201.22	NL	AchE* inhibitor
Pentachloro-		3.32	14	266	L	UOP**
1-Octanol	H ₃ C-(CH ₂) ₆ -CH ₂ OH	2.97	587	130.22	NL	Narcotic***

^aPartition coefficient in 1-Octanol:water

^bL = listed on U.S. EPA Priority List (1980), NL = not listed on U.S. EPA Priority List

*acetylcholinesterase, **uncoupler of oxidative phosphorylation, ***have no specific mode of action

et al.,(1972) showed that in cells, PCP treatments caused a hypermetabolic state characterized by accelerated use of tissue energy reserves and subsequent loss of body weight in eels. If expressed in cladocerans, such effects should cause C. dubia (the smaller species) to be more sensitive than D. magna to PCP.

(3) 1-octanol. This chemical has a nonspecific mode of action referred to as narcosis. Narcosis is a reversible state of arrested activity of protoplasmic structures (Veith, et al., 1983). Ferguson (1939) proposed that with narcosis, an equilibrium exists between the toxicant concentration within the organism and the external environment. The physicochemical properties of 1-octanol ($\log P = 2.97$ and aqueous solubility = 587 mg/L) (Table II-1) permit passive diffusion of the chemical into an organism.

(4) 1-naphthyl-N-methylcarbamate (carbaryl) affects the central nervous system by inhibiting the enzyme acetylcholinesterase (McKim, et al., 1987). Inhibition of acetylcholinesterase at nerve endings results in continuous stimulation of neural-muscular junctions.

These four chemicals were used to probe the chronic endpoints, survival and reproduction, for the two species for each chemical. Two test durations were used to determine whether 7-d C. dubia and 14-d D. magna tests

would reliably predict the outcome of 18-d exposures to the toxicants.

Materials and Methods

General Cultures

Stock cultures of D. magna were initiated from animals obtained in 1986 from the Environmental Protection Agency (EPA) Laboratory, Newtown, Ohio. The cultures were maintained at $21 \pm 1^\circ\text{C}$ in 8 liter (L) aquaria containing 6 L of well/spring water. Subsequently, new cultures were started at $23 \pm 1^\circ\text{C}$ from the aquaria stock animals and were maintained in 3.8 L wide-mouth glass jars containing 3 L of water. The jars were placed in controlled-temperature water baths to maintain the temperature. Animals used in the tests were selected from the jars with a 5 mm bore glass pipette.

Stock cultures of C. dubia were obtained from the Aquatic Toxicology Laboratory, Oak Ridge National Laboratory (ORNL). These animals were maintained at $23 \pm 1^\circ\text{C}$ in 1 L glass beakers containing 900 mL of water. Ceriodaphnia dubia were transferred using 2 mm bore glass pipettes. Six to eight cultures of each species were maintained for testing. New cultures of each species were started every seven days to maintain healthy cultures.

The water used for the cultures and for testing was obtained from a well/spring water source located on the Oak Ridge Reservation. The water was pumped directly from the source through sand filters to remove coarse particulate matter; no additional treatment was used. Chemical analyses of the well/spring water were performed by the Analytical Chemistry Division, ORNL, following EPA Method 200.7 (1982) (Appendix A-1). Water quality was measured periodically throughout the test period. The well/spring water was aerated and allowed to equilibrate for 24-h before use. Light intensity to the cultures was approximately 100 foot-candle (ft-c); an automatic timer was used to provide a 16-h light/8-h dark daily photoperiod.

Food for C. dubia and D. magna consisted of a mixture of trout chow, cerophyl, and yeast (TCY) suspension prepared as described by Kszos, et al. (1989) in combination with the green unicellular alga, Selenastrum capricornutum. To prepare the TCY mixture, 13.3 g of Purina Trout Chow was added to 2.66 L of distilled water and blended. The mixture was then placed in a 4 L beaker and aerated for seven days. On the sixth day, 10 g of Fleishmann's dry yeast and 2 L of distilled water were blended at low speed for 5 minutes. The supernatant was poured into a 4 L beaker and refrigerated overnight. Then

11.1 g of cerophyl and 2.22 L of distilled water were blended at high speed for 5 minutes before being poured into a 4 L flask. A stir bar was added, and the flask was placed on a magnetic stirrer. The mixture was refrigerated and allowed to settle overnight. The next day, the decanted trout chow and the decanted cerophyl were strained twice through four layers of cheesecloth, and the yeast mixture was added to the trout chow-cerophyl mixture. The TCY was frozen in 250 mL plastic bottles and stored frozen until needed. Thawed food was refrigerated and used for no longer than one week. The S. capricornutum was obtained from the National Fisheries Contaminant Research Center in Columbia, Missouri; cultures of this algae were prepared following the methods described by Goulden, et al. (1982b). Nutrient stock solutions that supported the growth of the algae (Appendix A-2) were prepared using ACS reagent grade chemicals. The stock solutions were refrigerated and used for not more than three months. The culture medium was prepared by adding 1 mL of a 1 g/L stock of each nutrient, except ferric chloride (2 mL), to 900 mL of distilled water. The media was then inoculated with 50 mL of the previous algae culture. The new cultures were placed under fluorescent lights and stirred on a magnetic stirrer for seven to eight days. New cultures of S. capricornutum were

prepared weekly. Approximately 2 mL of TCY and 1 mL of S. capricornutum suspension were added to each D. magna culture every other day; 1 mL of TCY and 0.5 mL of S. capricornutum were added to the C. dubia cultures twice each day.

The trout chow and cerophyl used for the food preparations were analyzed for the presence of pesticide organics (Appendix A-3 and A-3.1). Thirty-gram samples were analyzed following the procedures outlined in EPA Method 625 (1984).

The glassware used in the culturing and testing of metals was hand washed in tap water with detergent. It was then rinsed twice in tap water, soaked in a 10% nitric acid solution, and rinsed twice in distilled water. All glassware used in the bioassays with the organic compounds was rinsed in hexane, washed in tap water with detergent, rinsed twice in tap water, then twice in distilled water.

Toxicant Concentrations

The toxicants used in the bioassays were purchased from Sigma Chemical Company (cadmium chloride, PCP, and 1-octanol); carbaryl was supplied by the EPA repository, Triangle Park, NC. Prior to each test date, 25 mL of a 1 mg/mL stock solution was prepared for all toxicants, except 1-octanol, by dissolving the toxicant in distilled

water (cadmium chloride) or in pesticide grade hexane (PCP and carbaryl). This stock was stored in glass vials capped with teflon and was used throughout the test period to prepare the test solutions. The appropriate volume of the stock solution was used to generate the highest exposure solution for a particular test.

Cadmium. The highest exposure solution was prepared by placing 60 μ L of the stock into a 2 L volumetric glass flask using a 100 μ L Hamilton syringe. Well/spring water was added to the flask, and the exposure solution was stirred overnight before used for testing.

PCP. The highest exposure solution was generated by placing the appropriate volume of stock solution into a 9-L glass jug with a 5.0-mL Kimax volumetric glass pipettes. The jug containing the PCP stock was place under a gentle nitrogen flow to evaporate the solvent, leaving the interior of the jug coated with the PCP. Well/spring water was added to bring the total volume to 8 L. The exposure solution was magnetically stirred at room temperature for 96-h.

1-octanol. The highest exposure solution was prepared by placing 0.30 mL of 1-octanol into a 9-L glass jug using

a 1-ml Kimax volumetric glass pipette. Four liters of well/spring water was added to the jug, and the solution was stirred for 1-h. Then well/spring water was added to bring the total volume to 9 L. The exposure solution was stirred at room temperature for 72-h.

Carbaryl. The highest exposure solution was generated by placing the appropriate volume of stock solution into a 4- L glass jar using a 150 μ L Hamilton syringe. The solvent was evaporated under a gentle flow of nitrogen. Then well/spring water was added to the jar, and the exposure solution was magnetically stirred at room temperature for 48-h.

For each toxicant, test dilutions in geometric series were prepared on the day of testing. The dilutions were made based on the chemical analysis of the highest toxicant exposure solution. Aliquots of the exposure solutions were measured in a graduate cylinder, then placed into a 1- or 2-L volumetric glass flask. Well/spring water was added to the flask volume. All test vials were rinsed in the appropriate exposure solution before they were filled for testing.

Analytical Techniques

The toxicant concentrations for the chronic studies were measured three times during a test period. Three samples of the highest and the second lowest exposure media and the well/spring water were collected for analyses. Each sample was taken before the introduction of the Ceriodaphnia and the Daphnia. The concentrations were verified by Inductively Coupled Plasma (ICP) atomic emission (cadmium chloride); Gas Chromatograph (GC) (PCP and 1-octanol); and High Pressure Liquid Chromatograph (HPLC) (carbaryl). Chemical analyses of the toxicants were performed by Tennessee Technological University Water Resources Center (Cookeville, TN) following standard EPA methods.

Cadmium. One hundred-mL samples of the cadmium test solution were collected for chemical analyses. Ultra-high purity grade, concentrated nitric acid was added to the samples to acidify them to a pH of 2. The samples were stored in a refrigerator at 4 °C for less than one month. For the determination of total cadmium, the sample preparations involved digesting the acid-preserved samples twice with concentrated nitric acid (3.0 mL and 5.0 mL, respectively). Then diluted hydrochloric acid was used to dissolve any precipitate or residue resulting from the

digestion process. The samples were filtered to remove insoluble materials that could clog the instrument. The cadmium concentrations were determined by ICP--Atomic Emissions Spectrometric methods (EPA Method 200.7, 1982). The instrument used in these analyses was a Perkin Elmer, ICP 6500.

PCP. The methods for analyses followed those given in EPA Method 604 (1982), and involved liquid/liquid extractions. One liter of the exposure solution was adjusted to a pH of 2 with concentrated sulfuric acid, then placed into a 2 L glass separatory funnel with teflon fittings. Sixty mL of methylene chloride were added to the separatory funnel, and the mixture was shaken by hand for two minutes. The methylene chloride was filtered through anhydrous sodium sulfate to remove excess water. The methylene chloride was then collected in a 250-mL volumetric flask with a ground glass stopper. The exposure solution was extracted twice more, and the methylene chloride extracts were combined. The extract was then concentrated using Kuderna-Danish apparatus. The solvent was exchanged to hexane, and the extract concentrated to a final volume of 1.0 mL. The extract was analyzed using a Hewlett Packard 5890 GC with ⁶³Ni electron capture detector. An HP-1 column, 5.0 m x 0.53

mm x 2.65 μ m, at a column temperature of 160 °C was used. The injector and detector temperatures were 250 °C. Nitrogen was used as both the carrier and the auxiliary gas flow, at flow rates of 20 mL/min and 40 mL/min, respectively.

1-octanol. One liter of the exposure solutions were extracted three times with methylene chloride. The methylene chloride extracts were dried with anhydrous sodium sulfate and concentrated to 10.0 mL using the Kuderna-Danish apparatus. Methylene chloride sample concentrates of the highest generated exposure solutions were diluted 10 times. The sample extracts were then analyzed using a Hewlett Packard 5890 GC with an HP-1, 5.0 m x 0.53 mm x 2.65 μ m, column at a temperature of 70 °C. The injector and detector temperatures were 200 °C. Helium was used as both the carrier and the auxiliary gas, at flow rates of 20 mL/min and 10 mL/min, respectively. A flame ionization detector was used, with a hydrogen flow rate of 30 mL/min and an air flow rate of approximately 400 mL/min.

Carbaryl. One liter samples of the exposure solutions were collected for analyses. A Fisher C₁₈ solid phase extraction column was conditioned with acetonitrile and

HPLC grade water. The carbaryl from the exposure samples was concentrated by introducing a known sample volume onto the Fisher C₁₈ solid phase extraction column. Adsorbed carbaryl was eluted from the column with 2.0 mL of acetonitrile and the sample was diluted to a total of 2.0 mL and analyzed by HPLC. The instrumentation consisted of a Hewlett Packard 1090 Liquid Chromatograph with a diode array detector, and a Hewlett Packard Hypersil ODS, 5 μ , 4.6 mm x 200 mm column. The mobile phase was a gradient of acetonitrile: the water from 15:85 to 85:15 in 10 minutes at a flow rate of 2.0 mL/min. The column temperature was maintained at 35 °C; the detector was set at a wavelength of 220 nm.

Bioassay Procedures

Acute Bioassay. In these experiments, C. dubia and D. magna were exposed separately to a toxicant for 48-h to estimate the relative sensitivities of the species and the concentration of a toxicant to be used in the chronic tests. Tests for the Ceriodaphnia and the Daphnia began on the same day so that each species was exposed to the same toxicant preparation. The neonates used in the tests were collected from the third or fourth brood of second-generation animals from a single parent.

The C. dubia tests contained four replicates per concentration, while the D. magna tests had three replicates per concentration. The animals were distributed randomly into the test vessels. Neonates (12 $\pm$ 12-h old) from several females were placed into a glass crystallizing dish that contained well/spring water at the test temperature. Ten animals, randomly chosen two at a time, were pipetted from the crystallizing dish into a 100-mL beaker that contained the test solution. A total of 180 D. magna and 240 C. dubia were used in each experiment.

The toxic response measured was mortality. Animals were considered dead if, after swirling the medium and allowing it to settle, microscopic observations revealed no swimming or movement of appendages. Observations on mortality were made 24- and 48-h after the test had started.

For each test, five concentrations of the toxicant and a well/spring water control were tested. The test solutions were not renewed, and the animals were not fed during the test. Water quality parameters were measured at the beginning of each test. The concentration of the chemical that caused half of the organisms to die within 48-h (48-h LC₅₀) was calculated by probit analysis.

Chronic Bioassay. Tests were conducted for both species with each of the four toxicants. The neonates (12 ± 12 -h old) were randomly selected and distributed individually into 25 mm x 95 mm glass vials that contained 30 mL of test solution or well/spring water. The test vessels were placed in a water bath to maintain a constant temperature ($23 \pm 1^\circ\text{C}$). Each concentration of each chemical was tested using 20 animals/concentration.

The C. dubia were fed 0.28 mL of both TCY and S. capricornutum per day; the D. magna were fed daily 0.56 mL of TCY suspension and 0.28 mL of S. capricornutum. Individual animals were observed daily; the young produced and any molts present were removed and recorded. The test solutions were renewed every other day. Water quality of the dilution water, at each toxicant renewal, was measured following standard methods, EPA Method 130.1 (pH, conductivity, and alkalinity), and EPA Method 130.2 (water hardness).

The endpoints measured for individual animals were survival and reproduction. The reproductive endpoints included the total number of viable young, the time from 12 ± 12 -h to the first brood release, the number of young per brood, and the number of broods per female. Other anomalies (e.g., embryonic development and male production) microscopically observed during a test were

also recorded. The total number of viable young was used to calculate the fertility value for each female (i.e., the number of offspring per female per day). This value is used in all graphs and its importance is explained in Chapter III.

The data via chronic testing were analyzed at 7-d and 14-d test durations for C. dubia and D. magna, respectively. These test durations are the characteristic times required for the release of three broods for each species. Additionally, tests were continued until 18-d to permit comparisons of longer term exposures.

For each toxicant, a test consisted of five concentrations and a well/spring water control. Duplicate tests were run for cadmium and PCP; however, the data collected for the second tests of these two chemicals were too variable for comparison with the first test. Thus, a single test using 1-octanol and carbaryl was run, and the results of the first test for cadmium and PCP were used for data analyses.

Statistical Analyses

The concentration of each toxicant at which one half of the test organisms died within 48-h (48-h LC_{50}) was determined statistically by probit analysis using the

EPistat BasicA statistical package for the IBM PC, Version 3.1 (Gustafson, 1984).

The data obtained during chronic exposures were analyzed by: (1) a test for a significant difference between the survival of the original test organisms in the exposure concentrations and the control. Significant differences in survival were identified using a Tukey-type test for multiple comparisons among proportions transformed to arcsine values (Zar, 1984; pp. 240 and 402).

$$p = \frac{1}{2} \left[\arcsin \sqrt{\frac{x}{n+1}} + \arcsin \frac{\sqrt{x+1}}{n+1} \right] \quad (\text{II-1})$$

where p = proportion transformed, x = the number of survivors, and n = the number of animals in the sample.

Then significant differences were determined using Dunnett's test statistic:

$$q = \frac{p_B - p_A}{SE} \quad (\text{II-2})$$

calling the control group B, and each other group A, using as the appropriate standard error for each comparison:

$$SE = \sqrt{\frac{410.34}{n+0.5}} \quad (\text{II-3})$$

The standard error for each comparison is in degrees (Zar, 1984). The constant 410.35 results from the variance in the transformed proportions for equal sample sizes.

(2) Reproductive data were analyzed by analysis of variance (ANOVA) followed by Dunnett's procedure. An ANOVA was performed using the general linear models (GLM) procedure on PC SAS, version 6.0 (Statistical Analysis Systems, Cary, NC). An ANOVA was used to determine if there were significant differences between the concentrations, i.e., to determine if the test chemical had significant effects on the reproductive parameters. Dunnett's *t*-test (Zar, 1984; p 194) was used to test for significant differences between the exposed groups and the controls when the ANOVA indicated a difference did exist. The word "significant" throughout this study implies that a statistical test was performed using a 0.05 α level. A no-observed-effect concentration (NOEC) and a lowest-observed-effect concentration (LOEC) was determined according to Horning and Weber (1985). The LOEC is defined as the lowest concentration determined to be significantly different from the control; the NOEC is the concentration below the LOEC that caused no effect to the test organism.

Results

Water Quality. The well/spring water quality changed little during the tests (Table II-2). Water hardness ranged from 80.2 to 90.7 mg/L, which was less than that recommended by EPA test protocols (160 to 180 mg/L as calcium carbonate (CaCO_3); Horning and Weber, 1985). This may have affected the toxicity of cadmium to both species. Metals are generally more toxic in soft water than in hard water (Calamari, et al., 1980; Abel, 1974; Sprague, 1970). The pH (range 8.1 to 8.2) and dissolved oxygen (range 8.4 to 8.8 mg/L) were within the limits of the EPA recommendations. The water temperature ranged from 22.0 to 22.5 °C.

Chemical Analyses of Test Solutions. The measured toxicant concentrations are presented in Table II-3. Except for carbaryl, the highest measured concentrations were similar to the predicted nominal concentrations. All toxicants measured at the lower concentrations were less than the predicted nominal concentrations, or below the detection limit of the instruments. The controls contained less than the detection limit for each of the toxicants.

Table II-2. Summary of water quality parameters measured during the chronic bioassays. Values are means ($\pm$ SD) for 11 sampling periods.

Parameters	Test Chemicals			
	Cadmium	PCP	1-Octanol	Carbaryl
Alkalinity (mg/L CaCO ₃)	74.8 (± 16.9)	80.7 (± 14.5)	72.8 (± 8.3)	88.9 (± 7.2)
Conductivity (μ mhos/cm)	146.2 (± 31.2)	156.7 (± 20.6)	146.4 (± 12.4)	158.9 (± 13.7)
Dissolved oxygen (mg/L)	8.4 ($\pm .135$)	8.5 ($\pm .123$)	8.7 ($\pm .225$)	8.8 ($\pm .323$)
Hardness (mg/L CaCO ₃)	85.6 (± 19.0)	90.7 (± 9.7)	80.2 (± 7.7)	90.2 (± 7.0)
Temperature ($^{\circ}$ C)	22.5 ($\pm .412$)	22.4 ($\pm .313$)	22.0 ($\pm .452$)	22.0 ($\pm .980$)
p ^H	8.2 ($\pm .124$)	8.2 ($\pm .107$)	8.2 ($\pm .088$)	8.1 ($\pm .186$)

Table II-3. Measured water concentrations for the four modes of action chemicals taken during the chronic bioassays. Values are means ($\pm$ SD) of 3 sampling periods. Less than (<) values reflect the detection limits of the instruments.

Toxicant	Nominal Concentration ($\mu\text{g/L}$)	Measured Concentration ($\mu\text{g/L}$)
Cadmium	30	25 (± 1.2)
	3.75	<8.0
	Control	<8.0
PCP	200	239 (± 38.7)
	6.25	3.2 (± 2.9)
	Control	<0.5
1-Octanol	28000	24500 (± 3775)
	3500	3033 (± 702)
	Control	<100
Carbaryl	20	7.8 (± 2.6)
	1.25	.53 ($\pm .16$)
	Control	<0.4

Acute Toxicity

The results of the cadmium, PCP, 1-octanol, and carbaryl acute toxicity tests for both species are presented in Table II-4. Control mortality was $\leq 10\%$ in every test. As determined by probit analysis, C. dubia was more sensitive than D. magna to all four chemicals.

The response of C. dubia to the four chemicals, in the order of the most toxic to the least toxic, was carbaryl > PCP > cadmium > 1-octanol. For D. magna, the order of toxicity (most toxic to least toxic) was carbaryl > cadmium = PCP > 1-octanol. Based on the responses of the two species, the relative toxicities of the chemicals showed carbaryl to be the most toxic chemical to both species, with less than a two-fold difference in LC_{50} values.

Chronic Toxicity

Results of survival and the reproductive parameters determined for both species exposed to all toxicants, are presented in Table II-5. In all test exposures, control mortality was $\leq 15\%$.

Cadmium. Survival of C. dubia (Tukey-type test for multiple comparisons among proportions) and reproduction (ANOVA and Dunnett's *t* test) were significantly reduced

Table II-4. The 48-h LC₅₀ values ($\pm$ 95% confidence intervals) for the acute bioassays with Ceriodaphnia dubia and Daphnia magna exposed to four chemicals having different modes of action.

Species	Toxicant	48-h LC ₅₀ (95% CI)
<u>C. dubia</u>	Cadmium	32.1 (9.0-66.0)
	PCP	17.9 (12.1-40.0)
	1-Octanol	8700 (7520-10060)
	Carbaryl	14.0 (10.8-17.4)
<u>D. magna</u>	Cadmium	249 (214-335)
	PCP	249.5 (207-308)
	1-Octanol	26160 (-)
	Carbaryl	22.5 (16-31)

Table II-5. Summary of the survival frequencies and the reproductive parameters for Ceriodaphnia dubia in 7- and 18-d exposures, and Daphnia magna in 14- and 18-d exposures. Significant differences in reproductive parameters (*) were determined by Analysis of Variance (GLM) and Dunnett's *t* test, $\alpha = 0.05$.

Species	Test duration(d)	Toxicant	Toxicant Concentration ($\mu\text{g/L}$)	Survival (%)	Reproductive Parameters (mean $\pm$ SD)		
					Time to first brood(d)	No. of broods	Brood Size
<u>C. dubia</u>	7	Cadmium	0	99	4 (.37)	3 (.31)	7 (1.8)
			1.5	89	4 (.59)	3 (.79)	5 (2.3)*
			3.0	91	4 (.44)	3 (.61)	5 (1.9)*
			6.0	79	5 (.85)	3 (.65)	4 (1.9)*
			13	44	5 (.58)	1 (0)	3 (.58)*
	18	Cadmium	25	44	6 (.71)	1 (0)	2 (.71)*
			0	94	4 (.37)	9 (2.0)	11 (3.1)
			1.5	90	4 (.59)	8 (3.2)	9 (4.5)*
			3.0	91	4 (.44)	8 (3.0)	8 (3.7)*
			6.0	72	5 (.85)	7 (1.6)*	6 (1.8)*
<u>D. magna</u>	14	Cadmium	13	17	5 (.58)	1 (0)*	3 (.58)*
			25	17	6 (.71)	-1 (0)*	2 (.71)*
	18	Cadmium	0	100	8 (.91)	3 (.64)	7 (1.4)
			1.5	98	7 (.41)	2 (.77)	8 (3.5)
			3.0	69	7 (.70)	1 (.50)*	7 (3.9)
			6.0	53	9 (1.1)	1 (0)*	4 (3.4)*
			13	43	-	-	-
	18	Cadmium	25	30	-	-	-
			0	100	8 (.91)	4 (.55)	7 (1.6)
			1.5	98	7 (.41)	3 (2.4)	10 (3.5)
			3.0	54	7 (.70)	1 (.50)*	7 (3.9)
			6.0	41	9 (1.1)	1 (0)*	4 (3.4)*
			13	33	-	-	-
			25	23	-	-	-

Table II-5 (Continued).

Species	Test duration(d)	Toxicant	Toxicant Concentration ($\mu\text{g/L}$)	Survival (%)	Reproductive Parameters (mean $\pm$ SD)		
					Time to first brood(d)	No. of broods	Brood Size
<u>C. dubia</u>	7	PCP	0	97	4 (.31)	3 (.45)	8 (1.5)
			4	100	4 (.47)	3 (.57)	9 (2.1)
			8	91	4 (.37)	3 (.64)	7 (1.7)
			15	90	4 (.46)	3 (.92)	7 (2.0)
			30	90	4 (0)	3 (.49)	7 (2.0)
			60	89	4 (.32)	3 (.58)	7 (1.8)
	18		0	87	4 (.31)	10 (2.9)	11 (3.8)
			4	95	4 (.47)	10 (1.6)	11 (2.2)
			8	81	4 (.37)	8 (1.5)*	9 (2.5)
			15	79	4 (.46)	8 (2.9)*	10 (3.9)
<u>D. magna</u>	14	PCP	0	100	8 (.99)	3 (.68)	5 (2.2)
			15	89	10 (1.5)	1 (.81)*	6 (5.3)
			30	95	9 (2.2)	1 (.83)*	1 (2.5)*
			60	95	9 (1.4)	1 (.67)*	3 (3.7)
			120	89	11 (2.6)*	1 (.75)*	1 (1.2)*
			239	45	12 (2.4)*	1 (.50)*	4 (1.7)
	18		0	99	8 (.99)	4 (.85)	6 (1.8)
			15	81	10 (1.5)	2 (.80)	4 (2.5)*
			30	95	9 (2.2)	1 (1.0)	5 (4.2)
			60	93	9 (1.4)	1 (1.0)	5 (4.5)
			120	76	11 (2.6)*	1 (.67)	1 (1.5)*
			239	35	12 (2.4)*	1 (.50)	4 (1.7)*

Table II-5 (Continued).

Species	Test duration(d)	Toxicant	Toxicant Concentration ($\mu\text{g/L}$)	Survival (%)	Reproductive Parameters (mean $\pm$ SD)		
					Time to first brood(d)	No. of broods	Brood Size
<u>C. dubia</u>	7	1-Octanol	0	100	4 (.50)	2 (.44)	7 (2.0)
			569	100	4 (.51)	2 (.41)	7 (2.7)
			1138	100	5 (.46)	2 (.32)	7 (2.0)
			2275	100	5 (.51)	2 (.55)	6 (2.8)
			4550	99	5 (.60)	2 (.51)	4 (1.3)*
			9100	81	-	-	-
	18		0	98	4 (.50)	9 (.76)	8 (.84)
			569	100	4 (.51)	9 (.51)	9 (.84)
			1138	98	5 (.46)	9 (1.1)	10 (1.6)
			2275	92	5 (.51)	9 (2.1)	10 (3.2)
			4550	87	5 (.60)	7 (2.3)*	7 (3.2)
<u>D. magna</u>	14	1-Octanol	0	44	10 (0)*	1 (.41)*	3 (2.2)*
			1531	95	8 (.51)	2 (.58)	7 (2.7)
			3062	95	9 (1.3)	3 (.67)	5 (1.7)*
			6125	89	10 (.92)*	2 (.56)	6 (2.7)
			12250	77	10 (4.5)*	1 (.84)*	7 (4.4)
	18		24500	33	-	-	-
			0	10	-	-	-
			1531	95	8 (.51)	4 (.46)	8 (1.5)
			3062	95	9 (1.3)	4 (.81)	5 (1.2)*
			6125	87	10 (.92)*	4 (.70)	6 (1.7)
			12250	75	10 (4.5)*	3 (.86)	7 (2.8)
			24500	26	-	-	-
				8	-	-	-

Table II-5 (Continued).

Species	Test duration(d)	Toxicant	Toxicant Concentration ($\mu\text{g/L}$)	Survival (%)	Reproductive Parameters (mean $\pm$ SD)		
					Time to first brood(d)	No. of broods	Brood Size
<u>C. dubia</u>	7	Carbaryl	0	100	4 (.44)	3 (.31)	6 (.91)
			0.25	99	4 (.50)	3 (.64)	5 (1.5)
			0.50	100	4 (.58)	3 (.45)	4 (1.2)
			1.0	100	4 (.51)	3 (.49)	4 (1.1)
			2.0	99	5 (.79)	2 (.51)	3 (.91)*
			4.0	15	-	-	-
	18		0	100	4 (.44)	9 (.64)	9 (1.1)
			0.25	96	4 (.50)	9 (1.6)	7 (1.7)*
			0.50	100	4 (.58)	9 (.52)	7 (.64)*
			1.0	100	4 (.51)	9 (.39)	6 (.55)*
			2.0	99	5 (.79)	9 (1.2)	5 (.87)*
			4.0	6	-	-	-
<u>D. magna</u>	14	Carbaryl	0	100	9 (.22)	3 (.66)	6 (1.1)
			0.5	100	11 (1.7)*	3 (.79)	3 (.93)*
			1.0	100	11 (1.6)*	2 (.88)*	3 (1.5)*
			2.0	99	11 (1.8)*	2 (.73)*	2 (1.2)*
			4.0	99	12 (1.2)*	1 (.43)*	2 (1.7)*
			8.0	35	-	-	-
	18		0	100	9 (.22)	4 (.66)	6 (1.0)
			0.5	100	11 (1.7)*	4 (1.1)	3 (.87)*
			1.0	100	11 (1.6)*	3 (.97)*	3 (1.3)*
			2.0	98	11 (1.8)*	2 (.92)*	3 (1.6)*
			4.0	98	12 (1.2)*	2 (.73)*	2 (1.2)*
			8.0	27	-	-	-

after 7-d and 18-d exposures to 6.0, 13, and 25 $\mu\text{g/L}$ (Fig. II-1). At 13 and 25 $\mu\text{g/L}$, less than 50% of the exposed adults survived the 7-d exposure, and less than 20% survived the 18-d exposure. The C. dubia that survived exposure at the higher concentrations did not appear smaller or less mobile than the animals in the lower concentrations and controls. At the end of the 18-d exposure, reproduction for the controls, 1.5, and 3.0 $\mu\text{g/L}$ was twice that of the 7-d exposure. This was attributed to an increased number of broods and an increased number of neonates per brood (Table II-5). At concentrations >6.0 $\mu\text{g/L}$ cadmium, reproduction (total number of young produced) declined significantly ($F = 16.46$, $P < 0.0001$) for 7-d and 18-d exposures (Fig. II-1) (ANOVA Appendix B-1). This was probably due to increased adult mortality at these concentrations. Additionally, dead C. dubia neonates were observed in test vessels at concentrations of 1.5 to 6.0 $\mu\text{g/L}$. The percent of dead neonates ranged from 0.4 to 5.6% (Table II-6).

The time to the first brood release of C. dubia was delayed one to two days at cadmium concentrations of 6.0, 13, and 25 $\mu\text{g/L}$, but was not significantly less than the controls (Appendix B-1) (Table II-5). Both survival and reproduction decreased with increasing cadmium

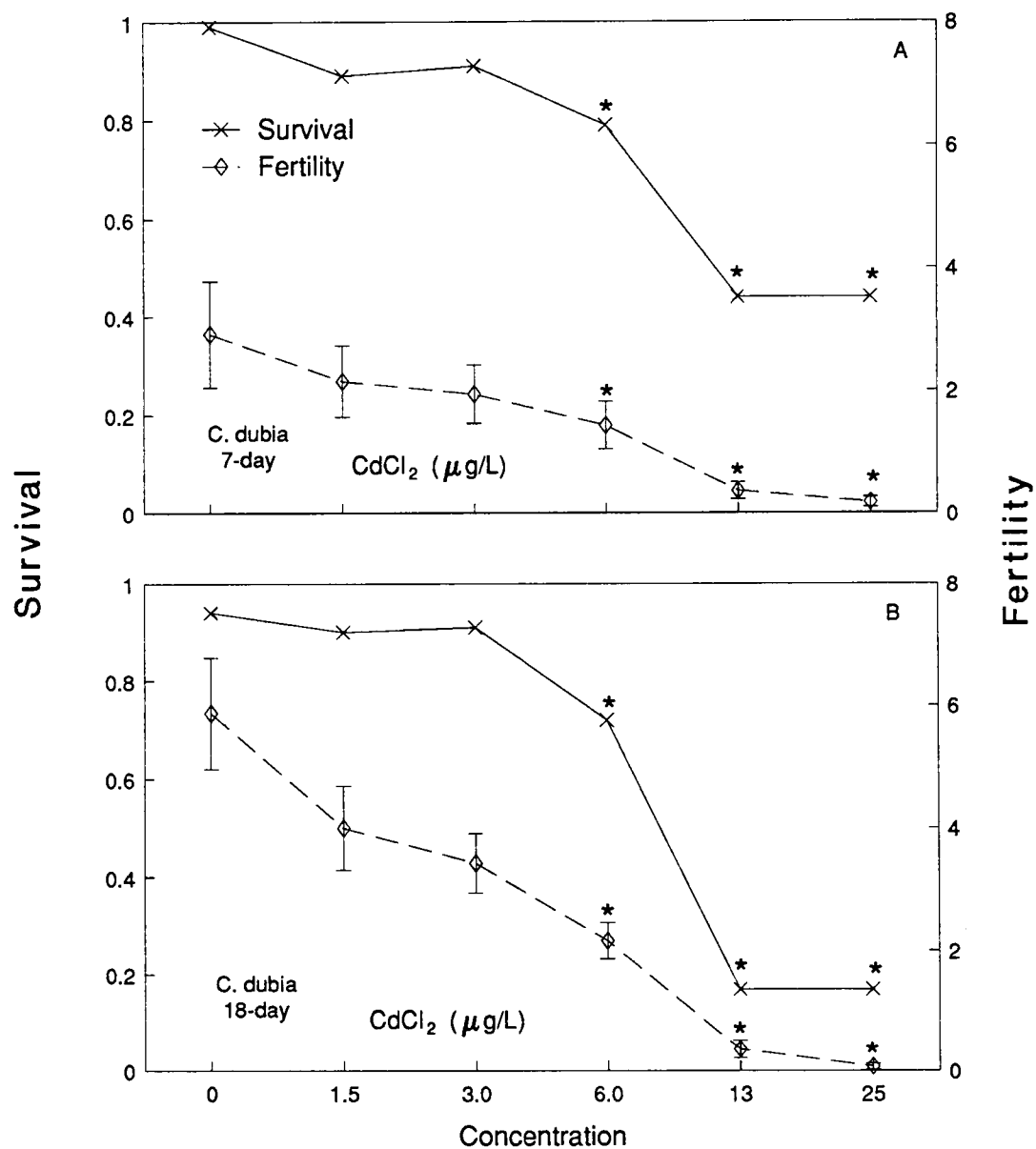


Figure II-1. Survival and fertility of Ceriodaphnia dubia exposed to cadmium for 7-d (panel A) and 18-d (panel B). Significantly different (*) from control ($\pm$ SE); $n = 20$.

Table II-6. The percent of dead neonates produced by Ceriodaphnia dubia and Daphnia magna exposed to cadmium and pentachlorophenol in chronic bioassays.

Species	Toxicant	Toxicant Concentration ($\mu\text{g/L}$)	Percent dead neonates			
			Test		duration	
			7-d	18-d	14-d	18-d
<u>C. dubia</u>	Cadmium	0	0	0		
		1.5	0	.4		
		3.0	0	3.3		
		6.0	0	5.6		
		13	0	----		
		25	0	----		
<u>D. magna</u>	Cadmium	0			0	0
		1.5			13	16
		3.0			7	7
		6.0			50	50
		13			--	--
		25			--	--
<u>C. dubia</u>	PCP	0	0	0		
		4	0	5		
		8	8	26		
		15	7	12		
		30	8	18		
		60	7	23		
<u>D. magna</u>	PCP	0			0	0
		15			7	30
		30			7	60
		60			51	62
		120			91	93
		239			37	48

concentrations in the 7-d and 18-d exposure durations.

Survival of D. magna was significantly reduced after 14-d and 18-d exposures to 3.0, 6.0, 13, and 25 $\mu\text{g/L}$ cadmium (Fig. II-2). After 14-d, >50% adult mortality occurred at concentrations $\geq 13 \mu\text{g/L}$. The exposed D. magna in cadmium concentrations greater than 1.5 $\mu\text{g/L}$, were small and whitish in color.

Reproduction of D. magna was significantly reduced at the lowest cadmium concentration (1.5 $\mu\text{g/L}$) ($F = 68.41$, $P < 0.0001$) at 14-d (Fig. II-2A). An additional brood produced at 1.5 $\mu\text{g/L}$ between the 14-d and the 18-d exposure resulted in significantly less reproduction at 3.0 $\mu\text{g/L}$ cadmium (Fig. II-2B). Other than the one additional brood at 18-d exposure of D. magna to cadmium, the reproductive parameters (Table II-5) were the same for both exposure periods, since there was only a 4-d difference in the exposure durations.

The D. magna test vessels contained aborted eggs and dead neonates at 1.5 $\mu\text{g/L}$ cadmium and greater (Table II-6). During the 14-d exposure, 13% of the total number of young produced were dead at 1.5 $\mu\text{g/L}$; this percentage increased to 50% at 6.0 $\mu\text{g/L}$. The time to the first brood release (ANOVA and Dunnett's test) for exposed D. magna was not significantly different from the controls (Table II-5). Survival and reproduction of D. magna decreased

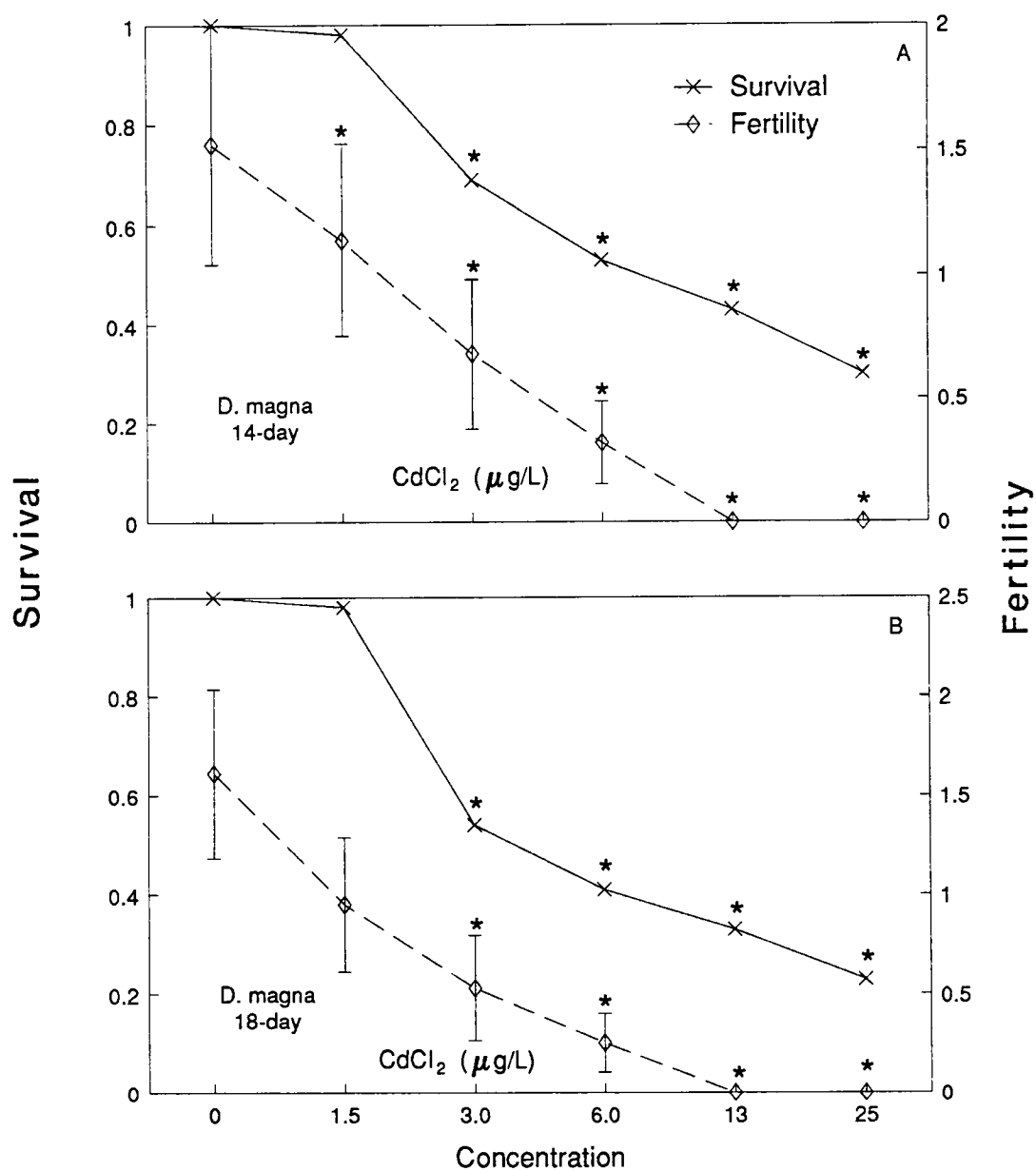


Figure II-2. Survival and fertility of *Daphnia magna* exposed to cadmium for 14-d (panel A) and 18-d (panel B). Significantly different (*) from control ($\pm$ SE); $n = 20$.

with increasing cadmium concentrations after 14-d exposures.

PCP. The results of 7-d and 18-d exposures to C. dubia and D. magna are presented in Figures II-3 and II-4, respectively. The 7-d survival (Tukey's test) and reproduction (Dunnett's test) of C. dubia was not significantly less than the controls for any of the exposure concentrations (Fig. II-3A). In fact, reproduction was stimulated at 4.0 $\mu\text{g/L}$, as evidenced by increased number of neonates per brood. The 18-d exposure resulted in a significant reduction in survival, beginning at 8.0 $\mu\text{g/L}$, and a significant reduction in reproduction at 4.0 $\mu\text{g/L}$ ($F = 14.64$, $P < 0.0001$) (Fig. II-3B). The reduced reproduction at 4.0 $\mu\text{g/L}$ PCP was attributed to the percent of dead neonates (5%) observed in test vessels (Table II-6). As the concentrations increased, the percent of dead neonates increased to 23% for 60 $\mu\text{g/L}$ PCP. The 18-d exposure to PCP did not affect the time to the first brood release for C. dubia. The number of broods were significant less ($F = 4.11$, $P < 0.0019$) than the control at 8 $\mu\text{g/L}$ (Table II-6); significant differences in brood size ($F = 4.00$, $P < 0.0022$) were observed only at 60 $\mu\text{g/L}$.

Survival of D. magna was significantly reduced after 14-d exposure to 120 and 239 $\mu\text{g/L}$ PCP (Tukey's test)

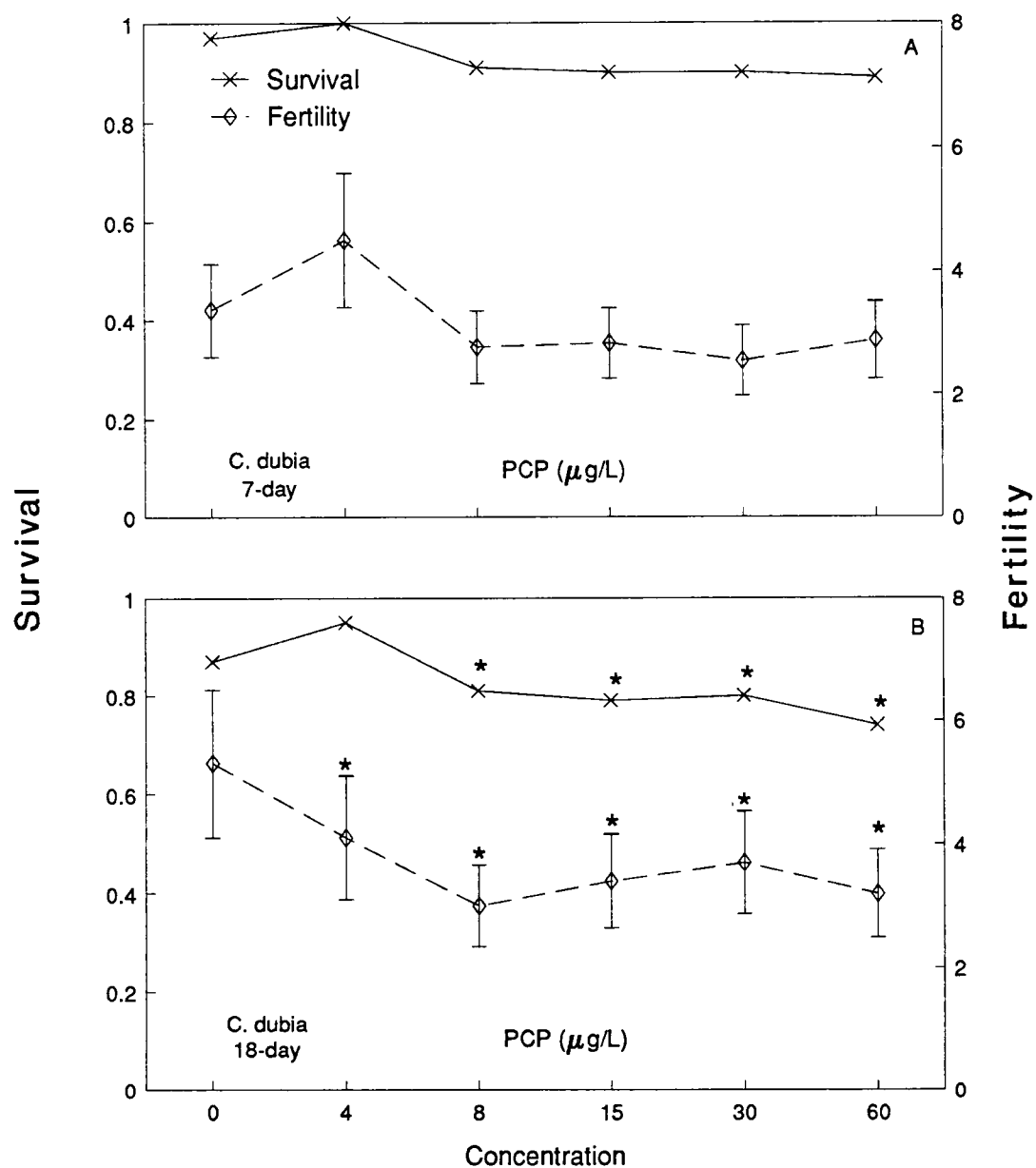


Figure II-3. Survival and fertility of Ceriodaphnia dubia exposed to pentachlorophenol for 7-d (panel A) and 18-d (panel B). Significantly different (*) from control ($\pm$ SE); n = 20.

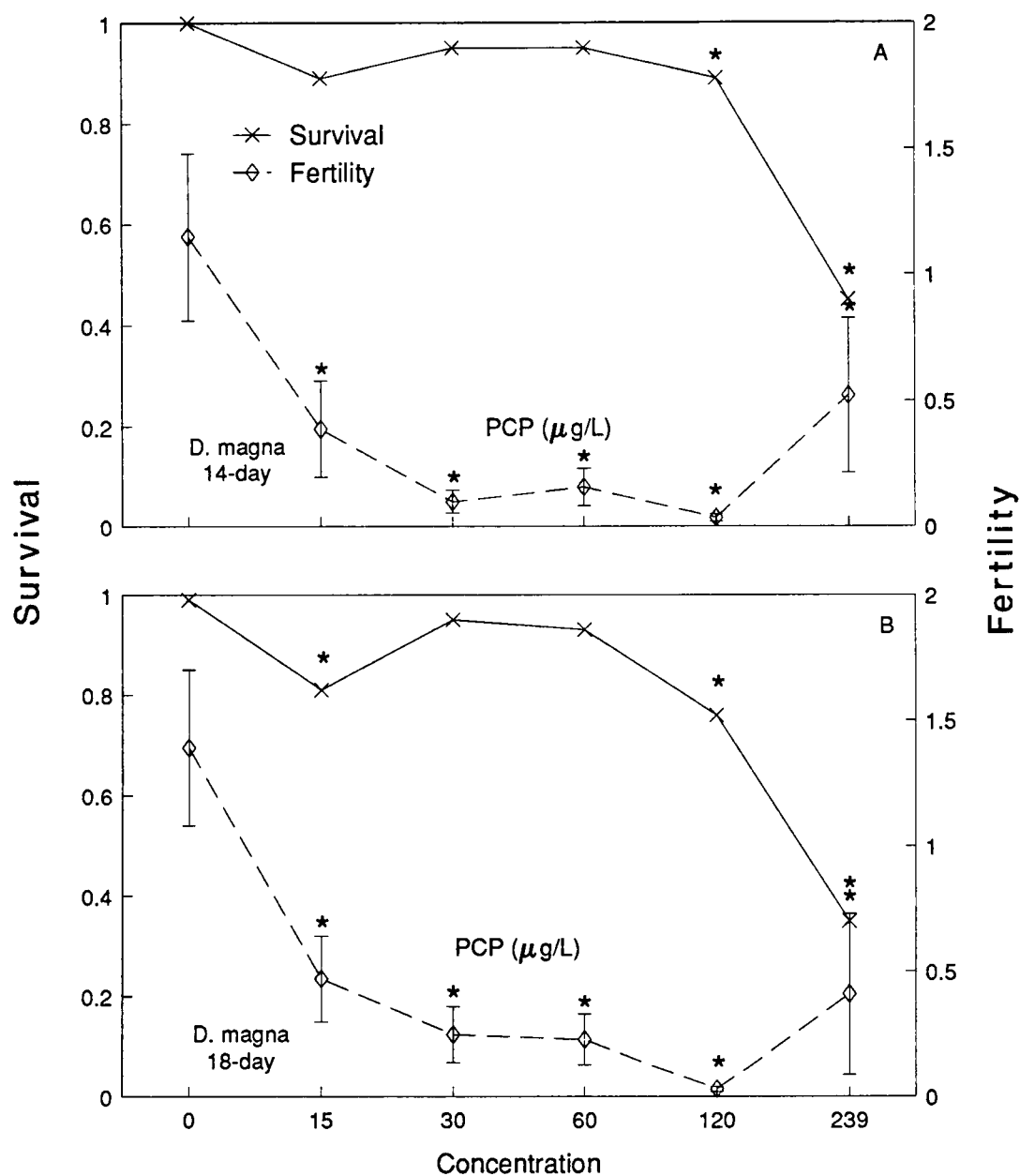


Figure II-4. Survival and fertility of *Daphnia magna* exposed to pentachlorophenol for 14-d (panel A) and 18-d (panel B). Significantly different (*) from control ($\pm$ SE); $n = 20$.

(Fig. II-4A). At 18-d exposure, survival at 15 $\mu\text{g/L}$ was significantly less than controls, but not at 30 and 60 $\mu\text{g/L}$ (Fig. II-4B). The reason for this was not determined.

A significant reduction in reproduction occurred at concentrations $\geq 15 \mu\text{g/L}$ for both 14-d and 18-d exposures ($F = 134.27$, $P < 0.0001$) (Fig. II-4). At these concentrations, the time to the first brood release was delayed by 1 to 5-d. A delay of >3 days was significantly different from the control ($F = 2.40$, $P < 0.0567$) (Table II-5). Both the number of broods and the brood sizes were reduced at all PCP concentrations (Table II-5 and Appendix B-1). The percent of dead neonates observed at 15 $\mu\text{g/L}$ PCP for 14-d and 18-d exposures were 7% and 30%, respectively (Table II-6). Survival and reproduction decreased with increasing concentration of PCP after 14-d.

1-octanol. Results of the 7-d and 18-d exposures are presented in Figure II-5. The 7-d survival of C. dubia was significantly reduced only at the highest concentration, 9100 $\mu\text{g/L}$ (Fig. II-5A). Although adult survival was high, reproduction was considerably lower than for any other test chemical. The reduced reproduction was attributed to a decreased number of broods (2) at 7-d (Table II-5). The reason for this discrepancy could have been a slight change in light

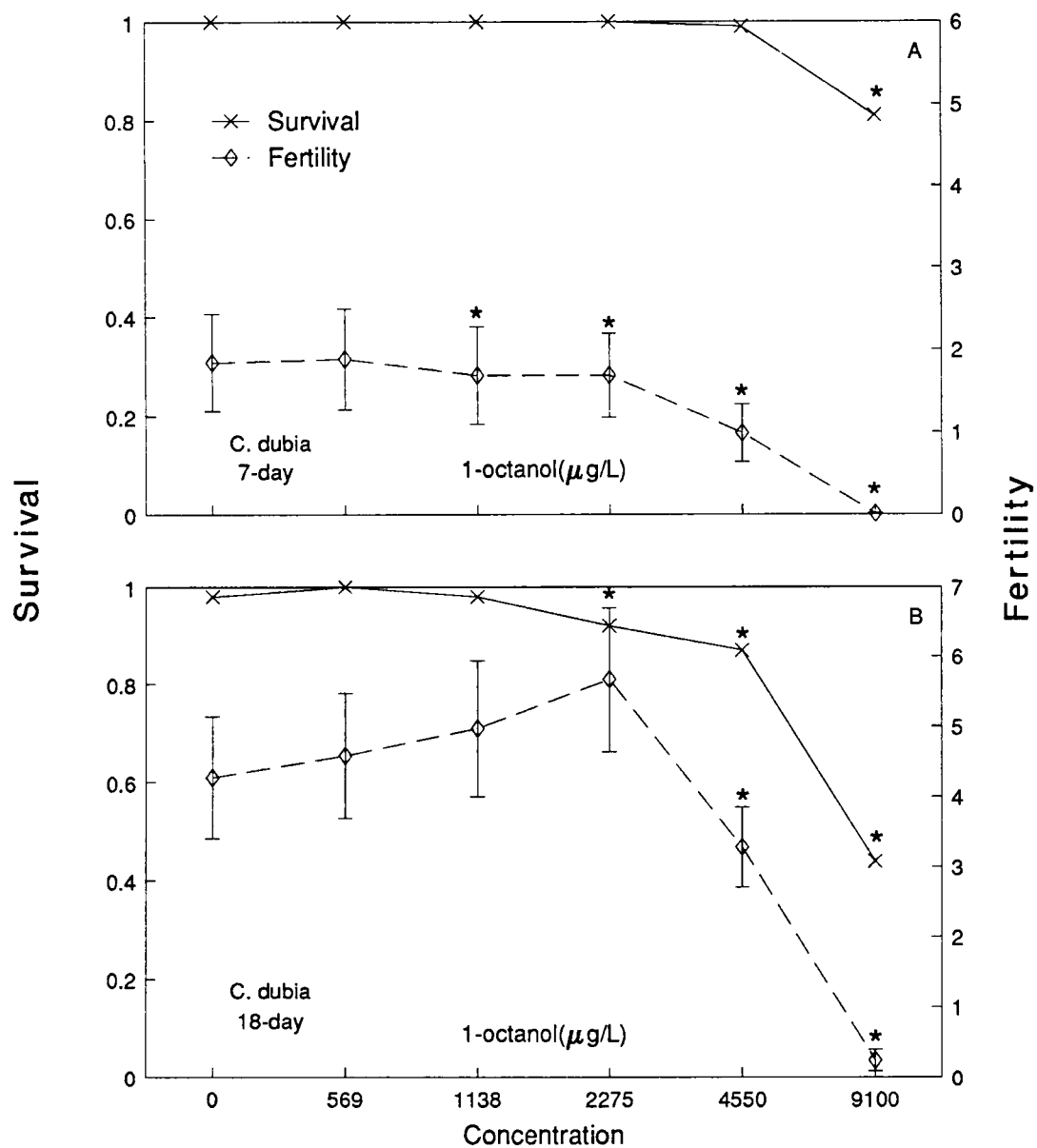


Figure II-5. Survival and fertility of *Ceriodaphnia dubia* exposed to 1-octanol for 7-d (panel A) and 18-d (panel B). Significantly different (*) from control ($\pm$ SE); $n = 20$.

intensity; there was a malfunction of the timer on day two of the experiment.

The 18-d exposure resulted in a significant reduction in survival at 2275 $\mu\text{g/L}$ 1-octanol, and reproduction was significantly reduced ($F = 31.32$, $P < 0.0001$) at 4550 $\mu\text{g/L}$ (Fig. II-5B). Interestingly, the concentration which reduced survival showed an increase in reproduction. Apparently, the decrease in adult survival was compensated for by an increase in reproduction for the survivors. The C. dubia test vessels did not contain dead neonates. The time to the first brood release was 10 days at 9100 $\mu\text{g/L}$ 1-octanol during the 18-d exposure (Table II-5). This was significantly longer than the control ($F = 5.16$, $P < 0.0008$). There was no reproduction at this concentration during the 7-d exposure. Only one brood was produced during the 18-d exposure (Table II-5). Increased concentrations of 1-octanol decreased survival and reproduction. Reproduction was affected to a greater degree than survival, and that was observed after 7-d exposures (Fig. II-5A).

The 14-d and 18-d exposure of D. magna to 1-octanol significantly reduced survival (Tukey's test) at 3062 $\mu\text{g/L}$, and reproduction was significantly reduced ($F = 70.54$, $P < 0.0001$) at 12250 $\mu\text{g/L}$ (Fig. II-6). No reproduction occurred at the two higher concentrations

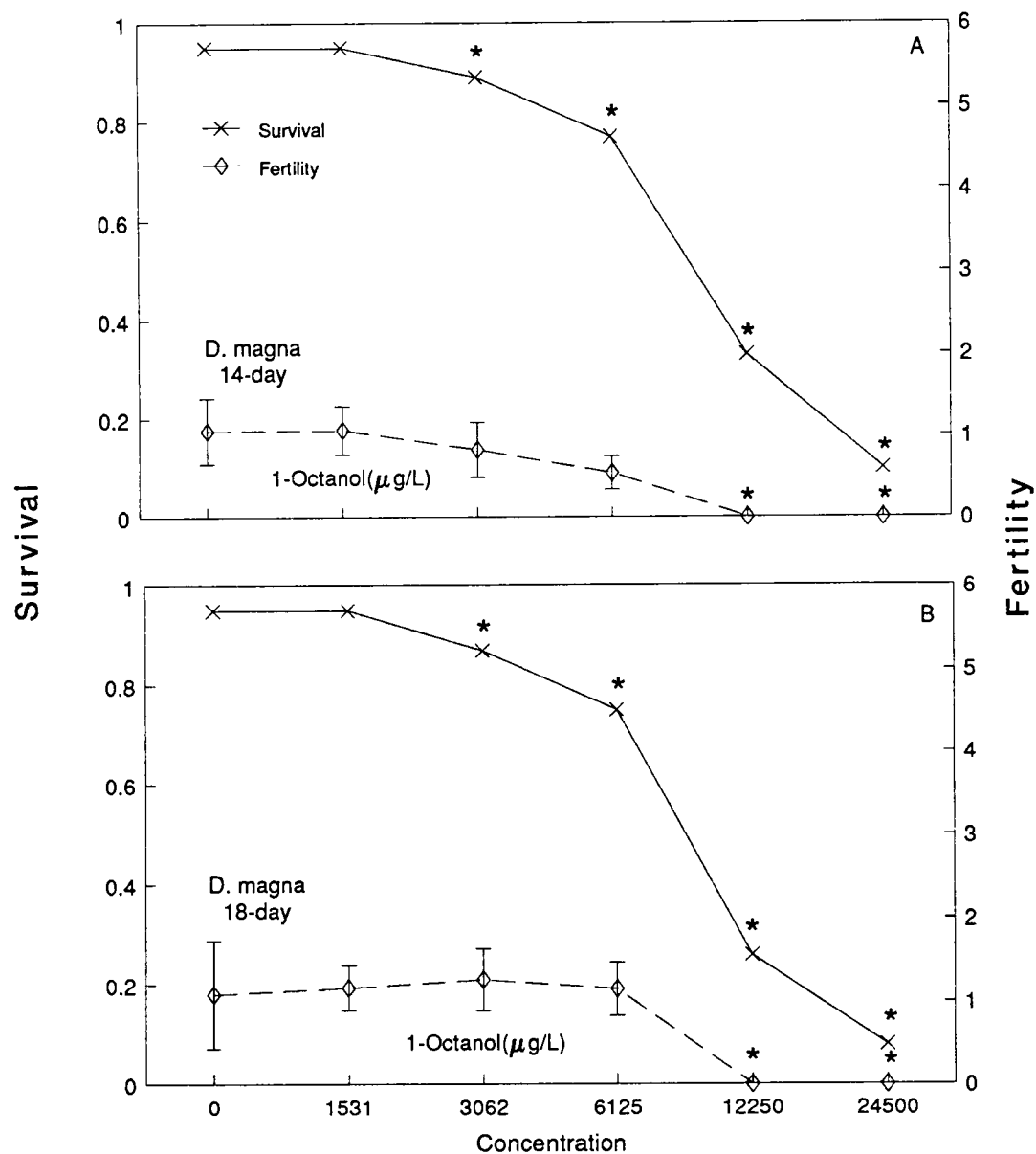


Figure II-6. Survival and fertility of *Daphnia magna* exposed to 1-octanol for 14-d (panel A) and 18-d (panel B). Significantly different (*) from control ($\pm$ SE); $n = 20$.

(12250 and 24500 $\mu\text{g/L}$). The time to the first brood release was delayed by 1 to 2 days, and was significantly different from the control after a 2 day delay ($F = 42.36$, $P < 0.0001$). At 18-d, the number of broods for exposed animals was not significant, but brood sizes were significantly less than controls at 1531 $\mu\text{g/L}$ ($F = 3.72$, $P < 0.0155$). The decreased survival and reproduction with increased 1-octanol concentrations were apparent after 14-d exposures.

Carbaryl. Results for C. dubia are presented in Figures II-7. For 7- and 18-d exposures, survival was significantly less than controls (Tukey's test) only at the highest exposure concentration, while reproduction was significantly less at all exposure concentrations ($F = 99.10$, $P < 0.0001$) (Fig. II-7A and 7B). Carbaryl did not significantly delay the time to the first brood release, nor reduce the number of broods for C. dubia, but brood size decreased with increasing carbaryl concentrations (Table II-5). An increase in reproduction was apparent at the 18-d exposure due to a three-fold increase in number of broods between 7- and 18-d (Table II-5 and Fig. II-7B).

Survival for D. magna 14-d and 18-d exposures to carbaryl was significantly less than controls only at the highest concentration (8 $\mu\text{g/L}$) (Tukey's test), but

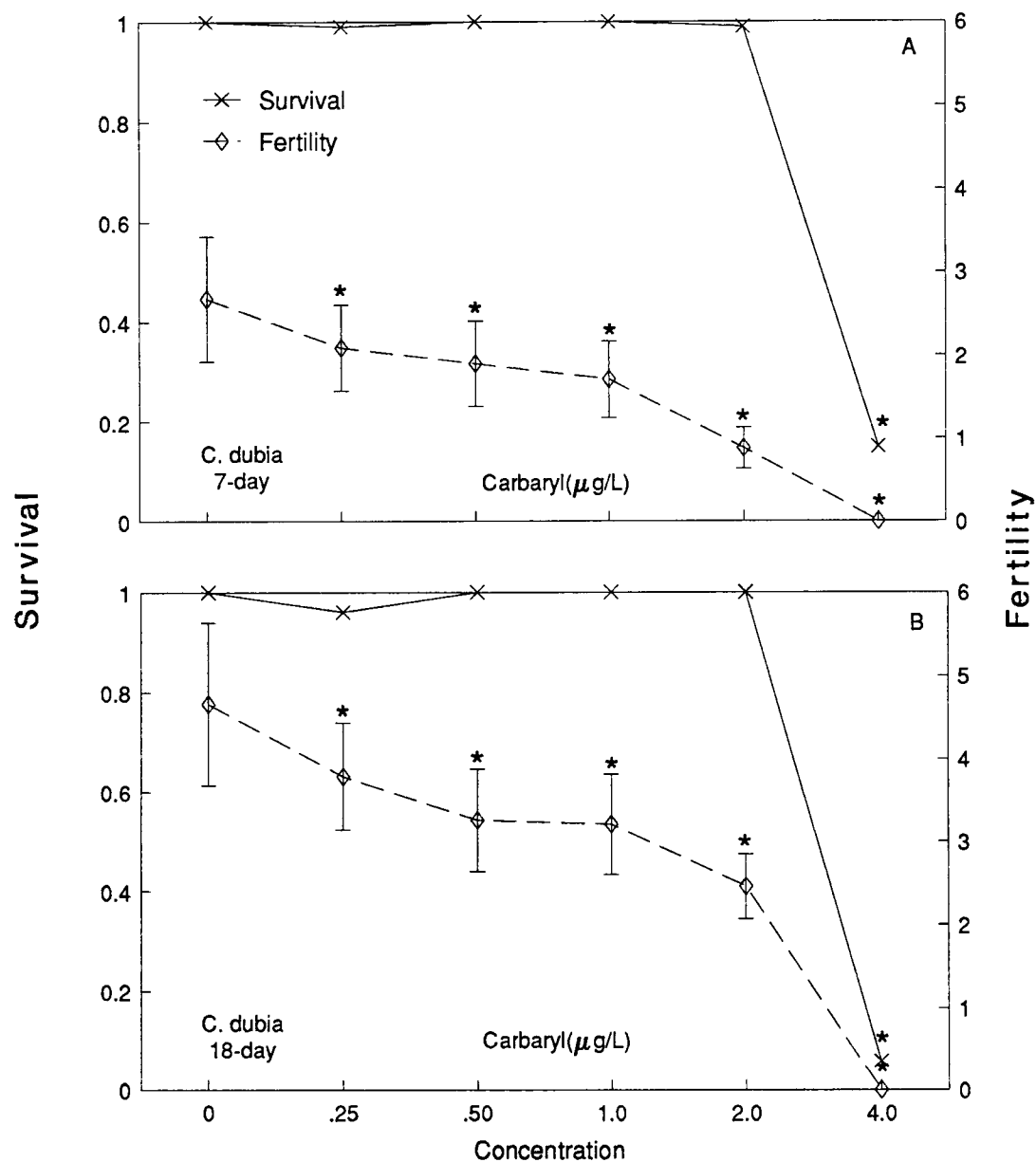


Figure II-7. Survival and fertility of *Ceriodaphnia dubia* exposed to carbaryl for 7-d (panel A) and 18-d (panel B). Significantly different (*) from control ($\pm$ SE); $n = 20$.

reproduction was significantly less at all exposure concentrations ($F = 46.60$, $P < 0.0001$) (Fig. II-8A and II-8B). The 14-d and 18-d carbaryl exposures delayed the time of the first brood release by 2- to 4-d, which resulted in a significant reduction in number of broods at $1.0 \mu\text{g/L}$ ($F = 28.93$, $P < 0.0001$) and decreased brood sizes at $0.05 \mu\text{g/L}$ ($F = 19.54$, $P < 0.0001$) (Table II-5). Although the carbaryl concentrations to which D. magna were exposed differed slightly from the C. dubia exposure concentrations, the response of D. magna was remarkably similar to C. dubia (Fig. II-7 and II-8).

Chemical effects on endpoint sensitivity. Results of the more sensitive endpoints for each species exposed to each chemical are summarized in Table II-7 as NOEC and LOEC limits. For cadmium, 7-d and 18-d exposures of C. dubia, survival and reproduction were equally sensitive endpoints, with an NOEC of $3.0 \mu\text{g/L}$ and LOEC of $6.0 \mu\text{g/L}$ (Table II-7). For D. magna 14-d exposure to cadmium, reproduction was more sensitive than survival, with LOEC of $1.5 \mu\text{g/L}$. Survival and reproduction were equally sensitive, at 18-d exposures with an LOEC of $3.0 \mu\text{g/L}$.

For PCP C. dubia 7-d exposures, survival and reproduction were not significantly different from the controls; therefore, the NOEC and LOEC limits were not determined. However, at 18-d, reproduction was more

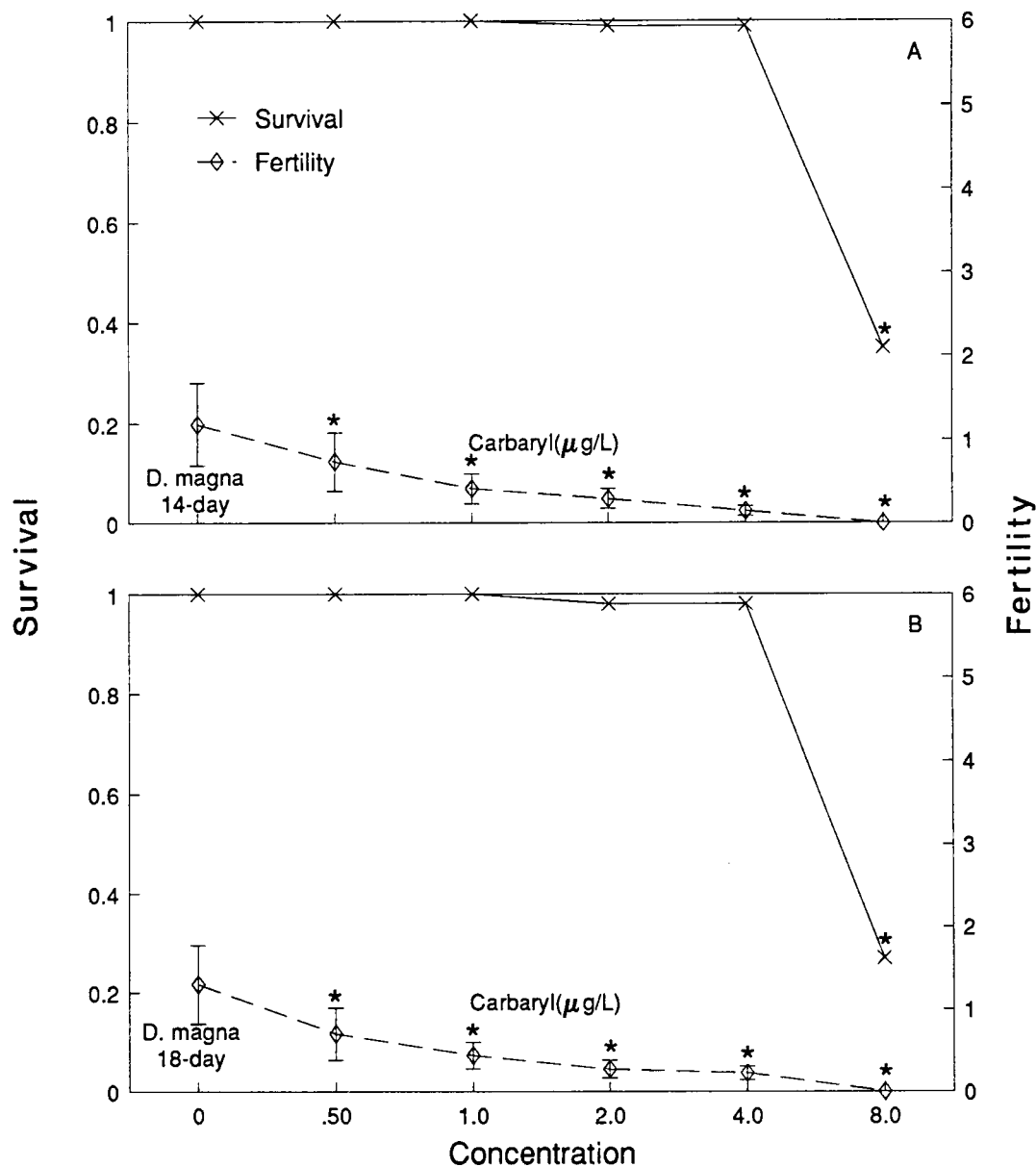


Figure II-8. Survival and fertility of *Daphnia magna* exposed to carbaryl for 14-d (panel A) and 18-d (panel B). Significantly different (*) from control ($\pm$ SE); n = 20.

Table II-7. Endpoint sensitivity as determined by NOEC and LOEC limits for Ceriodaphnia dubia and Daphnia magna exposures to four chemicals having different modes of action. S = survival; R = reproduction.

Species	Toxicant	Test duration(d)	Endpoint	Limits ($\mu\text{g/L}$)	
				NOEC	LOEC
<u>C. dubia</u>	Cadmium	7	S	3.0	6.0
			R	3.0	6.0
		18	S	3.0	6.0
			R	3.0	6.0
<u>D. magna</u>	Cadmium	14	S	1.5	3
			R	<1.5	1.5
		18	S	1.5	3.0
			R	1.5	3.0
<u>C. dubia</u>	PCP	7	S	<4.0	>60
			R	<4.0	>60
		18	S	4.0	8.0
			R	<4.0	4.0
<u>D. magna</u>	PCP	14	S	60	120
			R	<15	15
		18	S	60	120
			R	<15	15
<u>C. dubia</u>	1-Octanol	7	S	4550	9100
			R	569	1138
		18	S	1138	2275
			R	2275	4550
<u>D. magna</u>	1-Octanol	14	S	1531	3062
			R	6125	12250
		18	S	1531	3062
			R	6125	12250
<u>C. dubia</u>	Carbaryl	7	S	2.0	4.0
			R	<.25	.25
		18	S	2.0	4.0
			R	<.25	.25
<u>D. magna</u>	Carbaryl	14	S	4.0	8.0
			R	<.50	.50
		18	S	4.0	8.0
			R	<.50	.50

sensitive than survival. The LOEC was 4.0 $\mu\text{g/L}$.

Reproduction was the more sensitive endpoint for D. magna exposed to PCP, with an LOEC of 15 $\mu\text{g/L}$ for both endpoints (Table II-7).

For 1-octanol, C. dubia reproduction was the more sensitive endpoint after the 7-d exposure (LOEC of 1138 $\mu\text{g/L}$), but survival was more sensitive at 18-d (LOEC of 2275 $\mu\text{g/L}$). For D. magna 14-d and 18-d exposures, survival was the more sensitive endpoint. The LOEC was 3063 $\mu\text{g/L}$.

Carbaryl exposures indicated that reproduction was the more sensitive endpoint for both C. dubia and D. magna after 7 or 14-d and 18-d exposures (Table II-7).

The sensitivity of the two species varied depending upon the endpoint (Table II-7). Considering the LOEC for survival and reproduction separately, D. magna was more sensitive to cadmium (both endpoints) than C. dubia. For all other chemicals, C. dubia was the more sensitive species.

Discussion

Acute Toxicity. The LC_{50} values estimated in this study varies from those reported by other investigators. This is probably due to differences in test conditions

among laboratories. Acute toxicity data from this study and other studies are compared in Appendix B-2. Water hardness, pH, and temperature data included in the table ranged from 32 to 240 mg/L as CaCO₃, 7.4 to 8.6, and 16 to 25 °C, respectively. The range of LC₅₀ values was less for cadmium and PCP exposures to Ceriodaphnia spp. (32 to 184 µg/L and 18 to 900 µg/L, respectively) than for D. magna exposures to these chemicals (26 to 320 µg/L and 143 to 1050 µg/L, respectively. Kuhn, et al., (1989) reported a 24-h EC₅₀ for D. magna exposed to 1-octanol of 260 µg/L. I found C. dubia to be three times more sensitive to 1-octanol than D. magna. The acute toxicity of carbaryl obtained here agrees well with the LC₅₀ values reported in other studies with cladocerans.

While the acute bioassays were conducted to set the levels for chronic exposure concentrations, the acute results showed that C. dubia was more sensitive than D. magna to all four test chemicals (Table II-4). This agrees with the results obtained by Cowgill, et al. (1985) when the same two species were exposed to four organic chemicals.

In my study, the toxic order of the chemicals were carbaryl > PCP > cadmium > 1-octanol for C. dubia, and carbaryl > PCP = cadmium > 1-octanol for D. magna.

Chronic Toxicity. The chronic toxicity tests demonstrated that the current chronic data interpretations may be misleading due to the consideration of two separate endpoints (survival and reproduction), and that when reproductive parameters other than the total number of young produced are measured, the data is even more difficult to interpret.

Chronic studies that measure survival and reproduction are too numerous to report; however, the sensitive chronic toxicity endpoints for some species of cladocerans (Appendix B-2.1) indicate that decisions concerning water quality regulations and risk assessment procedures would benefit from endpoint integration when using chronic data to predict environmental effects of chemicals.

Species Sensitivities. Perhaps the most important generalization to be drawn from this study is that C. dubia appears to be more sensitive than D. magna to chemicals having different modes of action. This result was true of all chronic experiments except those involving cadmium, for which D. magna was more sensitive. Consequently, the results do not support the null hypothesis of no difference in the sensitivity between the two species.

While this study was not designed to identify specific morphological and physiological species differences, the most obvious reason for differences in species sensitivity relates to difference in body size. Every aspect of an animal's life is influenced by its body size -- its interactions with the abiotic environment, its encounters with other organisms, and the rate of its physiological processes (Naganuma and Roughgarden, 1990). The length of an adult Ceriodaphnia is about 0.88 mm (Berner, 1986); an adult D. magna is 4.5 mm (Goulden, et al., 1982a). This difference in size can be associated with the amount of energy reserved as lipid in the adult bodies, the surface-to-volume ratio, and metabolic activities.

The amount of maternal lipid carried by a neonate increases with the body size of a species (Goulden, et al., 1982a). This implies that C. dubia neonate energy reserves were less than those of D. magna at the beginning of a test. Thus, C. dubia put its energy into maintenance rather than reproduction, which could explain its greater sensitivity.

The generally accepted inverse relationship between body size and metabolic rate of an organism has been demonstrated in studies with D. magna (MacArther and Baillie, 1929 and Buikema, 1972). The metabolic rate

decreased with increasing size of D. magna. Additionally, C. dubia has a greater surface-to-volume ratio than D. magna, so the rate of uptake of a chemical was faster. Unless the rate of depuration during the exposure period exceeded the net bioaccumulation rate, then the amount of chemical in the target tissue of C. dubia most likely was greater than that in the target tissue of D. magna. Both of these factors can explain why C. dubia was the more sensitive species.

For cadmium exposures where D. magna was more sensitive, the sensitivity may be explained by adsorption of cadmium to food particles and food-size selection of the cladocerans. In a study of cadmium toxicity in highly organic pond water, Giesy, et al., (1977) noted the ability of organics to complex cadmium. Studies of food-size selection in cladocerans (Geller and Muller, 1981; Gophen and Geller, 1984; Hessen, 1985) have shown that morphological differences in filtering appendages account for the size of particle ingested by an animal. The area of the filtering combs of the thoracic limbs is among the factors influencing the rate of filtering of Daphnia (Arruda, 1983). Total filtering comb area has been related to daphnid body length. Porter, et al., (1983) studied the morphology and filtering rates of Daphnia and Ceriodaphnia fed natural bacteria. They examined the

morphological differences between the species by measuring the structural units of the filtering combs on the third thoracic appendage, including the intersetular distance, i.e., the distance between the setae and setules on the filtering combs. Intersetular distance for Ceriodaphnia (0.25 μm) was one fourth that for D. magna (1.00 μm). Thus, the size of particles ingested by C. dubia are smaller than the size of particles ingested by D. magna. The affinity of cadmium for the algae and the TCY particles and the selection of larger food particles by D. magna could have increased cadmium accumulation and toxicity to the animal.

Another explanation for the greater sensitivity of D. magna exposed to cadmium relates to growth of the organisms. While growth was not an endpoint measured during this study, there was evidence of cadmium effects on growth of D. magna. Animals were small and pale in color. In chronic studies, Geiger, et al., (1980) measured growth as an endpoint, and found that the prereproductive body length of D. pulex was a good indicator of the number of young produced in 21-d. The small size of the exposed D. magna can explain why the number of broods and brood sizes were low during cadmium exposures, and why D. magna was more sensitive to cadmium than C. dubia.

Endpoint Sensitivity. In terms of adult and embryo/neonate mortality, the exposure of the two species to cadmium lead to similar results. Greater than 50% adult mortality occurred for both species after exposure to 13 $\mu\text{g/L}$ cadmium. This suggests that the effects of cadmium on the first instar stages of the two species were similar. This was probably due to the susceptibility of the species during molting. Lee and Buikema (1979) showed that the susceptibility of Daphnia to toxicants varies with the molt cycle and that the susceptibility increases around the molt stage. During molting two activities could have occurred; first, cadmium interfered with the molt cycle, and second cadmium bioaccumulation was greater during the molt stage. Either activity could result in mortality.

Measurements of the reproductive parameters (Table II-5) show variation for all chemical exposures and identifies the complications associated with endpoint selection for setting regulation. Except for time to the first brood release, the reproductive parameters differed for subchronic and chronic exposures and for each species exposed to a particular chemical. The time to first brood release did not change because the data were collected for the chronic duration, then truncated and analyzed at the subchronic durations. For C. dubia exposures, brood size

was usually the more sensitive parameter, whereas the sensitivity of the parameters during the D. magna exposures differed depending on the test chemical.

The survival and reproduction data, as measured by NOEC and LOEC limits (Table II-7) shows that survival was never the more sensitive endpoint for both species exposed to a particular chemical, but reproduction was more sensitive for both species with exposure to PCP at 18-d and with exposure to carbaryl at both exposure durations. These results partially support the hypothesis of no difference in the sensitivity of an endpoint for both species exposed to the same toxicant.

Species Response at Different Test Durations. The results for different test durations showed that survival and reproduction, measured at 7-d, 14-d and 18-d, were similar for the two species (Figures II-1 to 8). However, when the sensitivity of the endpoints were evaluated statistically, some differences were apparent. Based on the LOEC's, C. dubia 7-d and 18-d exposures to PCP and 1-octanol differed, as did D. magna exposures to cadmium. The difference in C. dubia 7-d and 18-d exposures to PCP was 8-fold for survival and 15-fold for reproduction. This finding suggests that observations of significant effects on C. dubia survival and reproduction after exposure to PCP would require exposure periods longer than

7-d. The C. dubia 1-octanol exposures differed 4-fold for both endpoints. However, use of the 7-d test and the more sensitive endpoint would probably be sufficient to protect the organism. The same is true for D. magna 14-d exposures to cadmium.

Although there were slight differences in the sensitivities of the endpoints, this study supports the use of the 7-d C. dubia and 14-d D. magna chronic tests. Since statistical differences in toxicological data are used for regulatory purposes and the regulations are based on the most sensitive endpoint, the differences observed here are not large enough to discredit these test durations.

Conclusions concerning the objectives of this study are as follows:

- (1) the two cladoceran species have similar sensitivities to the chemicals used in these tests,
- (2) subchronic tests using C. dubia or D. magna can be used to reliably predict chronic effect, and
- (3) neither survival nor reproduction is consistently the more sensitive endpoint during chronic toxicity tests. This finding suggests and supports the use of an integrated chronic toxicity endpoint for the management of environmentally hazardous chemicals in aquatic ecosystems.

CHAPTER III

INTEGRATION OF CHRONIC TOXICITY ENDPOINTS INTO AN ECOLOGICALLY RELEVANT VARIABLE

Introduction

Accurate predictions of the sublethal effects of chemicals on populations within ecosystems are not currently possible; however, regulators and managers must address this problem in aquatic environments based upon available ecological information and toxicological tools. At its simplest level, this involves the quantification of biological responses of organisms in relation to concentrations of chemicals for designated exposure durations (Gentile, et al., 1982). Initially, management decisions were based on acute toxicity data using lethality as the endpoint. Later it became apparent that chronic exposure to sublethal concentrations could affect an organism's growth and reproduction without causing death. Toxicologists agree that data from chronic studies are essential to the development of predictive toxicology (Buikema, et al., 1982; Kenaga, 1982; Gentile, et al., 1982; and Francis, et al., 1986). Current regulatory approaches have attempted to combine acute and chronic

toxicity data into a management tool. While this approach is integrative, it does not allow predictions of the potential consequences at the population level.

When estimating chronic toxicity of water to cladocerans, both survivorship and fecundity data are obtained. These data can be evaluated in terms of potential population growth rates. Separate measures of survival and fecundity may be integrated and used to estimate the intrinsic rate of natural increase, r , based on the Lotka (1925) equation:

$$\sum l_x m_x e^{-rx} = 1 \quad (\text{III-1})$$

where l_x is the probability of a female surviving to age x , m_x (fertility) is the number of female offspring per female of age x produced during the interval x to $x+1$, e is the exponential constant (2.71828), r is the intrinsic rate of natural increase, and x is the age interval (Keyfitz, 1968 and Krebs, 1978).

Stephan (1989) stated that the use of r as a chronic endpoint is a step in the right direction because r : (1) provides a direct link between aquatic toxicology and population biology by increasing the ability of laboratory tests to provide information that is ecologically relevant, (2) provides a more objective basis for

determining the appropriate duration of chronic toxicity tests in terms of survival and fecundity data, (3) decreases the concern for the kind of reproductive data to analyze (e.g., total number of young, mean brood sizes, or mean number of broods), and (4) resolves the questions about which endpoint (survival or reproduction) to consider for risk assessment. Instead of basing decisions on the lowest concentrations producing a significant impairment of the most sensitive endpoint, calculation of r combines and integrates the data for survival and fecundity and generates one endpoint.

The use of r to assess the chronic effects of chemicals on populations has been investigated (Marshall, 1962; Hummon, 1974; Winner and Farrell, 1976; Bertram and Hart, 1979; Hatakeyama and Yasuno, 1981; Daniels and Allen, 1981; Walton, et al., 1982; Gentile, et al., 1982, 1983; and van Leeuwen, et al., 1985, 1987; Bodar, et al., 1988; Chandini, 1989). Although r has been used to assess chronic data, only recently have attempts been made to estimate the uncertainty (variance) associated with the calculations of r (Lenski and Service, 1982; Rago and Dorazio, 1984; Meyer, et al., 1986; Barbour, et al., 1989) without performing duplicate tests.

Regulatory decisions in environmental toxicology require objective statistical criteria to evaluate the

potential population level effects of human activities. Therefore, it is important to develop and test procedures for estimating variance associated with population growth rates determined from toxicity tests so that levels of confidence can be assigned to observed differences (Meyer, et al., 1986). Currently, computer-intensive procedures for estimating variance are popular. Lenski and Service (1982) used the statistical procedure, jackknifing, to calculate finite growth rates and their standard errors for populations of aphid. Survival and reproduction for a cohort of 18 aphids, Uroleucon rudbeckiae, was observed daily. The authors concluded that jackknifing could be used to reliably estimate the variance in finite growth rates; for this purpose, they recommended that large cohorts be used to assure normal-based sample distributions.

Rago and Dorazio (1984) suggested that normal-based statistical procedures for comparing experimental estimates of the finite growth rate may be misleading when observed mortality produces skewed distributions of the finite growth rate. To address this concern, they used life-table data from Daphnia pulex cohorts and a procedure for pairwise comparison of sampling distributions to test for significant differences among confidence intervals. They compared the results of this analysis to a Monte

Carlo simulation, and recommended the pairwise comparison method over the jackknife procedure (Lenski and Service, 1982).

Another computer-intensive technique, bootstrapping, has been used to estimate variance about a statistic (Diaconis and Efron, 1983 and Meyer, et al., 1986). This procedure uses the data of a single sample. Meyer, et al.(1986) examined estimates of variance in r by comparing the jackknife and bootstrap techniques. For this, they used data from laboratory cohorts of D. pulex (real and hypothetical) populations. The authors concluded that the jackknife and the bootstrap techniques estimated the variance in r for D. pulex populations with about equal levels of success.

With the goal of improving current risk assessment approaches to include measurements of population effects from chronic bioassays, the primary objective of this study was to integrate chronic toxicity endpoints of the Ceriodaphnia and Daphnia tests into a population measurement, r , for use as an index for environmental hazard assessment. The calculations of r involved integrating survival and reproduction for all organisms within a treatment. The variance in r was then calculated using a bootstrap technique. An estimate of r and its associated variance was calculated for 7 or 14-d

(subchronic) and 18-d (chronic) exposure durations for C. dubia and D. magna. Two null hypotheses were formulated and tested: (1) there is no difference in r measured at 7-d or 14-d and 18-d exposure durations, and (2) there is no difference in the sensitivity between r and the separate chronic endpoints (survival and reproduction). The ecological variable, r , can be used as a chronic endpoint during daphnid toxicity testing (Daniels and Allan, 1981), and the bootstrap estimates of the variance in r should permit hypothesis testing to determine significant difference between control and chemical concentrations.

Materials and Methods

Cladoceran Chronic Toxicity Tests

Survival and reproduction data for C. dubia and D. magna were taken from the laboratory studies on the toxic effects of cadmium, PCP, 1-octanol, and carbaryl (Chapter II). The data were reanalyzed by (1) calculating the intrinsic rate of natural increase (r) with estimates of the variance associated with it, using a bootstrap statistical technique, (2) determining the significance level for the estimates of r for the control and the exposed groups within each test, and (3) comparing the significance levels of r (the integrated endpoint) with the

significance levels of survival and reproduction considered separately (Chapter II) for each species and each chemical at subchronic and chronic exposures.

In these studies, tests were initiated with neonates (12 ± 12 -h old) and the animals were observed for 18-d. The animals were maintained individually in separate vials that contained 30 mL of test media or well/spring water. The temperature was maintained at $23 \pm 1^\circ\text{C}$ under a 16-h light/8-h dark photoperiod. Survival and reproduction of 20 females per chemical concentration were observed daily. The new offspring were removed from the vials each day, and the adults were transferred to fresh test media every other day.

Survival and reproduction data for the control and the exposed cohorts were summarized in life-table/fertility-table form for 7 or 14-d and 18-d exposure durations. Both survivorship and fertility are included in the same table, which is hereafter referred to as a life-table. The r values were calculated from the life-table data.

The chronic toxicity data required to construct a life-table (Appendix C-1) were age interval in days (x), the proportion of organisms surviving to the start of age interval x (l_x), the number of offspring per female aged x per day (m_x), and the product of l_x and m_x . The other information in the appendix (e.g., number of animals,

number of survivors, and reproduction (number of young per female per day)) were obtained directly from the chronic experiments; they were necessary for the calculations of the life-table parameters.

A bootstrap statistical procedure was used to calculate an estimate of the variance associated with r to test the hypothesis that r is an endpoint that is as sensitive as survival and reproduction for chronic toxicity data.

Intrinsic Rate of Natural Increase Calculations

The intrinsic rates of natural increase were calculated according to the Lotka (1925) equation (Eq III-1). This calculation involves a summation over several age classes; therefore, r cannot be isolated to one side of the equation to provide a closed-form algebraic solution (Meyer, et al., 1986). Iterative calculations were performed to determine an r value that satisfied Eq III-1. To begin computation for a data set, an approximation of r was calculated using the equations given by Ricklefs (1979:423-445):

$$r_1 = \frac{\ln R_0}{T_c} \quad (\text{III-2})$$

where R_o is the net reproductive rate (i.e., the number of offspring that an individual may be expected to produce during its lifetime), and is mathematically expressed as:

$$R_o = \sum l_x m_x \quad (\text{III-3})$$

and T_c is the mean generation time (i.e., the time required for a population to increase by the factor R_o); the mathematical notation is:

$$T_c = \frac{\sum x l_x m_x}{R_o} \quad (\text{III-4})$$

After calculating the approximate r values, I refined the estimates of r until the value on the left side of Eq III-1 was equal to 1.00. The steps for the calculation of r (Table III-1) were done following the procedures of Tanner (1978). The general scheme began by using the chronic toxicity data (step 1; columns 1, 2, and 3 of Table III-1) to calculate the products in step 2. Using the products, a calculation was made of the first trial estimate of r (r_1) (Eq III-2). The values for column 6 were calculated using the r_1 value. Then each product in column 7 and 8 were calculated and summed. If the sum of column 7 equalled 1.00, the r value used in column 6 was assigned as the r value for the cohort at that

Table III-1. The procedures for calculating r from the chronic bioassay data. (Source: Tanner, 1978)

Step 1			Step 2		Step 3	Step 4	Step 5	
1	2	3	4	5	----- $\Sigma l_x m_x$	6	7	8
x	l_x	m_x	$l_x m_x$	$x l_x m_x$	$\Sigma x l_x m_x$	e^{-rx}	$l_x m_x e^{-rx}$	$x l_x m_x e^{-rx}$
0	1	0	0	0		1	0	0
1	l_1	m_1	$l_1 m_1$	$1 l_1 m_1$		e^{-r}	$l_1 m_1 e^{-r}$	$1 l_1 m_1 e^{-r}$
2	l_2	m_2	$l_2 m_2$	$2 l_2 m_2$		e^{-2r}	$l_2 m_2 e^{-2r}$	$2 l_2 m_2 e^{-2r}$
3	l_3	m_3	$l_3 m_3$	$3 l_3 m_3$		e^{-3r}	$l_3 m_3 e^{-3r}$	$3 l_3 m_3 e^{-3r}$
.	.	.	.	.		.	.	.
.	.	.	.	.		.	.	.
.	.	.	.	.		.	.	.
n	l_n	m_n	$l_n m_n$	$n l_n m_n$		e^{-nr}	$l_n m_n e^{-nr}$	$n l_n m_n e^{-nr}$
			$\Sigma l_x m_x$	$\Sigma x l_x m_x$			$\Sigma l_x m_x e^{-rx}$	$\Sigma x l_x m_x e^{-rx}$

concentration. When the sum of column 7 did not equal 1.00, a second approximation of r (r_2) was calculated:

$$r_2 = r_1 + \ln \Sigma 7 \frac{\Sigma 7}{\Sigma 8} \quad (\text{III-5})$$

Steps 6 to 8 were repeated until the sum of the values in column 7 equalled 1.00. Before performing the bootstrap calculations, r for an original data set was computed.

Bootstrap Calculations

The bootstrap is a statistical technique that uses random sampling with replacement to mimic the original data set (Diaconis and Efron, 1983). The bootstrap samples are generated by organizing a data set into a matrix of values, with rows representing cases and columns representing variables. The bootstrap samples (replicates) are constructed by drawing at random, with replacement, entire rows from the matrix of values (Noreen, 1989).

The bootstrap program, rBOOT, (Appendix C-2) is a modification of the procedures of Meyer, et al. (1986). Briefly, the procedure involves: (1) recombining the original data, (2) calculating pseudo-values of r , and (3) estimating the mean and standard error of r from the frequency distribution of pseudo-values (Meyer, et al., 1986). The rBoot program, developed in this study, is

written in Fortran (Appendix C-2) and contains Statistical Analysis System (SAS) algorithms (Appendix C-2.1). It includes a simplified data input format to create a more user-friendly interface to facilitate the integration of standard chronic endpoints without additional data manipulation. The general format for a data set (Table III-2) contains the information obtained from chronic toxicity testing. The program allows specification of a maximum of 20 animals and 26-d of exposure. The number of animals or the number of days of exposure can be specified in the initialization statements. Survival (l_x) and reproduction (m_x) are calculated for each experimental cohort, and from these calculations an estimate of r is calculated, using the previously described iterative procedure. In cases where survival and reproduction were zero for an individual, the program calculates the innate capacity for increase (Krebs, 1978). The innate capacity for increase is defined as the exponential growth of a population under specified physical conditions, in an unlimited environment where density effects are not considered (Birch, 1948). Here, I assume that all animals respond instantaneously to the changes in chemical concentrations over time and that the rate of change of the population size is a function of the present population. This is expressed by the equation (Krebs, 1978; p. 162):

Table III-2. Example of the chronic toxicity data format used for calculating r with rBOOT. Conc = concentration

Conc	Animal	Reproduction/Day/Female											Last Day Alive
		1	2	3	4	5	6	7	.	.	.	.	n (days)
0	1												7
0	2												7
.	3												5
.	4												2
.	5												7
.	6												.
.	7												.
.	8												.
.	9												.
.	10												.
.	.												.
.	.												.
.	.												.
.	.												.
0	n												7

$$N_t = N_0 e^{-rt} \quad (\text{III-6})$$

where N_t is the number of individuals at time t , N_0 is the number of individuals at time 0, e is a constant (2.71828), r is the innate capacity for increase, and t is time. In this equation, all parameters were known except r . To calculate r , Eq (III-6) was rearranged:

$$r = \frac{\log_e(N_t) - \log_e(N_0)}{t} \quad (\text{III-7})$$

In this study, the bootstrap samples were generated by placing the original data set of 20 animals into a matrix of values (pool). The rows represented individuals and the columns contained the survival and reproduction data through time. From the pool, an individual was randomly selected with replacement (i.e., placed back into the pool before another animal was drawn). This allowed any animal of the data set to be chosen more than once or not at all in the bootstrap replicate. When 20 replicates had been chosen in this fashion, an estimate of r was calculated for this random recombination of the original data.

The precision of a bootstrap estimate depends on the number of times the original data are randomly recombined (Meyer, et al., 1986). Random sampling and a computation

of r were iteratively conducted 1000 times for each concentration of each toxicant. The mean and the standard error of r ($SE(r)$) were then calculated for each chemical concentration by issuing a SASbatch command (Appendix C-2.1). The bootstrap procedure was applied to each data set for 7- or 14-d and 18-d exposures of each species to a particular chemical. The value of r was plotted against exposure concentrations. The criteria for the interpretation of r values was that for $r < 0$ the population was decreasing, for $r = 0$ the population was replacing itself, and for $r > 0$ the population was increasing (Gentile, et al., 1982).

Determination of Significance Levels

Since r and $SE(r)$ were computed for cohorts and not for individuals, an analysis of variance (ANOVA) followed by Dunnett's procedure (Chapter II) could not be used to estimate differences between control and exposed r values. Instead, each concentration was compared relative to the control by computing a pooled standard error from the control and exposed $SE(r)$ values in a test (Meyer, et al., 1987):

$$SE(r)_p = \sqrt{\frac{1}{k} (\sum SE(r)^2)} \quad (III-8)$$

where k is the number of control and exposed levels tested, and p is the pooled standard errors. Pairwise comparisons were then computed (Meyer, et al., 1987) as:

$$t = \frac{r_c - r_e}{\sqrt{2} SE(r)_p} \quad (\text{III-9})$$

where r_c is the estimate of the control r , and r_e is the estimate of the exposed r . The calculated value of t was compared with the one-tailed value of Dunnett's t at $\alpha = 0.05$ to determine significant differences between control and exposed animals. Also, the distribution of r values (90% quantile limits) were examined by excluding the extreme 5% values at the lower and upper end of the distribution curve. The mean r values and the 90% quantile limits were plotted against exposure concentration.

The NOEC and LOEC for r values were determined based on significant differences as indicated by Dunnett's t test. The LOEC values for r were then compared with the LOEC values for survival and reproduction (Chapter II). Finally, the sensitivity of r at the lowest LOECs were used to clarify the interpretations of the chronic toxicity data.

Results

Intrinsic rates of natural increase

Estimates of r by the bootstrap simulations resulted in a measure of the variance associated with r . The variance in r , expressed as standard error (SE), was small due to the large number (1000) of bootstrap samples; therefore, the 90% quantile limits on r values provided a statistical picture of the estimated differences in r .

Cadmium. Mean r values and the 90% quantile limits of r for *C. dubia* 7-d and 18-d exposures are shown in Figure III-1. The intrinsic rate of natural increase decreased with increasing cadmium concentrations. The r values for the control, 1.5, 3.0, and 6.0 $\mu\text{g/L}$ concentrations of cadmium were all positive, which indicated an increasing population, while the values for r in the 13 and 25 $\mu\text{g/L}$ concentrations of cadmium decreased to -0.179 and -0.290, respectively (Table III-3). This characterized a population with almost no reproductive potential, which would most likely be eliminated with continuous exposure at these concentrations. Variation associated with r was greater at the higher concentrations of cadmium when survival was low and reproduction approached zero.

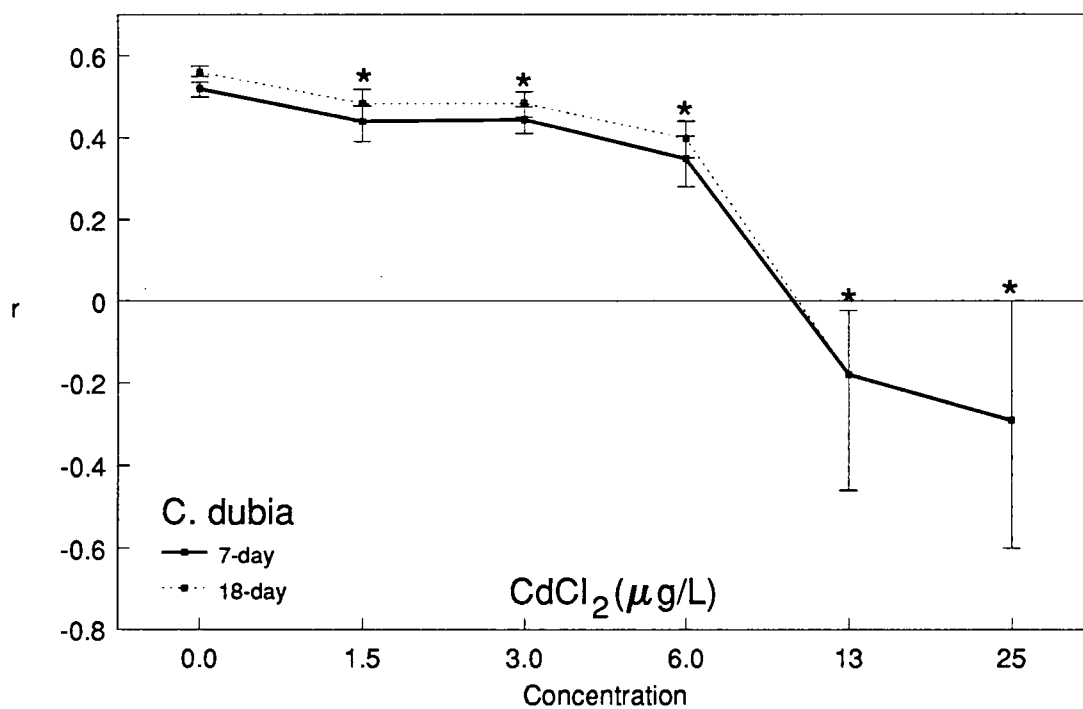


Figure III-1. Mean r values and concentrations of cadmium for 7- and 18-d *Ceriodaphnia dubia* tests. Significant (*) at $\alpha = 0.05$.

Table III-3. Mean r values ($\pm$ SE) and 90% quantile limits for Ceriodaphnia dubia and Daphnia magna exposed to cadmium, pentachlorophenol, 1-octanol, and carbaryl. Means and SE values are for 1000 bootstrap replicates.

Species	Toxicant	Test duration(d)	Toxicant Concentration (μ g/L)	r Values ($\pm$ SE)	90% quantile limits	
					Lower	Upper
<u>C. dubia</u>	Cadmium	7	0	.520 (0.00)	.504	.536
			1.5	.440 (.001)	.393	.478
			3.0	.444 (.001)	.406	.475
			6.0	.348 (.001)	.282	.403
			13	-.179 (.004)	-.461	-.024
			25	-.290 (.005)	-.599	0
		18	0	.560 (0.00)	.546	.575
			1.5	.484 (.001)	.444	.518
			3.0	.484 (.001)	.452	.512
			6.0	.397 (.001)	.346	.440
			13	-.179 (.004)	-.461	-.024
			25	-.290 (.005)	-.599	0
<u>D. magna</u>	Cadmium	14	0	.322 (0.00)	.306	.337
			1.5	.325 (0.00)	.309	.339
			3.0	.244 (.001)	.215	.269
			6.0	-.037 (.005)	-.230	.072
			13	--	--	--
			25	--	--	--
		18	0	.325 (0.00)	.310	.340
			1.5	.325 (0.00)	.310	.340
			3.0	.244 (.001)	.215	.269
			6.0	-.037 (.005)	-.230	.072
			13	--	--	--
			25	--	--	--

Table III-3 (Continued).

Species	Toxicant	Test duration(d)	Toxicant Concentration ($\mu\text{g/L}$)	r Values ($\pm\text{SE}$)	90% quantile limits	
					Lower	Upper
<u>C. dubia</u>	PCP	7	0	.625 (0.00)	.603	.644
			4.0	.648 (0.00)	.626	.670
			8.0	.562 (.001)	.527	.592
			15	.561 (.001)	.525	.594
			30	.552 (.001)	.527	.575
			60	.532 (.001)	.496	.560
		18	0	.651 (0.00)	.631	.668
			4.0	.668 (0.00)	.650	.686
			8.0	.591 (.001)	.560	.618
			15	.593 (.001)	.562	.621
			30	.585 (0.00)	.564	.603
<u>D. magna</u>	PCP	14	60	.563 (.001)	.532	.589
			0	.274 (0.00)	.262	.286
			15	.149 (.001)	.111	.183
			30	.021 (.002)	-.079	.098
			60	.097 (.001)	.033	.147
			120	-.095 (.002)	-.211	-.017
			239	-.046 (.002)	-.136	.022
		18	0	.284 (0.00)	.273	.294
			15	.165 (.001)	.136	.190
			30	.109 (.001)	.072	.144
			60	.129 (.001)	.090	.165
			120	-.053 (.001)	-.119	0
			239	-.046 (.002)	-.136	.022

Table III-3 (Continued).

Species	Toxicant	Test duration(d)	Toxicant Concentration ($\mu\text{g/L}$)	r Values ($\pm\text{SE}$)	90% quantile limits	
					Lower	Upper
<u>C. dubia</u>	1-Octanol	7	0	.467 (0.00)	.451	.483
			569	.465 (0.00)	.445	.485
			1138	.438 (0.00)	.415	.457
			2275	.426 (0.00)	.401	.447
			4550	.344 (0.00)	.318	.367
			9100	-.971 (.023)	-1.60	-.474
		18	0	.530 (0.00)	.522	.537
			569	.531 (0.00)	.522	.541
			1138	.514 (0.00)	.496	.527
			2275	.505 (0.00)	.493	.515
			4550	.432 (0.00)	.419	.445
			9100	-.032 (.001)	-.130	.038
<u>D. magna</u>	1-Octanol	14	0	.253 (0.00)	.237	.266
			1531	.264 (0.00)	.243	.281
			3062	.227 (.001)	.201	.250
			6125	.130 (.001)	.097	.158
			12250	--	--	--
			24500	--	--	--
		18	0	.275 (0.00)	.263	.284
			1531	.273 (0.00)	.257	.288
			3062	.246 (0.00)	.224	.265
			6125	.180 (0.00)	.156	.201
			12250	--	--	--
			24500	--	--	--

Table III-3 (Continued).

Species	Toxicant	Test duration(d)	Toxicant Concentration ($\mu\text{g/L}$)	r Values ($\pm\text{SE}$)	90% quantile limits	
					Lower	Upper
<u>C. dubia</u>	Carbaryl	7	0	.603 (.001)	.553	.649
			0.25	.495 (.001)	.442	.556
			0.50	.453 (0.00)	.431	.472
			1.0	.435 (0.00)	.415	.455
			2.0	.309 (0.00)	.285	.329
			4.0	--	--	--
		18	0	.632 (.001)	.595	.669
			0.25	.552 (.001)	.515	.590
			0.50	.511 (0.00)	.489	.534
			1.0	.496 (0.00)	.476	.514
			2.0	.404 (0.00)	.391	.417
			4.0	--	--	--
<u>D. magna</u>	Carbaryl	14	0	.241 (0.00)	.234	.246
			0.5	.190 (0.00)	.179	.201
			1.0	.141 (0.00)	.124	.158
			2.0	.116 (.001)	.091	.138
			4.0	.051 (.001)	.024	.074
			8.0	--	--	--
		18	0	.241 (0.00)	.234	.246
			0.5	.190 (0.00)	.179	.201
			1.0	.141 (0.00)	.124	.158
			2.0	.116 (.001)	.091	.138
			4.0	.051 (.001)	.024	.074
			8.0	--	--	--

A decrease in r values for populations of C. dubia at higher concentrations of cadmium (compared pairwise to the control (Meyer, et al., 1987); Dunnett's t at $\alpha = 0.05$ (Zar, 1984), p. 194)) was detected for all cadmium 7-d and 18-d exposures ($t = 11.43$ and 12.7 , respectively). The calculated LOEC for r was $1.5 \mu\text{g/L}$ (Table III-4).

For D. magna, estimates of r for 14-d and 18-d exposures to cadmium are shown in Figure III-2 and Table III-3. Estimates of r did not differ for the two exposure durations. I found no significant difference between the control and the $1.5 \mu\text{g/L}$ concentrations; the 90% quantile limits for r at these two concentrations overlapped. Mean values of r decreased as cadmium concentrations exceeded $3.0 \mu\text{g/L}$. Exposure to cadmium concentrations that were $\geq 3.0 \mu\text{g/L}$ lead to r values that were significantly lower than those of the control ($t = 19.5$, $\alpha = 0.05$). No animals reproduced at 13 and $25 \mu\text{g/L}$ cadmium. The LOEC for r was $3.0 \mu\text{g/L}$ for both exposure durations (Table III-4).

PCP. For C. dubia 7-d and 18-d exposures, the mean r values were very similar (Figure III-3). The r values for $4.0 \mu\text{g/L}$ were slightly greater than those for the control (Figure III-3 and Table III-3). Concentrations of PCP up

Table III-4. The sensitivity of r values as determined by NOEC and LOEC limits for all four modes of action chemical exposures to Ceriodaphnia dubia and Daphnia magna.

Species	Toxicant	Test duration(d)	Endpoint	Limits ($\mu\text{g/L}$)	
				NOEC	LOEC
<u>C. dubia</u>	Cadmium	7	r	<1.5	1.5
		18	r	<1.5	1.5
<u>D. magna</u>	Cadmium	14	r	1.5	3.0
		18	r	1.5	3.0
<u>C. dubia</u>	PCP	7	r	4.0	8.0
		18	r	4.0	8.0
<u>D. magna</u>	PCP	14	r	<15	15
		18	r	<15	15
<u>C. dubia</u>	1-Octanol	7	r	569	1138
		18	r	569	1138
<u>D. magna</u>	1-Octanol	14	r	1531	3062
		18	r	1531	3062
<u>C. dubia</u>	Carbaryl	7	r	<.25	.25
		18	r	<.25	.25
<u>D. magna</u>	Carbaryl	14	r	<.50	.50
		18	r	<.50	.50

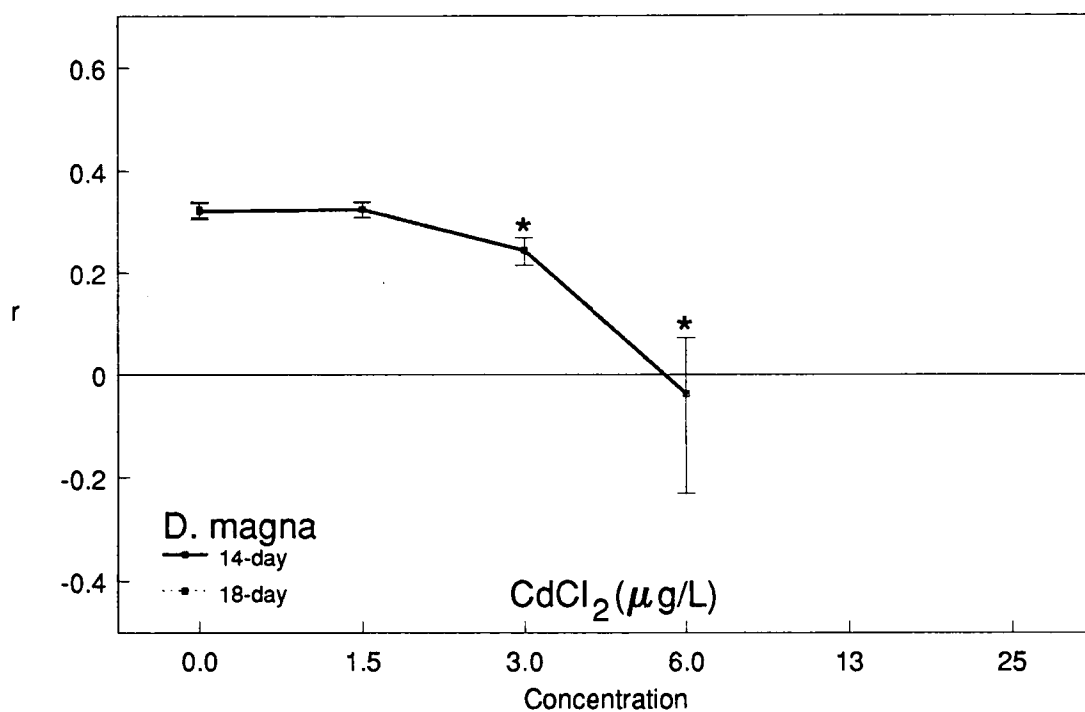


Figure III-2. Mean r values and concentrations of cadmium for 14- and 18-d *Daphnia magna* tests. Significant (*) at $\alpha = 0.05$.

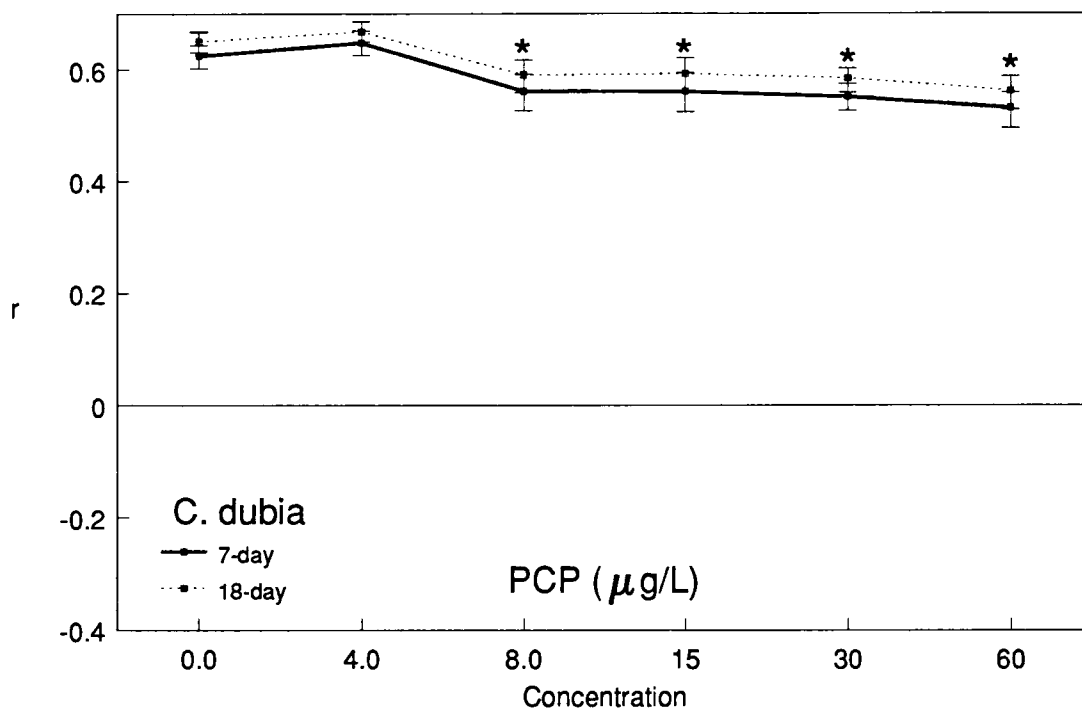


Figure III-3. Mean r values and concentrations of pentachlorophenol for 7- and 18-d *Ceriodaphnia dubia* tests. Significant (*) at $\alpha = 0.05$.

to 60 $\mu\text{g/L}$ only slightly reduced the r values of C. dubia in 7-d and 18-d exposures. A significant difference ($t = 31.5$, $\alpha = 0.05$) was found between the control and concentrations ≥ 8.0 $\mu\text{g/L}$ PCP. However, r values for animals in PCP concentrations ≥ 8.0 $\mu\text{g/L}$ were reduced only slightly.

The r values for D. magna exposed to PCP were more variable than those for C. dubia (Figure III-4). Variations in r with PCP concentration appeared similar to the reproductive curves for D. magna observed in the chronic toxicity studies (Figure II-4). The distribution of r values varied with the reproductive trend as well. All concentrations of PCP significantly decreased the r values for D. magna in both 14-d and 18-d exposures ($t = 25.0$ and 39.6 , $\alpha = 0.05$, respectively).

1-octanol. The r values for 7-d and 18-d exposure of C. dubia to 1-octanol remained nearly constant until the concentration of 1-octanol exceeded about 4550 $\mu\text{g/L}$ (Figure III-5 and Table III-3). At the highest tested concentration (9100 $\mu\text{g/L}$), 7-d survival was $>80\%$, but reproduction (fertility) was zero (Figure II-5). At the 18-d exposure, survival was $<50\%$, but those animals that did survive produced some offspring (0.240 offspring/

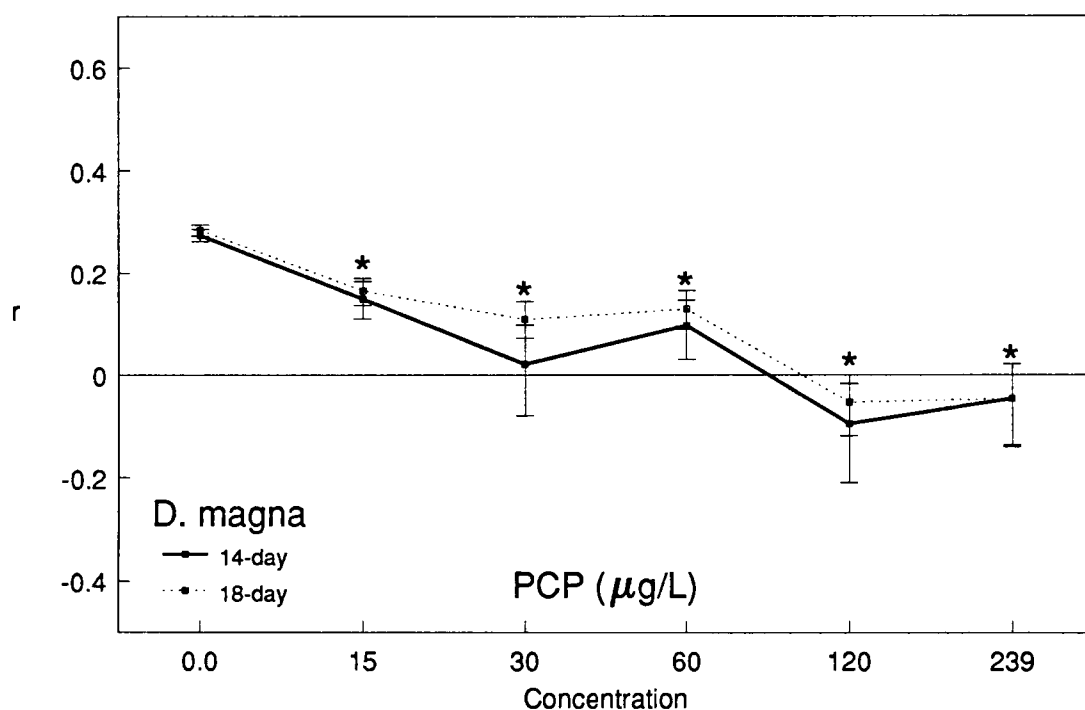


Figure III-4. Mean r values and concentrations of pentachlorophenol for 14- and 18-d Daphnia magna tests. Significant (*) at $\alpha = 0.05$.

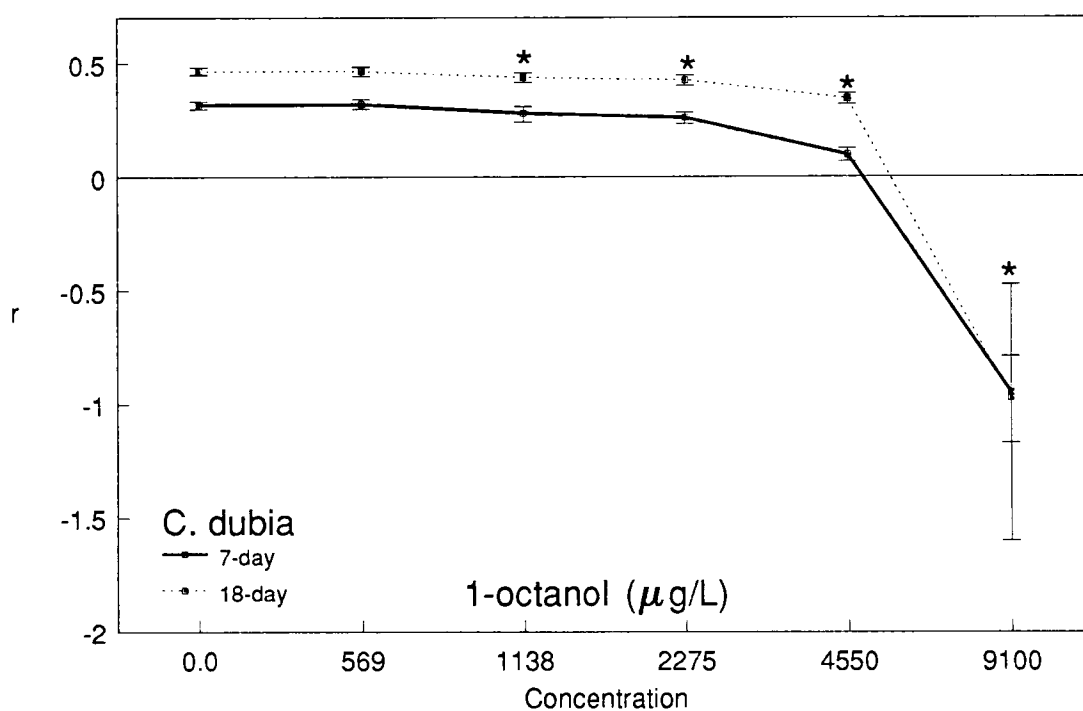


Figure III-5. Mean r values and concentrations of 1-octanol for 7- and 18-d *Ceriodaphnia dubia* tests. Significant (*) at $\alpha = 0.05$.

day/individual; Figure II-5). This reduced the variability in r (Figure III-5). Significant differences between the control and the exposure concentrations ($t = 8.0, \alpha = 0.05$) were detected at 1138 $\mu\text{g/L}$; thus, this concentration was the LOEC for both exposure durations (Table III-4).

The mean r values for 14-d and 18-d exposure of D. magna to 1-octanol were similar (Figure III-6 and Table III-3). The intrinsic rate of natural increase decreased when the concentration of 1-octanol was $\geq 3062 \mu\text{g/L}$. The r values for D. magna 14-d and 18-d exposures were similar. Survival and reproduction were too low (<40% and zero, respectively) (Figure II-6) at concentrations $> 6125 \mu\text{g/L}$ to permit calculations of r . The significance level, 3062 $\mu\text{g/L}$, was the LOEC for both exposure durations (Table III-4).

Carbaryl. Both 7-d and 18-d exposures of C. dubia resulted in less than two-fold decreases in r values with increasing concentrations (Figure III-7 and Table III-3). The r (offspring/day/individual) values for the control were > 0.60 , and the r values for populations exposed to carbaryl concentrations up to 2.0 $\mu\text{g/L}$ were very similar. A significant difference between control and exposure

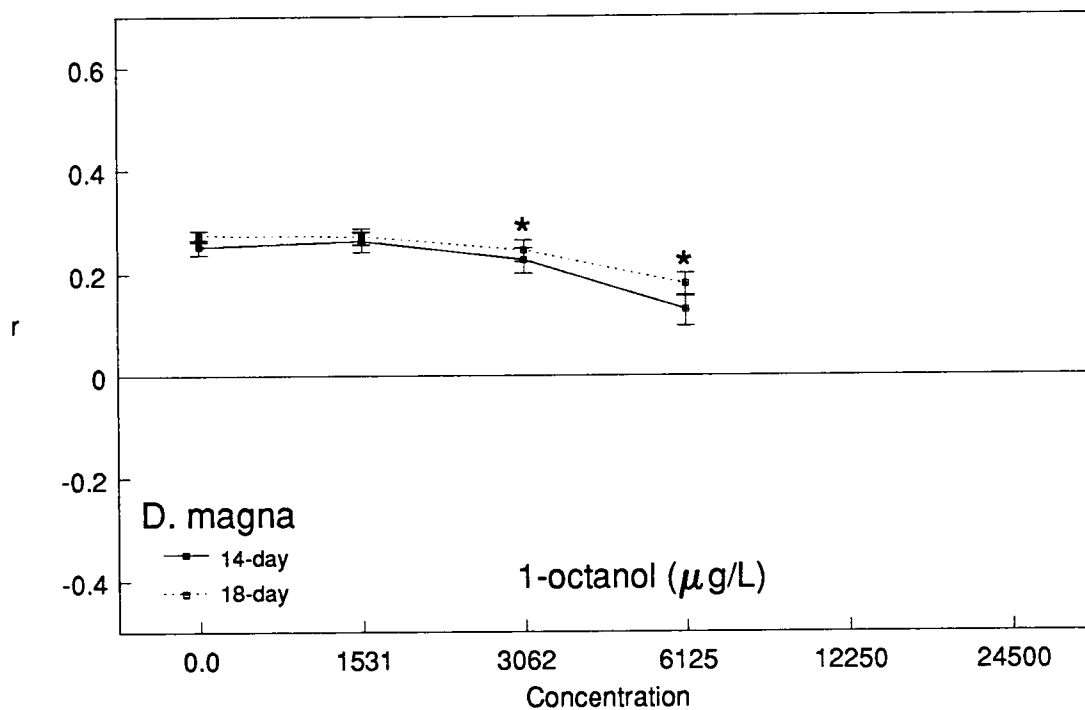


Figure III-6. Mean r values and concentrations of 1-octanol for 14- and 18-d *Daphnia magna* tests. Significant (*) at $\alpha = 0.05$.

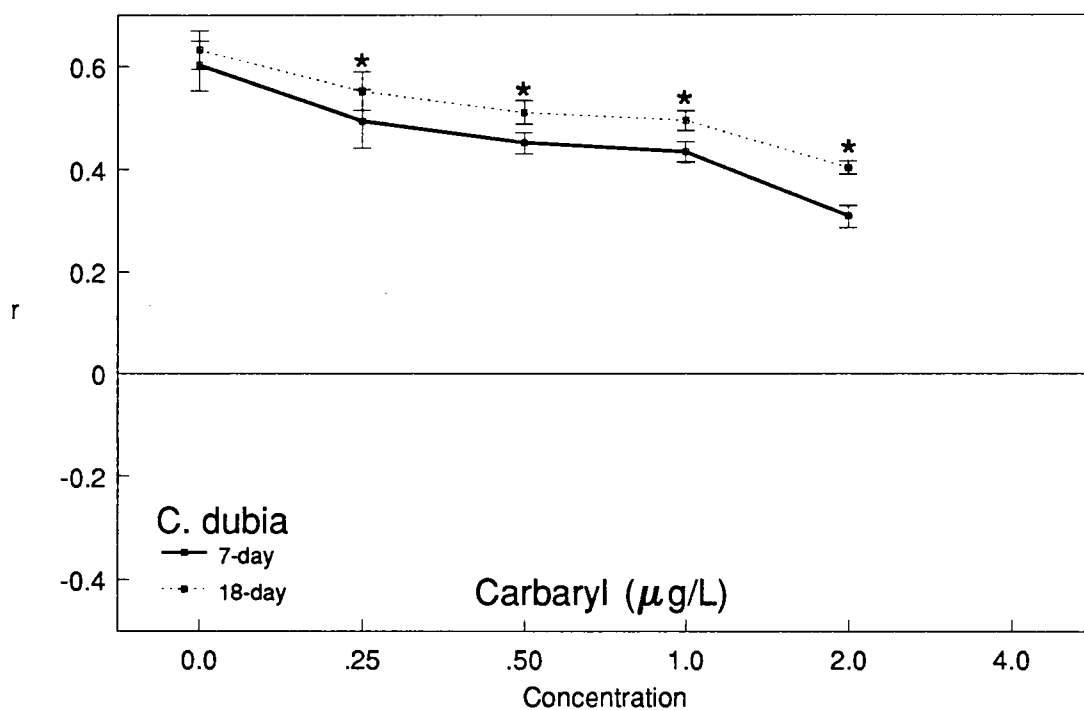


Figure III-7. Mean r values and concentrations of carbaryl for 7- and 18-d *Ceriodaphnia dubia* tests. Significant (*) at $\alpha = 0.05$.

concentrations was detected at the 0.25 $\mu\text{g/L}$ concentration ($t = 54.0$, $\alpha = 0.05$); this concentration was the LOEC for C. dubia in both exposure durations (Table III-4). Low survival (<20%) and no reproduction at 4.0 $\mu\text{g/L}$ carbaryl (Figure II-7) precluded effective computation of r at this concentration.

For D. magna 14-d and 18-d exposures to carbaryl, the mean r values were the same (Figure III-8 and Table III-3). The variation associated with r at various concentrations of carbaryl also closely agreed. A significant difference between the control and exposed cohorts ($t = 63.7$, $\alpha = 0.05$) indicated that the LOEC for carbaryl was 0.50 $\mu\text{g/L}$. As for C. dubia, no data were available to calculate r at the highest exposure concentration (8.0 $\mu\text{g/L}$).

Sensitive test endpoints

A summary of the sensitivity of r as determined by NOEC and LOEC is shown in Table III-4. The NOEC and LOEC values were the same for subchronic and chronic exposures of each species exposed to all four test chemicals. For the two species and the two test durations, the LOEC for each chemical differed by no more than two-fold. Based on the LOEC values for r , C. dubia was more sensitive than D. magna in every case; the order of toxicity of the

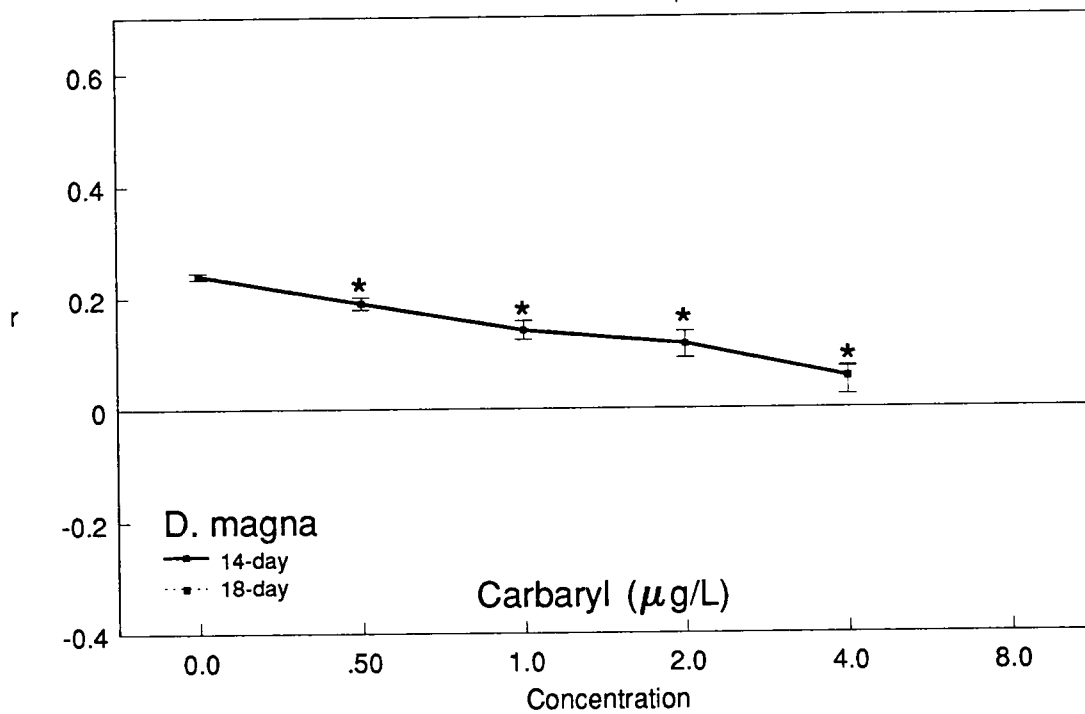


Figure III-8. Mean r values and concentrations of carbaryl for 14- and 18-d Daphnia magna tests. Significant (*) at $\alpha = 0.05$.

chemicals to both test organisms was carbaryl > cadmium > PCP > 1-octanol.

The LOEC values for all three endpoints (survival, reproduction, and r) are given in Table III-5. The r was more sensitive than survival or reproduction for C. dubia exposed to cadmium for 7- and 18-d. Reproduction and r were equally sensitive for C. dubia exposures to 1-octanol after 7-d, and carbaryl exposures after 7- and 18-d.

The D. magna cadmium exposures revealed that reproduction was the more sensitive endpoint after 14-d, but that all endpoints were equally sensitive at 18-d. Survival and r were equally sensitivity endpoints for D. magna 1-octanol exposures, while reproduction and r were equal in sensitivity for carbaryl exposures. Neither endpoint was consistently the most sensitive for either species exposed to the four chemicals.

When the criteria used by ecologists to indicate adverse effects of environmental stressors to populations (Gentile, et al., 1982) were considered, the data show that statistical interpretations may have little, if any, relevance to the ecological resources they propose to protect. For example, the r values for C. dubia exposed to PCP and carbaryl, and the r values of D. magna exposed to 1-octanol and carbaryl never declined below zero. This

Table III-5. Endpoint sensitivities based on LOEC for cadmium, pentachlorophenol, 1-octanol, and carbaryl exposures to Ceriodaphnia dubia and Daphnia magna. S = survival, R = reproduction, and r = intrinsic rate of natural increase.

Species	Toxicant	Test duration(d)	Endpoint	LOEC ($\mu\text{g/L}$)
<u>C. dubia</u>	Cadmium	7	S	6.0
			R	6.0
			r	1.5
		18	S	6.0
			R	6.0
			r	1.5
<u>D. magna</u>	Cadmium	14	S	3.0
			R	1.5
			r	3.0
		18	S	3.0
			R	3.0
			r	3.0
<u>C. dubia</u>	PCP	7	S	>60
			R	>60
			r	8.0
		18	S	8.0
			R	4.0
			r	8.0
<u>D. magna</u>	PCP	14	S	120
			R	15
			r	15
		18	S	120
			R	15
			r	15
<u>C. dubia</u>	1-Octanol	7	S	9100
			R	1138
			r	1138
		18	S	2275
			R	4550
			r	1138
<u>D. magna</u>	1-Octanol	14	S	3062
			R	12250
			r	3062
		18	S	3062
			R	12250
			r	3062
<u>C. dubia</u>	Carbaryl	7	S	4.0
			R	.25
			r	.25
		18	S	4.0
			R	.25
			r	.25
<u>D. magna</u>	Carbaryl	14	S	8.0
			R	.50
			r	.50
		18	S	8.0
			R	.50
			r	.50

indicates that the populations would continue to increase during the exposure to concentrations used here.

Discussion

The survival and reproduction data presented in Chapter II for C. dubia and D. magna exposed to four chemicals with different modes of action afforded the opportunity to assess the effects of chronic toxicant exposures on r for the two cladoceran species. This integrated endpoint was examined with respect to its application for environmental hazard assessment. The estimation of r is intended to provide an ecologically relevant account of the biological data obtained from chronic bioassays (i.e., a measure of the potential for population growth).

The use of r as a chronic endpoint for aquatic toxicity testing involved an examination of: (1) the duration of chronic test exposures, (2) the relative sensitivities of two commonly used test organisms, and (3) the quantification of the variance in r for use in hypothesis testing to determine significant differences between exposed and control groups.

Exposure Durations. The results showed that when C. dubia and D. magna were exposed to a chemical for subchronic durations compared to longer chronic durations, the response curves were similar, and in many cases identical (Fig. III-1 to 8). Daniels and Allan (1981) reported that r estimated after 21-d is indistinguishable from r estimated for the entire life span of D. pulex and Eurytemora affinis. Meyer, et al., (1987) found that after the first three to four broods, survival and reproduction did not change the estimate of r for D. pulex. This study supports the observation that what happens during the first three broods often affects r more than the remaining broods (Cole, 1954). My results clearly show that 7-d C. dubia and 14-d D. magna exposures are appropriate chronic test durations to obtain data for estimates of r . This finding supports the null hypothesis that r does not differ for short- and long-term exposures. These results were consistent for all four chemicals. Thus, the results of this study suggest that C. dubia or D. magna may be used to study the effects of chemicals on r .

Species Sensitivities. In all tests, the r values for C. dubia were greater than those for D. magna (Table III-4). When species with different body sizes are compared,

there is a tendency for r to decrease with increasing body size (Fenchel, 1968 and Pianka, 1970). In a study of the chronic toxicity of copper to four species of Daphnia, Winner and Farrell (1976) found that under control conditions, D. magna had smaller r values than D. pulex, D. parvula, and D. ambigua. They attributed the smaller r values to differences in species size, which relates to differences in frequency of reproduction and age of reproduction. In my study C. dubia and D. magna showed similar trends. As expected, r values for D. magna were lower than those for C. dubia under control conditions. Also, the r values for D. magna in controls for this study are similar to those found in other studies (Table III-6).

The lower r values for D. magna were probably due to their longer maturation time, longer embryonic periods, and smaller brood sizes. The D. magna produced their first brood at 8- or 9-days, while C. dubia produced their first brood at 3- or 4-days. The embryonic period for D. magna was 2- to 3-days, but 1- to 2-days for C. dubia. Additionally, C. dubia produced larger broods than D. magna (Table II-5).

Barbour, et al., (1989) reported r values for C. dubia exposed to effluents for 7-d. The control r values in that study ranged from 0.59 to 1.61, which slightly exceeded the r values I obtained in this study. The

Table III-6. Literature reported r values (not bootstrapped) and test durations for the control groups of Daphnia spp. exposed to various toxicants.

Species	r Value	Test duration(d)	Reference
<u>D. pulex</u>	0.33	40	Marshall, 1962
<u>D. magna</u>	0.373	140	Winner & Farrell, 1976
<u>D. pulex</u>	0.498	140	"
<u>D. parvula</u>	0.423	140	"
<u>D. ambigua</u>	0.459	140	"
<u>D. pulex</u>	0.30	60	Daniels & Allan, 1981
<u>D. pulex</u>	0.31	21	Walton, et al., 1982
<u>D. magna</u>	0.356	28	Goulden, et al., 1982b
<u>D. magna</u>	0.31	21	Meyerhoff, et al., 1985
<u>D. magna</u>	0.302	21	Van Leeuwen, et al., 1985
<u>D. magna</u>	0.362	21	"
<u>D. magna</u>	0.354	21	"
<u>D. magna</u>	0.362	21	Francis, et al., 1986
<u>D. magna</u>	0.332	21	Van Leeuwen, et al., 1987
<u>D. magna</u>	0.312	21	"
<u>D. magna</u>	0.325	25	Bodar, et al., 1988

differences probably reflect the test conditions, which were not specified by Barbour, et al., (1989). The value of r for any species is dependent on environmental factors (Fenchel, 1974; Krebs, 1978; Threlkeld, 1986a); therefore, reported r values should always be accompanied by a statement of such factors. Nevertheless, the study showed that r for D. magna is innately lower than that of C. dubia. Although the r values for D. magna were lower than the r values for C. dubia, the sensitivity of the r values as determined by the LOEC for each chemical exposure to each organism (Table III-5) show that C. dubia was the more sensitive species, only by a factor of about 2 times.

Endpoint Comparisons. The use of r as an assessment criterion requires methods for reliably estimating uncertainty in the values. The bootstrap procedure meets this need. In this study, a bootstrap statistical method was used to estimate the variance in r to determine the usefulness of r as a chronic toxicity test endpoint, and to determine the efficacy of using r in hypothesis testing to determine significant differences between the exposed and control groups. Considering all tests (i.e., the four chemicals, the two species, and the two test durations), r was more sensitive than survival or reproduction 25% of the time, and as sensitive as survival and reproduction

30% and 50% of the time, respectively (Table III-5). When r was the more sensitive endpoint, a 2 to 4-fold difference in LOEC values was observed. In each case, C. dubia was the test organism. As expected, r was never less sensitive than survival or reproduction alone, since r integrates the two endpoints.

A potential concern in the application of r involves the relative variability of this integrated endpoint, compared to that of the endpoints used to calculate it. However, the coefficients of variation (CV) for r for C. dubia were 10% to 20% as large as the CV in the means of total reproduction (Table III-7). For example, cadmium CV[r] was 11% and CV[total reproduction] was 53%; PCP, CV[r] was 4.0%, while the CV[total reproduction] was 36% (Table III-7). Coefficients of variation for r for D. magna were 20% to 50% as large as the CV for reproduction. This trend was observed by Meyer, et al. (1986), and it suggests that if r and reproduction are equally sensitive endpoints, then r should be a more sensitive indicators of the potential for population growth because the variability in the estimate of r is lower than the variability in the estimate of total reproduction (Meyer, et al., 1986).

Table III-7. Coefficients of variation (CV) in r (bootstrap) and means of total reproduction for Ceriodaphnia dubia and Daphnia magna exposed to cadmium, pentachlorophenol, 1-octanol, and carbaryl. Data represents the test concentration with the highest CV value.

Species	Toxicant	CV (%)	
		CV [r]	CV [total reproduction]
<u>C. dubia</u>	Cadmium	10.5	53.0
	PCP	3.8	36.1
	1-Octanol	4.3	43.5
	Carbaryl	7.3	36.1
<u>D. magna</u>	Cadmium	6.7	34.4
	PCP	20.1	91.2
	1-Octanol	14.0	28.7
	Carbaryl	30.3	57.0

Interpretation of r. The criteria for the interpretation of r used by ecologists ($r > 0$ = increasing populations; $r = 0$ = constant populations; $r < 0$ = decreasing populations) are not statistical. Thus, the r values were evaluated for ecological relevance based on the relative magnitude of change. The percent change in r from the control for C. dubia and D. magna are presented in Table III-8. The r -based LOEC for each chemical closely agrees with a 10-15% reduction in r from the control. This shows that the evaluation of these data by hypothesis testing procedures and a relative magnitude-of-change were not remarkably different. However, the concentrations that were significantly different from controls and that produced 10-15% reduction in r did not result in negative r values. Based on the criteria for r , C. dubia populations would have decreased at concentrations ≥ 13 , 9100, and 4.0 $\mu\text{g/L}$ for cadmium, 1-octanol, and carbaryl, respectively. In contrast, none of the PCP exposures would have caused population growth decreases. The D. magna populations would have decreased at concentrations ≥ 6 , 120, 6125, and 4 $\mu\text{g/L}$ for cadmium, PCP, 1-octanol, and carbaryl, respectively.

The statistical interpretations of the data did not yield results similar to the ecological interpretation of population effects. Thus, it may not be appropriate to

Table III-8. The relative magnitude of change in r from the control for Ceriodaphnia dubia and Daphnia magna exposed to cadmium, pentachlorophenol, 1-octanol, and carbaryl. Significance (*) is the LOEC for r.

Species	Toxicant	Concentration ($\mu\text{g/L}$)	% Change @ Exposure duration(d)			
			7	18	14	18
<u>C. dubia</u>	Cadmium	1.5 *	15	14		
		3.0	15	14		
		6.0	33	29		
		13	134	131		
		25	156	151		
<u>D. magna</u>	Cadmium	1.5			-.93	0
		3.0 *			24	25
		6.0			111	111
		13			-----	---
		25			-----	---
<u>C. dubia</u>	PCP	4.0	-3.7	-2.6		
		8.0 *	10	9		
		15	10	9		
		30	12	10		
		60	15	14		
<u>D. magna</u>	PCP	15.0 *			46	42
		30.0			92	62
		60.0			65	55
		120.0			134	119
		239.0			117	116
<u>C. dubia</u>	1-Octanol	569	.43	-.19		
		1138	6.2	3		
		2275	9.0	4		
		4550	26	18		
		9100	307	106		
<u>D. magna</u>	1-Octanol	1531			-4.3	.72
		3062 *			10	11
		6125			49	35
		12250			-----	---
		24550			-----	---
<u>C. dubia</u>	Carbaryl	.25 *	18	12		
		.50	25	19		
		1.0	28	22		
		2.0	49	36		
		4.0	---	---		
<u>D. magna</u>	Carbaryl	.50 *			21	21
		1.0			41	41
		2.0			52	52
		4.0			79	79
		8.0			---	---

set standards based solely on statistical differences between the exposed and control groups. If r is used as an index for hazard assessment, perhaps the data should be interpreted in a practical biological context. An acceptable amount of reduction in r must be agreed upon before the magnitude of the adverse effects of toxicants to the population at risk can be identified.

There are caveats to be considered before results of laboratory-based estimates of r are extrapolated to field applications. These laboratory experiments demonstrated the relative importance of some of the factors involved in the interpretation of chronic toxicity data in an ecological context. Observations in the field may not be supported by these laboratory data because many of the factors that affect r in the field were controlled in the laboratory. In nature, actual rates of population increase are continuously varying from positive to negative in response to changes in environmental factors (e.g., weather, food availability, predation, and disease). Threlkeld (1986b) studied midsummer shifts in community structure of zooplankton. Population growth rates of Ceriodaphnia and Daphnia spp. were examined. Natural seasonal variations in r values were observed and attributed to slower individual growth during certain seasons that resulted in delays in time to the first

reproduction and reduced numbers of total young produced. Positive r values observed in the spring and early summer were followed by negative r values in mid to late summer. Ceriodaphnia r values varied, but were always positive, whereas Daphnia r values were sometimes negative. The changes in population growth rates in the field study were influenced by high algal blooms at high water temperatures. Lake population and *in situ* population studies of copepods (Wyngaard, 1983) showed that summer temperatures influenced the developmental rates of the organisms and that was reflected in their r values.

While variation in r of natural populations makes it difficult to interpret for comparisons with laboratory studies, r is still useful as an endpoint for chronic toxicity tests. The importance of this measure is that it gives us a model to improve our interpretations of chronic data and thus, make predictions that better relate to population-level effects.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The results of this study indicate that survival, reproduction, and population growth were all affected by exposure to four chemicals with different modes of action. In the acute bioassays, 48-h LC_{50} s were used to compare the species sensitivities. Ceriodaphnia dubia was more sensitive than D. magna to all four chemicals. Carbaryl was the most toxic chemical in all tests.

Cladoceran chronic toxicity data can be used to estimate population growth rates from sample cohort schedules of survivorship and fecundity; the variation inherent in these estimates can be calculated using a bootstrap statistical procedure. During the chronic studies, tests were designed to evaluate the duration of chronic exposures, the sensitivity of the endpoints (survival, reproduction, and r), and the use of r as an index for hazard assessment.

Chronic test durations of 7-d for C. dubia and 14-d for D. magna were compared to 18-d exposures. The 7-d C. dubia and the 14-d D. magna exposures were similar to the 18-d exposures for each of the test chemicals. Generally, survival decreased and reproduction increased over time;

however, the r values for the different exposure periods were similar, showing less than a 2-fold difference. Survival, reproduction, and r were all reduced with increased concentrations of each chemical.

The sensitivity of the endpoints were examined based on the LOEC for each chemical. Neither survival nor reproduction was consistently the more sensitive endpoint for either C. dubia or D. magna exposures to the four chemicals.

The three measures of reproduction (mean time to first brood release, mean number of broods, mean brood size) had varying degrees of sensitivity depending on the species, test duration, and exposure concentration. In the 7-d C. dubia tests, the mean brood size was the most sensitive measure for all four chemicals. For 18-d exposures to PCP and 1-octanol, mean number of broods was the most sensitive measure. The mean number of broods was the most sensitive measure of reproduction for D. magna 14- and 18-d exposures to cadmium and PCP; the mean brood size was the most sensitive measure for exposure to 1-octanol. When D. magna was exposed to carbaryl, the mean time to first brood release and the mean brood size were equally sensitive measures of reproduction. The difference in the sensitivities of these variables clearly demonstrated the problem associated with the use of

separate chronic toxicity endpoints for regulatory decisions.

The integration of survival and reproduction data produced a single r value for each chemical concentration. Thus, estimates of the variance in r were calculated using a bootstrap statistical method. The bootstrap is a practical, nonparametric method that can be used to estimate the sampling distribution of a parameter (Solow, 1989).

A bootstrap program (rBoot) was written in Fortran and SAS algorithms, and based on the bootstrap procedures described by Meyer, et al., (1986). The rBoot is flexible; it can calculate an r value for data sets of up to 20 organisms, and up to 26 days of exposure. Additionally, when the organisms within an exposure concentration fail to reproduce an r value is calculated on the bases of the number of animals surviving at the time. In this study, the calculations of r based on the number of animals surviving at a given time, and by the life-table method produced similar and sometimes identical results. This indicated that biases in the estimates of r for populations without a stable age was small and not in the concentration range that affected r . The statistical accuracy of r is estimated from the data obtained from a single chronic toxicity test. A Monte

Carlo simulation selected (sampling with replacement) 1000 samples of size 20 in order to find the variance in r .

The estimated variance in r (standard error) was small due to the large sample sizes. The 90% quantile limits provided a statistical picture of the data (Fig. III-1 to 8). Pairwise comparisons and Dunnett's t test identified differences (reductions) between the exposed and control groups, and the LOEC for each chemical was determined.

The LOECs for r were the same for both exposure durations for each species. This result supports the hypothesis that r measured at short-term test durations do not differ from longer term exposures. Ceriodaphnia dubia was more sensitive than D. magna in all tests. When the LOECs for survival, reproduction, and r were compared, r was more sensitive or equally as sensitive as survival and reproduction considered alone. The coefficients of variation in r for C. dubia and D. magna were less than the coefficients of variation in mean total reproduction for all test chemicals (Table III-7). This shows that r can be used as a chronic toxicity endpoint, and it suggest that when r and reproduction are equally sensitive, r may be the more environmentally protective endpoint. Although r was not always the most sensitive endpoint in this study, these results indicate that the calculation of r

from chronic toxicity data can be used to eliminate conflicting effects of toxicants on separate endpoints.

To use r as an index for hazard assessment, considerations should be given to practical biological evaluations as well as statistical analysis of the data. Additionally, an acceptable amount of reduction in r should be determined as a guideline for assessing the adverse effects of toxicants to a population.

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LITERATURE CITED

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APPENDICES

APPENDIX A

A-1. Chemical analysis of well/spring water.

Determination	Concentration (mg/L)
Aluminum	.26
Arsenic	<.06
Cadmium	<.002
Calcium	27.
Chromium	<.005
Cobalt	<.003
Copper	<.01
Iron	.054
Lead	<.05
Magnesium	8.1
Manganese	<.002
Molybdenum	<.004
Nickel	<.005
Nitrate	<.200
Phosphorus	<.3
Selenium	<.06
Silicon	3.7
Sodium	.8
Zinc	<.008

A-2. Nutrients for algal media for growing daphnid food
(Source: Goulden, 1982b).

Macronutrients (g/L stock)

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	36.76
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	36.97
NaHCO_3	12.60
K_2HPO_4	8.71
NaNO_3	85.01
$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	28.42

Micronutrients:

Na_2EDTA	4.36
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	1.58
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	.01
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	.01
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	.022
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	.18
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	.006
H_3BO_3	1.0

A-3. Analysis of Purina trout chow for pesticide organics.

CAS NO.	COMPOUND	CONCENTRATION UNITS:	
		(ug/L or ug/Kg)	ug/L
319-84-6-----	alpha-BHC		8.00
319-85-7-----	beta-BHC		8.00
319-86-8-----	delta-BHC		8.00
58-89-9-----	gamma-BHC (Lindane)		8.00
76-48-8-----	heptachlor		8.00
309-00-2-----	aldrin		8.00
1024-57-3-----	heptachlor epoxide		8.00
959-98-8-----	endosulfan I		8.00
60-57-1-----	dieldrin		16.00
72-55-9-----	4,4'-DDE		16.00
72-20-8-----	endrin		16.00
33213-65-9-----	endosulfan II		16.00
72-54-8-----	4,4'-DDD		16.00
1031-07-8-----	endosulfan sulfate		16.00
50-29-3-----	4,4'-DDT		16.00
72-43-5-----	methoxychlor		80.00
53494-70-5-----	endrin ketone		16.00
5103-71-9-----	alpha-chlordane		80.00
5103-74-2-----	gamma-chlordane		80.00
8001-35-2-----	toxaphene		160.00
12674-11-2-----	Aroclor-1016		80.00
11104-28-2-----	Aroclor-1221		80.00
11141-16-5-----	Aroclor-1232		80.00
53469-21-9-----	Aroclor-1242		80.00
12672-29-6-----	Aroclor-1248		80.00
11097-69-1-----	Aroclor-1254		160.00
11096-82-5-----	Aroclor-1260		160.00

A-3.1. Analysis of cerophyl for pesticide organics

CAS NO.	COMPOUND	CONCENTRATION UNITS:	
		(ug/L or ug/Kg)	ug/L
319-84-6-----	alpha-BHC		8.00
319-85-7-----	beta-BHC		8.00
319-86-8-----	delta-BHC		8.00
58-89-9-----	gamma-BHC (Lindane)		8.00
76-44-8-----	heptachlor		8.00
309-00-2-----	aldrin		8.00
1024-57-3-----	heptachlor epoxide		8.00
959-98-8-----	endosulfan I		8.00
60-57-1-----	dieldrin		16.00
72-55-9-----	4,4'-DDE		16.00
72-20-8-----	endrin		16.00
33213-65-9-----	endosulfan II		16.00
72-54-8-----	4,4'-DDD		16.00
1031-07-8-----	endosulfan sulfate		16.00
50-29-3-----	4,4'-DDT		16.00
72-43-5-----	methoxychlor		80.00
53494-70-5-----	endrin ketone		16.00
5103-71-9-----	alpha-chlordane		80.00
5103-74-2-----	gamma-chlordane		80.00
8001-35-2-----	toxaphene		160.00
12674-11-2-----	Aroclor-1016		80.00
11104-28-2-----	Aroclor-1221		80.00
11141-16-5-----	Aroclor-1232		80.00
53469-21-9-----	Aroclor-1242		80.00
12672-29-6-----	Aroclor-1248		80.00
11097-69-1-----	Aroclor-1254		160.00
11096-82-5-----	Aroclor-1260		160.00

APPENDIX B

B-1.1 through 1.21. General Linear Models (GLM) procedures (ANOVA tables) for chronic bioassay reproductive data.

B-1.1. Total reproduction for 7-d Ceriodaphnia dubia exposure to cadmium.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	147410.3417	29482.0683	43.00	0.0001
Error	114	78153.4500	685.5566		
Corrected Total	119	225563.7917			

R-Square	C.V.	Root MSE	REPRO Mean
0.653520	64.71630	26.18314	40.4583333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	147410.3417	29482.0683	43.00	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	147410.3417	29482.0683	43.00	0.0001

B-1.2. Total reproduction for 18-d Ceriodaphnia dubia exposure to cadmium.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	50768.05000	16922.68333	16.46	0.0001
Error	76	78127.90000	1027.99868		
Corrected Total	79	128895.95000			

R-Square	C.V.	Root MSE	REPRO Mean
0.393868	52.97384	32.06242	60.5250000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	3	50768.05000	16922.68333	16.46	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	3	50768.05000	16922.68333	16.46	0.0001

B-1.3. The time to the first brood release for Ceriodaphnia dubia exposure to cadmium.

Dependent Variable: REPRO					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1.48960135	0.49653378	1.53	0.2155
Error	65	21.11909430	0.32490914		
Corrected Total	68	22.60869565			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.065886	13.24261	0.570008	4.30434783	
Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	3	1.48960135	0.49653378	1.53	0.2155
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	3	1.48960135	0.49653378	1.53	0.2155

B-1.4. Total reproduction for 14-d Daphnia magna exposure to cadmium.

Dependent Variable: REPRO					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	5044.433333	2522.216667	68.41	0.0001
Error	57	2101.500000	36.868421		
Corrected Total	59	7145.933333			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.705917	34.43443	6.071937	17.6333333	
Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	2	5044.433333	2522.216667	68.41	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	2	5044.433333	2522.216667	68.41	0.0001

B-1.5. Total reproduction for 18-d Ceriodaphnia dubia exposure to pentachlorophenol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	97396.37500	19479.27500	14.64	0.0001
Error	114	151709.95000	1330.78904		
Corrected Total	119	249106.32500			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.390983	42.25888	36.47998	86.3250000	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	97396.37500	19479.27500	14.64	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	97396.37500	19479.27500	14.64	0.0001

B-1.6. The number of broods for 18-d Ceriodaphnia dubia exposure to pentachlorophenol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	107.8661581	21.5732316	4.11	0.0019
Error	109	571.9251462	5.2470197		
Corrected Total	114	679.7913043			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.158675	26.66228	2.290637	8.59130435	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	107.8661581	21.5732316	4.11	0.0019
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	107.8661581	21.5732316	4.11	0.0019

B-1.7. The brood sizes for 18-d Ceriodaphnia dubia exposure to pentachlorophenol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	254.1666667	50.8333333	4.00	0.0022
Error	114	1449.8000000	12.7175439		
Corrected Total	119	1703.9666667			

R-Square	C.V.	Root MSE	REPRO Mean
0.149162	38.83303	3.566167	9.1833333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	254.1666667	50.8333333	4.00	0.0022

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	254.1666667	50.8333333	4.00	0.0022

B-1.8. Total reproduction for 14-d and 18-d Daphnia magna exposure to pentachlorophenol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	11990.50000	3996.83333	134.27	0.0001
Error	76	2262.30000	29.76711		
Corrected Total	79	14252.80000			

R-Square	C.V.	Root MSE	REPRO Mean
0.841273	42.62441	5.455924	12.8000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	3	11990.50000	3996.83333	134.27	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	3	11990.50000	3996.83333	134.27	0.0001

B-1.9. The time to the first brood release for 14-d Daphnia magna exposure to pentachlorophenol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	212.5938961	53.1484740	2.40	0.0567
Error	81	1794.2433132	22.1511520		
Corrected Total	85	2006.8372093			

R-Square	C.V.	Root MSE	REPRO Mean
0.105935	59.69898	4.706501	7.88372093

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	4	212.5938961	53.1484740	2.40	0.0567

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	4	212.5938961	53.1484740	2.40	0.0567

B-1.10. The number of broods for 14-d Daphnia magna exposure to pentachlorophenol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	46.64871345	15.54957115	25.50	0.0001
Error	71	43.29795322	0.60983033		
Corrected Total	74	89.94666667			

R-Square	C.V.	Root MSE	REPRO Mean
0.518626	59.76401	0.780916	1.30666667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	3	46.64871345	15.54957115	25.50	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	3	46.64871345	15.54957115	25.50	0.0001

B-1.11. The brood sizes for 14-d Daphnia magna exposure to pentachlorophenol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	337.0780670	67.4156134	5.97	0.0001
Error	85	959.0538012	11.2829859		
Corrected Total	90	1296.1318681			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.260065	98.28631	3.359016	3.41758242	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	337.0780670	67.4156134	5.97	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	337.0780670	67.4156134	5.97	0.0001

B-1.12. Total reproduction for 7-d Ceriodaphnia dubia exposure to 1-octanol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2700.141667	540.028333	31.32	0.0001
Error	114	1965.650000	17.242544		
Corrected Total	119	4665.791667			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.578710	43.51875	4.152414	9.54166667	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	2700.141667	540.028333	31.32	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	2700.141667	540.028333	31.32	0.0001

B-1.13. The time to the first brood release for 7-d Ceriodaphnia dubia exposure to 1-octanol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.54000000	1.38500000	5.16	0.0008
Error	95	25.50000000	0.26842105		
Corrected Total	99	31.04000000			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.178479	11.16581	0.518094	4.64000000	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	4	5.54000000	1.38500000	5.16	0.0008
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	4	5.54000000	1.38500000	5.16	0.0008

B-1.14. Total reproduction for 14-d Daphnia magna exposure to 1-octanol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	118589.4417	23717.8883	70.54	0.0001
Error	114	38333.1500	336.2557		
Corrected Total	119	156922.5917			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.755719	28.67813	18.33728	63.9416667	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	118589.4417	23717.8883	70.54	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	118589.4417	23717.8883	70.54	0.0001

B-1.15. The time to the first brood release for Daphnia magna exposure to 1-octanol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1171.375000	234.275000	42.36	0.0001
Error	114	630.550000	5.531140		
Corrected Total	119	1801.925000			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.650069	31.46271	2.351838	7.47500000	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	1171.375000	234.275000	42.36	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	1171.375000	234.275000	42.36	0.0001

B-1.16. The brood sizes for 18-d Daphnia magna exposure to 1-octanol.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	97.23109885	32.41036628	3.72	0.0155
Error	65	565.58049536	8.70123839		
Corrected Total	68	662.81159420			
	R-Square	C.V.	Root MSE	REPRO Mean	
	0.146695	45.94475	2.949786	6.42028986	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	3	97.23109885	32.41036628	3.72	0.0155
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	3	97.23109885	32.41036628	3.72	0.0155

B-1.17. Total reproduction for 7-d Ceriodaphnia dubia exposure to carbaryl.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4400.066667	880.013333	99.10	0.0001
Error	114	1012.300000	8.879825		
Corrected Total	119	5412.366667			

R-Square	C.V.	Root MSE	REPRO Mean
0.812965	27.80625	2.979903	10.7166667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	4400.066667	880.013333	99.10	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	4400.066667	880.013333	99.10	0.0001

B-1.18. The brood sizes for 7-d and 18-d Ceriodaphnia dubia exposure to carbaryl.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	96.34000000	24.08500000	16.79	0.0001
Error	95	136.30000000	1.43473684		
Corrected Total	99	232.64000000			

R-Square	C.V.	Root MSE	REPRO Mean
0.414116	26.26765	1.197805	4.56000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	4	96.34000000	24.08500000	16.79	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	4	96.34000000	24.08500000	16.79	0.0001

B-1.19. Total reproduction for 14-d Daphnia magna exposure to carbaryl.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	14565.86667	2913.17333	46.60	0.0001
Error	114	7126.00000	62.50877		
Corrected Total	119	21691.86667			

R-Square	C.V.	Root MSE	REPRO Mean
0.671490	57.01622	7.906249	13.8666667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	5	14565.86667	2913.17333	46.60	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	5	14565.86667	2913.17333	46.60	0.0001

B-1.20. The number of broods for 14-d Daphnia magna exposure to carbaryl.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	75.10000000	18.77500000	28.93	0.0001
Error	95	61.65000000	0.64894737		
Corrected Total	99	136.75000000			

R-Square	C.V.	Root MSE	REPRO Mean
0.549177	37.46850	0.805573	2.15000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	4	75.10000000	18.77500000	28.93	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	4	75.10000000	18.77500000	28.93	0.0001

B-1.21. The brood sizes for 14-d Daphnia magna exposure to carbaryl.

Dependent Variable: REPRO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	141.9118056	35.4779514	19.54	0.0001
Error	91	165.2444444	1.8158730		
Corrected Total	95	307.1562500			
	R-Square	C.V.	Root MSE		REPRO Mean
	0.462018	39.56090	1.347543		3.40625000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CONC	4	141.9118056	35.4779514	19.54	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	4	141.9118056	35.4779514	19.54	0.0001

B-2. Literature reported acute toxicity of the test chemicals to cladoceran species.

Toxicant	Species	LC ₅₀	Duration (h)	Conditions	Reference
Cadmium	<u>C. dubia</u>	32	48	22/8.2/86	Present Study
	<u>C. reticulata</u>	90	48	22/7.7/200	Hall, et al., 1986
	<u>C. reticulata</u>	184	48	23/8.0/246	Elnabarawy, et al., 1986
	<u>C. reticulata</u>	66	48	25/7.4/45	Mount & Norberg, 1984
	<u>D. magna</u>	249	48	22/8.2/86	Present Study
	<u>D. magna</u>	320	48	20/8.4/150	Bodar, et al., 1990
	<u>D. magna</u>	26	48	24/7.7/80	Hatakeyama & Sugaya, 1989
	<u>D. magna</u>	178	48	23/8.0/240	Elnabarawy, et al., 1986
	<u>D. magna</u>	80	48	22/7.7/200	Hall, et al., 1986
	<u>D. magna</u>	24	48	20/8.5/170	Lewis & Weber, 1985
	<u>D. magna</u>	118	48	25/7.4/45	Mount & Norberg, 1984
	<u>D. magna</u>	40	48	20/ND/32	Schuytema, et al., 1983
	<u>D. magna</u>	65	48	18/7.7/45	Biesinger & Christenson, 1972
	<u>D. carinata</u>	345	48	25/7.0/ND	Chandini, 1989
	<u>D. pulex</u>	100	48	22/7.7/200	Hall, et al., 1986
	<u>D. pulex</u>	140	48	20/8.2/90	Lewis & Weber, 1985

Appendix B-2
(Continued)

Toxicant	Species	LC ₅₀	Duration (h)	Conditions	Reference
Cadmium	<u>D. pulex</u>	68	48	25/7.4/45	Mount & Norberg, 1984
	<u>D. pulex</u>	115	48	20/8.6/106	Ingersoll & Winner, 1982
	<u>D. pulex</u>	62	72	22/7.7/ND	Bertram & Hart, 1979
PCP	<u>M. macrocopa</u>	71	48	22/7.6/84	Hatakeyama & Yasuno, 1981
	<u>C. dubia</u>	18	48	22/8.2/91	Present Study
	<u>C. reticulata</u>	900	48	23/8.0/240	Elnabarawy, et al., 1986
	<u>C. reticulata</u>	220	48	22/7.7/200	Hall, et al., 1986
	<u>C. reticulata</u>	164	48	25/7.4/45	Mount & Norberg, 1984
	<u>D. magna</u>	249	48	22/8.2/91	Present Study
	<u>D. magna</u>	550	48	20/8.0/ND	Kuhn, et al., 1989
	<u>D. magna</u>	760	24	22/8.2/200	Devillers & Chambon, 1986
	<u>D. magna</u>	470	48	22/7.7/200	Hall, et al., 1986
	<u>D. magna</u>	620	48	20/8.5/170	Lewis & Weber, 1985
	<u>D. magna</u>	145	48	23/8.6/46	Thurston, et al., 1985
	<u>D. magna</u>	143	48	25/7.4/45	Mount & Norberg, 1984
	<u>D. magna</u>	1050	48	ND/8.0/Hard	Adema & Vink, 1981
	<u>D. magna</u>	680	48	22/8.0/173	LeBlanc, 1980
	<u>D. magna</u>	600	48	20/ND/ND	Adema, 1978

Appendix B-2
(Continued)

Toxicant	Species	LC ₅₀	Duration (h)	Conditions	Reference
PCP	<u>D. magna</u>	800	48	ND	Canton & Adema, 1978
	<u>D. pulex</u>	1080	48	22/7.7/200	Hall, et al., 1986
	<u>D. pulex</u>	540	48	20/8.2/90	Lewis & Weber, 1985
	<u>D. pulex</u>	246	48	25/7.4/45	Mount & Norberg, 1984
	<u>D. pulex</u>	2000	48	ND	Canton & Adema, 1978
	<u>D. cucullata</u>	1500	48	ND	Canton & Adema, 1978
1-Octanol	<u>C. dubia</u>	8700	48	22/8.2/80	Present Study
	<u>D. magna</u>	26160	48	22/8.2/80	Present Study
	<u>D. magna</u>	260	24	25/8.0/ND	Kuhn, et al., 1989
	<u>C. dubia</u>	14	48	22/8.1/90	Present Study
Carbaryl	<u>D. magna</u>	23	48	22/8.1/90	Present Study
	<u>D. magna</u>	10.2	26	21/8.1/204	Crosby, Tucker & Aharonson, 1966
	<u>D. pulex</u>	6.4	48	16/7.8/ND	Sanders & Cope, 1966
	<u>S. serrulatus</u>	7.6	48	16/7.8/ND	Sanders & Cope, 1966

NOTES: LC₅₀ Values are in µg/L.
Conditions are temperature (°C)/pH/hardness (mg/L as CaCO₃).
ND = No Data

B-2.1. Sensitive chronic toxicity endpoints reported in the literature.

Species	Exposure Duration (d)	Sensitive Endpoint	Reference
<u>D. magna</u>	28	No. broods Tot. Young	Parkhurst, et al., 1981
<u>D. magna</u>	21	Tot. Young	Poston, 1983
<u>D. magna</u>	21	Time to first brood	Meyerhoff, et al., 1985
<u>D. magna</u>	14	Reproduction	Elnabarawy, et al., 1986
<u>C. dubia</u>	7	Reproduction	Elnabarawy, et al., 1986

APPENDIX C

C-1. Example of the chronic toxicity data used to construct a life-table.

Day x	No. Ani	No. Surv	Pro Surv lx	Reprod	mx	lxmx
1.00	20.00	20.00	1.00	0.00	0.00	0.00
2.00	20.00	20.00	1.00	0.00	0.00	0.00
3.00	20.00	20.00	1.00	0.00	0.00	0.00
4.00	20.00	20.00	1.00	61.00	3.05	3.05
5.00	20.00	20.00	1.00	21.00	1.05	1.05
6.00	20.00	20.00	1.00	139.00	6.95	6.95
7.00	20.00	20.00	1.00	154.00	7.70	7.70
8.00	20.00	20.00	1.00	15.00	0.75	0.75
9.00	20.00	20.00	1.00	219.00	10.95	10.95
10.00	20.00	20.00	1.00	242.00	12.10	12.10
11.00	20.00	20.00	1.00	26.00	1.30	1.30
12.00	20.00	20.00	1.00	193.00	9.65	9.65
13.00	20.00	20.00	1.00	78.00	3.90	3.90
14.00	20.00	20.00	1.00	164.00	8.20	8.20
15.00	20.00	20.00	1.00	0.00	0.00	0.00
16.00	20.00	20.00	1.00	140.00	7.00	7.00
17.00	20.00	20.00	1.00	200.00	10.00	10.00
18.00	20.00	20.00	1.00	25.00	1.25	1.25

C-2. The bootstrap program, rBOOT, developed to calculate the variance in r.

```
C ON 5/1: THE PROGRAM WILL ATTEMPT TO FIND MONTE CARLO
C SETS WITH AT LEAST ONE NONZERO ROW. IF IT CANNOT, THE
C NUMBER OF REPS WILL BE LESS THAN MONTE. IF THE DATA SET
C HAS ALL ROWS ZERO, YOU MUST CHANGE PROGRAM TO ALLOW
C KREBS METHOD TO BE USED. IT IS NOW TURNED OFF THROUGH
C THE USE OF A GOTO 25 STATEMENT WHICH ROUTES PAST THE
C KREBS CODE.
c on 2/28 we redefine lxsum as the number of females
c capable of giving birth on that day k, whether or not
c they are alive on that day. we thus want to look at
c mat2(i,j,ie)+1, to give an extra day for birth.
c
c 5 minutes later- now we decided to make a special case
of those females giving birth on day past the last day of
life.
c*****declarations*****

c      parameter(maxanim=20, maxdays=26, maxcon=5,
1      maxmonte=1000)
c
c mat(indiv,maxdays+3)
      integer mat(maxanim,29)
c mat2(maxmonte,indiv,maxdays+3)
      real mat2(maxmonte,maxanim,29)
c lx(maxdays), mx(maxdays)      real lx(maxdays),mx(maxdays)

c from Krebs p 162
      real time,icfi,nsub0
      integer ndaymax,nsubt
c
c incrementers for loops
      integer ia,ib,ic,id,ie,if,ij,ik,il,im,concen
      integer i,j,k,l,monte,actdays
      INTEGER CNT,MONTCNT,KREBCNT
      integer nanimal,ndays,SEED,count,incr,iterate
c summers
      real sum,sum1,sum2,sum3,sum4,sum5,lxsum,mxsum
      integer random,ncon
      real step6,step7,r,r0,eps,r1,tc
C *****END DECLARATIONS*****

      OPEN(8, FILE='output.DAT', STATUS='NEW')

      OPEN(7, FILE='entcd1.DAT', STATUS='OLD')
```

```

C*****INITIALIZATIONS*****
C
C initializations
      nanimal=20
      ndays=18
C actdays is the actual number of days considered
C ndays is the number of days in the data set.
      actdays=18
      monte=1000
      iterate=10
      SEED=357571
      ncon=6

C
      Do 3 Concen=0,ncon-1
C change format number (e.g., 26(i2) to match ndays.
      Do 10 I=1, Nanimal
        read(7,1)(Mat(I,J),J=1,ndays+3)
1        format(i1,1x,i2,1x,26(i2),1x,i2)
10       continue
C
C      Do 100 I=1, Nanimal
C        write(8,101)(Mat(I,J),J=1,ndays+3)
C101      format(' ',18(i2,1x))
C
C        write(8,101)(Mat(I,J),J=1,ndays+3)
C101      format(' ',18(i2,1x))
C100      Continue
C
C      skip=1
C      if(skip .eq. 1) goto 3
      MONTCNT=0
C-----
      do 15 I=1,MONTE
        CNT=0
C
C3000      KREBCNT=0
C
C      do 20 K=1, nanimal
C
C if random = k, picks original data set

C IF YOU WANT ORIGINAL DATA SET, PUT "I .EQ. 1"
C THIS WILL PRODUCE THE ORIGINAL R VALUE FOR THE FIRST
C MONTE CARLO OBSERVATION IN THE OUTPUT DATA SET; THE
C REMAINING MONTE-1 OBSERVATIONS ARE R VALUES GENERATED
C FROM RANDOM ROWS OF THE DATA SETS.

```

```

        if(i .eq. 1) then
            random=k
        else
            random=int(1+((nanimal)*ran(SEED)))
        end if
C
        do 22 L=1,ndays+3
            mat2(I,K,L)= Mat(random,L)
C
            IF(L .GT. 2 .AND. L .LT. (ACTDAYS+3) )THEN
                KREBCNT=KREBCNT+ MAT2(I,K,L)
            END IF
C
C            write(8,8884)k,L,random,KREBCNT,mat2(i,k,l)
C8884        format(' ',3(i4,2x),i5,2x,f8.3)
22        Continue
C
20        Continue
C
        IF(KREBCNT .EQ. 0)THEN
            IF(CNT .LT. 100)THEN
                CNT=CNT+1
                GOTO 3000
            ELSE
                GOTO 15
            END IF
        END IF
C
        MONTCNT=MONTCNT+1
15        Continue
C-----

c        if(concen .lt. 4)goto 3
C
C        do 420 ic=1,5
C        Do 400 I=1, Nanimal
C            write(8,401)(Mat2(ic,I,J),J=1,15)
c401        format(' ',15(f4.1,1x))
C            write(8,4012)(Mat2(ic,I,J),J=16,ndays+3)
c4012        format(' ',29(f4.1,1x))
c400        Continue
c420        continue
C
        WRITE(6,3001)MONTCNT
3001        FORMAT(' ','MONTCNT',I6)
        25 i=1,MONTCNT
C
        do 500 ib=1, ndays
            lx(ib)=0.0
            mx(ib)=0.0

```

```

500      continue
c
      Count=0
c
      Do 26 K=1, actdays
          Lxsum=0
          Count=count+1
          Mxsum=0
c
          ie=ndays+3
c
          Do 27 J=1, nanimal
c              write(8,519)ie,mat2(i,j,ie),k
c519      format(' ','ie,mat2(i,j,ie),day',i4,2x,f9.4,2x,i3)
c
          If( Mat2(I,J,ie) .ge. K .or. mat2(i,j,k+2) .ne. 0)
              Then
                  Lxsum=Lxsum+1
                  Mxsum=Mxsum + Mat2(I,J,k+2)
              End if
27      Continue
c
c          write(8,910)lxsum,mxsum
c910      format(' ','lxsum,mxsum before assign',2(f10.2))

          Lx(k)=lxsum/float(nanimal)
          if(lxsum .le. 0)goto 26
          Mx(k)=Mxsum/lxsum
26      continue
c
c          do 430 id=1,actdays
c              write(8,432)id,lx(id),mx(id)
c432      format(' ','day,lx,mx',i3,2x,2(f9.4,1x))
c430      continue
c
          Sum1=0
          Sum2=0
          Do 30 Ia=1,actdays
              Sum1=Sum1+(Lx(Ia)*Mx(Ia))
              Sum2=Sum2+(ia*Lx(ia)*Mx(Ia))
30      Continue
c
c      put safety net if all mx is zero, goto next monte.
c
c-----
c      use this code from Krebs Ecology p162 to calculate
c      innate capacity for decrease: both lx and mx are all
c      zero.
c      1000 krebs: TO TURN KREBS ON, COMMENT OUT GOTO 25
c      STATEMENT.

```

```

c          if(sum1 .le. 0)GOTO 25
c          if(sum1 .le. 0)then
c
c
c          ndaymax is the last day at which at least one animal is
c          alive.
c          find ndaymax
c              ndaymax=0
c              do 33 ia=1,nanimal
c                  if(mat2(i,ia,ndays+3) .gt. ndaymax)then
c                      ndaymax=mat2(i,ia,ndays+3)
c                  end if
33          continue
c              write(8,3399)ndaymax
c3399          format(' ','ndaymax...',i9)
c
c              nsub0=float(nanimal)
c              nsubt=0
c              time=float(ndaymax)
c              do 31 ia=1,nanimal
c                  if(mat2(i,ia,ndays+3) .ge. ndaymax)then
c                      nsubt=nsubt+1
c                  end if
31          continue
c
c
c              icfi= (log(nsubt/nsub0))/time
c              r=icfi
c              write(8,32)nsubt,ns0,time,r,icfi
c32          format(' ','krebs method...',i4,4(1x,f10.6))
c              WRITE(6,33991)CONCEN,I,R
33991          FORMAT(' ','KREBS METHOD ',2(I5,1X),F10.6)
c              goto 99
c              end if
c-----

c
c          r0=Sum1
c          Eps=.01
c          Tc=Sum2/r0
c          write(8,200)r0,sum2,tc
c200          format(' ','r0,xlxx,tc= ',3(f12.3,1x))
c          R1=log(R0)/Tc
c          R=R1
c
c          Do 35 Incr=1,iterate
c              Sum3=0
c              Sum4=0
c          Do 36 J=1,actdays

```



```

        Sum3=Sum3+(Lx(J)*Mx(J)*(exp(-R*J)))
        Sum4=Sum4+(J*Lx(J)*Mx(J)*(exp(-R*J)))
36      Continue
C
C      write(8,920)incr,sum3
C920    format(' ','incr,lxmxe=',i5,1x,f9.5)
        If(abs(Sum3-1) .lt. Eps)goto 99
C
C
        Step6=log(Sum3)
        Step7=R+(Step6*(Sum3/Sum4))
        R=Step7
35      Continue
C
99      write(8,40) Concen,I,R
40      Format(' ',i2,2x,i4,2x,f10.7)

c 25 is monte carlo loop
c 3 is concentration loop
25      Continue
3       Continue
C
        stop
        end

```

C-2.1. The Statistical Analysis System (SAS) algorithms used with rBOOT.

```

data one;
  infile output;
  input concen monte r;
cards;

***proc print;
  proc means n mean std var cv stderr;
    var r ; by concen;
  proc univariate plot;
    var r ; by concen;

run;

```

VITA

Willodean Delores Burton was born in Franklin, TN, on March 8, 1947. She attended elementary and high school in Robertson Co., TN, and was graduated from Bransford High School in May, 1964. She attended Tennessee State University (TSU) (Nashville, TN) and received a Bachelor of Science degree in Biology (May, 1972) and a Master of Science degree, with a major in Zoology (May, 1975).

The author's compassion for learning and sharing knowledge gained her a staff/instructor's position at TSU. She taught there for eleven years, and was coordinator of the freshman Animal Biology courses for three years.

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