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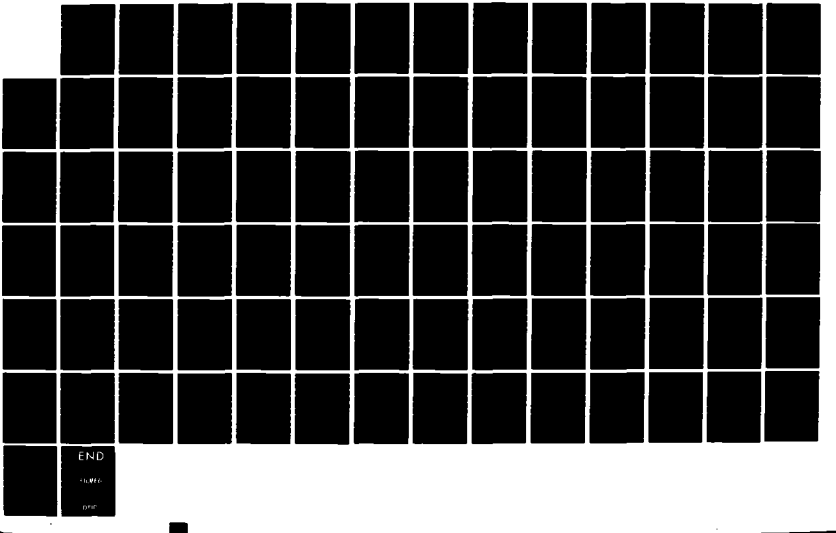

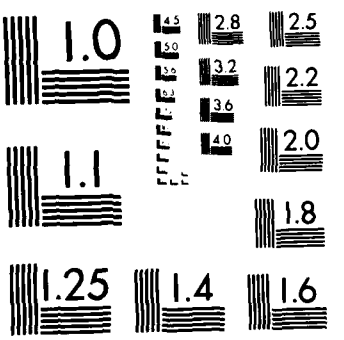

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TOXICITY OF TNT WASTEWATERS TO AQUATIC ORGANISMS

Final Report

Volume IV

Chronic Toxicity of 2,4-Dinitrotoluene and Condensate Water

By

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July 1984

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Early life stage tests were performed with 2,4-dinitrotoluene and synthetic condensate wastewater using rainbow trout, channel catfish, and fathead minnows as test organisms. Chronic toxicity studies were also performed with 2,4-dinitrotoluene and condensate water using fathead minnows and Daphnia magna as test organisms. Another chronic toxicity study was conducted with irradiated condensate water using Daphnia magna. Based on data from these studies and from previous acute studies, water quality criteria based on US EPA recommended procedures were developed for 2,4-dinitrotoluene and condensate water. For

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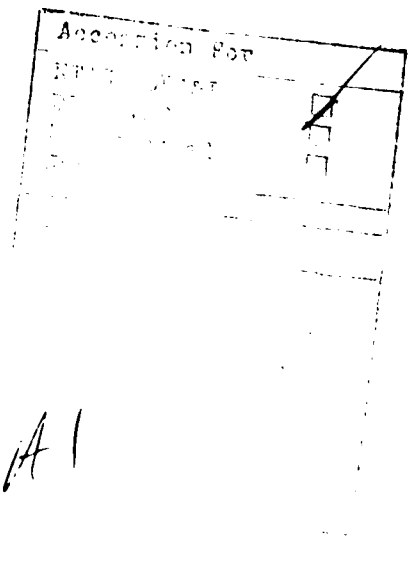
2,4-dinitrotoluene, 8.1 mg/L was calculated as the maximum allowable concentration and 0.12 mg/L as the 24-hr average allowable concentration. A concentration of 2.3 mg/L condensate water was calculated as the maximum allowable value, while 0.14 mg/L was derived as the 24-hr average allowable concentration.



FOREWORD

The U.S. Army Medical Research and Development Command, Ft. Detrick, Frederick, MD, is conducting a research program for the purpose of developing the scientific data base necessary for assessing the potential environmental hazards associated with compounds unique to the munitions industry. From these data, criteria will be developed that are qualitative or quantitative estimates of the concentrations of a pollutant in ambient waters that, if not exceeded, should ensure the protection of aquatic organisms and human health. These criteria, when compared to actual or estimated environmental concentrations, will form the basis of a hazard assessment. In addition, these criteria will be used to assess the adequacy of current pollution abatement technologies and thus influence research and development in this area.

This report represents a portion of the data base being developed on 2,4,6-trinitrotoluene and its associated wastewaters and should not be construed as a complete evaluation or as official policy of the U.S. Army Surgeon General.

This work was conducted under the technical control and review of the U.S. Army Medical Bioengineering Research and Development Laboratory: J. Gareth Pearson and William H. van der Schalie (Aquatic Toxicology), Jesse J. Barkley, Jr. (Analytical Chemistry), and Jerry W. Highfill (Statistical Analysis).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

EXECUTIVE SUMMARY

This report is the last in a series of four reports on the toxicity of 2,4,6-trinitrotoluene (TNT) wastewaters to aquatic organisms. The information presented in the four volumes was developed in a study performed by SRI International for the U.S. Army Medical Research and Development Command (USAMRDC) under Contract No. DAMD 17-75-C-5056. The study was undertaken to assist USAMRDC in developing a data base for assessing the potential hazards to aquatic life of wastewater from TNT manufacturing and processing plants.

This report presents and discusses the results of early life stage and chronic studies on 2,4-dinitrotoluene (2,4-DNT), condensate water, and photolyzed condensate water. 2,4-DNT is a major component of the condensate wastewater that results from treatment of the effluent (red water) that comes from the continuous production of TNT. Condensate water is a synthetic blend based on the actual condensate wastewater and developed by SRI under a separate contract.

Early life stage studies were conducted on 2,4-DNT and condensate water with rainbow trout, channel catfish and fathead minnows. Full life-cycle chronic studies were performed on 2,4-DNT and condensate water with fathead minnows and Daphnia magna and on irradiated condensate water with D. magna. For 2,4-DNT, the effect/no-effect concentrations from the chronic studies were 0.62 and 0.28 mg/L for fathead minnows and 0.39 and 0.19 mg/L for D. magna. For condensate water, the effect/no-effect concentrations for fathead minnows were 0.75 and 0.34 mg/L and for daphnids they were 3.68 and 2.09 mg/L. Irradiated condensate water was similarly or slightly less toxic to daphnids than non-irradiated condensate water. In comparing these results to those from earlier acute studies, it appears that 2,4-DNT caused chronic effects at concentrations 50 to 100 times less than concentrations that caused acute effects. Similarly, condensate water produced chronic effects at concentrations 4 to 8 times less than those that resulted in acute effects.

Based on the results of these studies, water quality criteria for 2,4-DNT and condensate water were calculated using USEPA recommended methods. The criteria comprise two parts; one is a concentration that cannot be exceeded in a 24-hour period, and the other is an average allowable concentration for a 24-hour period. For 2,4-DNT, the maximum allowable concentration is 8.1 mg/L and the average maximum allowable 24-hour average concentration is 0.12 mg/L. For condensate water, the maximum allowable concentration is 2.3 mg/L and the maximum allowable 24-hour average concentration is 0.14 mg/L.

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INTRODUCTION

The production of munitions compounds generates a significant volume of wastewater, which has historically been discharged into the environment with little or no treatment. To assess the hazard of these wastewaters to human health and to aquatic life, the U.S. Army Medical Research and Development Command (USAMRDC) funded a comprehensive investigation to develop a scientific data base comprising data from literature reviews, on-site field studies, and laboratory investigations in mammalian and aquatic toxicology.

Of the various kinds of wastewaters produced in the manufacture and processing of munitions compounds, condensate and LAP wastewater are of major concern to the USAMRDC. Condensate wastewater is produced during the continuous process for manufacturing 2,4,6-trinitrotoluene (TNT) and comprises at least 30 organic compounds, with 2,4-dinitrotoluene (2,4-DNT) accounting for almost 50 percent of the total dissolved organics (Spanggord et al. 1978). LAP wastewater is produced at load, assemble and pack (LAP) facilities during the washing of shells and other equipment. The composition of LAP wastewater depends on the particular kind of explosive formulation being processed by the LAP facility. The LAP wastewater of primary concern is that produced by LAP facilities handling an explosive formulation called Composition B (COMP B). That wastewater is composed primarily of TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX).

Under Contract DAMD 17-75-C-5056, SRI International conducted a laboratory study to determine the acute, subchronic, and chronic toxicity to aquatic organisms of condensate wastewater, LAP wastewater from COMP B processing plants, and selected organic components of both wastewaters. The study comprised four phases, each with several tasks, and followed the approach proposed by Pearson and co-workers (1979) for toxicological evaluation of complex industrial wastewaters. Based on the results of numerous chemical analyses, synthetic wastewaters containing representative quantities of the different constituent chemicals were formulated and used for most of the tests designed to assess the toxicity of the authentic wastewaters. These formulations were designated as condensate water and LAP water to differentiate them from the actual condensate and LAP wastewaters.

The results of SRI's study are presented in a series of four reports. This report is the fourth in the series; it presents and discusses the results of the early life stage and chronic studies performed on 2,4-DNT, condensate water, and photolyzed condensate water. Graphic displays of the data from the chronic studies on fathead minnows are contained in the appendix to Volume IV (Bailey et al. 1984).

The other reports in the series are Volume I, "Acute Toxicity of LAP Wastewater and 2,4,6-Trinitrotoluene" (Liu et al. 1984), which describes the overall testing approach and the facilities, equipment and procedures used to conduct acute toxicity and bioconcentration studies on TNT, LAP wastewater, and related materials; Volume II, "Acute Toxicity of Condensate Wastewater and 2,4-Dinitrotoluene" (Liu et al. 1984); and Volume III, "Chronic Toxicity of LAP Wastewater and 2,4,6-Trinitrotoluene" (Bailey et al. 1984).

Statistical Tests

The statistical tests were designed to detect statistically significant differences between control and treatment groups. It was assumed that any treatment effect would be detrimental to the organism, so that the tests were all one-tailed in the direction of greater mortality, smaller fry, or lower fertility. One of two types of tests was used, depending on the type of data analyzed:

(1) Proportional data, which included measures of egg and fry survival and fry deformity, were analyzed in an untransformed state using Fisher's Exact Test for analysis of 2 x 2 contingency tables. When the total sample size exceeded 40, the normal approximation to the hypergeometric distribution was used. Probability levels of less than 0.01 for each comparison (Miller, 1965) were flagged as statistically significant, yielding experiment-wise alpha levels of approximately 0.05.

(2) Nonproportional data were first subjected to an analysis of variance, using concentration and, where there were sufficient degrees of freedom, series and concentration-series interactions as independent variables. The mean square error from the ANOVA was then used to perform Dunnett's test of control-treatment differences (Dunnett, 1955).

Graphics

Two types of graphic displays were produced to aid in analyzing the data: detailed plots on the smallest level practical (chamber, breeding pair, or batch), and aquarium-level plots with error bars (where possible). For untransformed data, the error bar widths were the sample standard errors (e.g., the sample standard deviations divided by the square root of the sample size). In cases where the dependent variable underwent a variance-stabilizing transformation, the mean point for each series was plotted in its untransformed state, while the error bars represented the standard deviation of the mean point of the transformed data that were inversely transformed back into the original metric and bracketed around the untransformed point. The rationale for this approach was to provide a graphic illustration of the raw data while at the same time displaying error bars that corresponded to the statistical tests that were performed on the transformed data. For example, survival data were transformed by the Tukey arcsin method. A set of theoretically correct error bars for the transformed data, based on underlying distribution assumptions, is given by the expression

$$\arcsin \sqrt{X/(N+1)} \pm 1/\sqrt{2N},$$

where X is the number of fry alive and N is the number that was transferred originally into an aquarium. When inversely transformed back into the original metric, the error bars become

$$\sin\left[\arcsin \sqrt{X/(N+1)} \pm 1/\sqrt{2N}\right]^2.$$

Statistical Analyses

General

Up to 31 variables were analyzed for each compound, including survival and growth measurements, fertility measures, and, in the case of fathead minnow chronic studies, various global or summary types of measures. Most of these variables were tested for each series separately and for the pooled series. Statistical analyses and graphics were produced on an IBM 3033 computer using the SAS statistical package and the VERSATEC plotter.

Transformations

In cases where homogeneity of variance assumptions were unwarranted, variance-stabilizing transformations were applied to variables before statistical tests or error-bar calculations were performed. These transformations were of two types (Bishop et al., 1975):

(1) Tukey arcsin transformation for proportions,

$$Y = \arcsin \sqrt{X/(N+1)}$$

where X/N is the proportion to be transformed;

(2) Square root transformation,

$$Y = \sqrt{X}$$

used for the F_0 fertility measures, which were assumed to be Poisson-distributed.

Unit of Analysis

The unit of analysis is the smallest experimental unit that yields a single observation. For example, both fry length and fry survival are measured for each fish (note that fry survival is a Bernoulli random variable valued 0 or 1 for each death or survival); whereas F_0 biomass, being a product of a chamber mean and a chamber proportion, yields only one value per chamber. Likewise, the total survivability and total productivity indices can only be measured on the aquarium level because they include F_0 cumulative survival, which yields only one measurement per aquarium.

Series Included in the Analyses

In most cases, the data were analyzed for each series separately as well as for both series pooled. The exceptions were total survivability and total productivity indices, for which there were insufficient degrees of freedom to analyze the series separately.

one individual were measured with an ocular micrometer. Data obtained from each test included:

(1) Mortality at 7, 14, 21, and 28 days.
(2) Total reproduction at 7, 14, 21, and 28 days.
(3) Young produced per female at 7, 14, 21, and 28 days.
(4) Young produced per female per reproductive day.
(5) Length (to base of spine) at 28 days.
(6) Days until first young produced.

Chemical Analyses

Chemical concentrations were measured weekly in each treatment level, alternating between the replicates. Stock concentrations were determined before the stocks were added to the toxicant reservoirs. Twenty-four hours after the new stocks were added to the diluter, a sample was taken from the highest concentration to ensure that the proper concentrations were being delivered. In the event of a diluter malfunction, samples were taken from all concentrations to determine the extent to which the malfunction affected the test concentrations. Samples were also taken 24 hours after the malfunction was corrected to verify that the diluter was again working properly. Analytical methods are described in detail in Volume I (Liu et al., 1984) of the final report series.

Water Quality Analyses

Dissolved oxygen and pH were measured daily at all treatment levels for a period, usually a week, until we determined that the levels were stabilized. After this, pH and dissolved oxygen were determined at weekly intervals, alternating between the replicates. Dissolved oxygen was measured with a Yellow Springs Instrument dissolved oxygen probe and pH with an Orion 407A Ionanalyzer. Temperature was monitored hourly in one of the control tanks using a Honeywell thermograph and checked weekly at all concentrations with a glass mercury thermometer. Hardness, alkalinity, and acidity were determined weekly in the diluent water using titration techniques (Hach Chemical Company, Sunnyvale, CA). Residual chlorine was also determined weekly in the diluent water using a Fischer and Porter amperometric titrator (Arthur H. Thomas Co., Philadelphia, PA). The diluent water was also evaluated periodically for the presence of contaminants such as pesticides and PCBs. No detectable levels of these chemicals were found in any of the samples even though samples yielding suspicious peaks were further investigated by mass spectroscopy.

F_0 Survival

Egg
30-day fry
60-day fry
90-day fry
120-day fry
150-day fry
178-day fry

F_0 Growth

30-day length
60-day length
90-day length

F_0 Fry Deformities

F_0 Fertility Measurements

Breeding pair survival
Spawns per pair
Eggs per spawn
Eggs per pair per day
Eggs per pair

F_1 Survival

Egg
30-day
60-day

F_1 Deformities

F_1 Growth

30-day length
30-day weight
60-day length
60-day weight

Global Indices

F_0 90-day cumulative fry survival
F_0 178-day cumulative fry survival
F_0 60-day biomass
F_1 60-day cumulative fry survival
F_1 60-day standing crop
Total survivability index
Total productivity index

Daphnia magna. These tests were conducted in 80-L aquaria that containing approximately 28 L of water. The daphnids were housed in 400-ml beakers, each having a 2-cm-wide by 5-cm-long hole cut in the side. This hole was covered with 200-μ Nitex screen. Ten beakers were placed in each aquarium; seven of the 10 beakers received one daphnid each and the remaining three beakers received five daphnids each.

Young daphnids were reared in a colony maintained under the same conditions as the tests, except that individual beakers contained two adults each. Reproduction was carefully monitored in the colony to ensure that daphnids used in the tests did not come from the first brood. Twenty-four hours before a test was scheduled to begin, all young were removed from the beakers in the rearing colony. On the day of the test, the new young were removed from the beakers, pooled, and distributed randomly into the test beakers using a large bore pipet. This procedure was repeated the following day to start the replicate series. If a sufficient number of young was not available to initiate a test, this procedure was repeated until enough could be obtained within a 24-hour period.

The beakers were inspected daily for mortality and young. Dead daphnids were removed, and young were removed and counted. The daphnids exposed to 2,4-DNT and condensate water were fed algae (Selenastrum capricornutum) twice daily at the rate of 30,000 cells/mL. In the test on irradiated condensate water, the algal diet was supplemented with a daphnid formula (Biesinger and Christensen, 1972) to increase reproduction. The tests were terminated after 28 days of exposure, and surviving daphnids from the beakers that contained

frozen adult brine shrimp four times per day. The food mix varied, depending on the size of the fry. On Days 30, 60, and 90 post-hatch, the rearing chambers were removed from the tanks, placed on millimeter grid paper and photographed several times. These pictures were used to determine the total length of the fry during this period as well as provide an indication of fry survival. After the pictures were taken at 90 days, the fry were released from the rearing chambers into the aquaria. After release, we continued to observe the fry for signs of developing breeding characteristics in the males, such as dark banding, blunt snouts, and tubercles.

When several males were obvious in all of the tanks, the fish were removed from the tanks and carefully segregated according to sex. Males were determined on the basis of banding, blunt snouts, or tubercles. Females were differentiated by the presence of urogenital papillae. A third category was reserved for fry whose sex we could not readily determine. Four males and four females were then selected, weighed and measured, and randomly assigned as individual pairs in stainless-steel breeding cages located in the lower two-thirds of each tank. The remaining fish were anesthetized, weighed and measured, and preserved in 10% formalin according to sex.

Spawning substrates were made by coating the inside of a semicircular piece of PVC pipe with silicone sealant and embedding fine sand into the silicone sealant before it hardened. The pieces of PVC pipe were formed by cutting a 4-in. diameter Schedule 40 pipe in half lengthwise and then cutting each half into 3-in. sections. After the silicone sealant had dried, the excess sand was brushed off and the substrates were soaked for 24 hours in dechlorinated tap water. One substrate was added to each breeding chamber.

Substrates were inspected daily for eggs. If eggs were present, the substrate was replaced with a clean one and placed under a low-power microscope where the eggs could be removed and counted. A minimum of 35 eggs were selected at random and placed in an egg cup to determine hatchability. If the spawn did not contain at least 35 eggs, the eggs were counted and discarded. After the eggs hatched, the fry were counted and discarded or added to a rearing chamber if one was available. We attempted to rear two batches of F_1 fry to 30 days and two batches to 60 days in each tank. If possible, each set of fry selected for rearing was obtained from a different spawning pair.

In addition to hatchability and mortality, records of deformities and lengths (total) and weights were taken for the F_1 fry. Lengths and weights were taken at the end of the exposure period, and interim 30-day lengths were determined photographically on fry reared to 60 days.

Each test was terminated when no spawns occurred in any concentration for 1 week. The following data were prepared for statistical analysis:

chow ad libitum three times per day. Excess food and waste materials were siphoned from the bottom of the tanks as necessary. The surviving fry were measured (total length) at the end of the exposure period. Chemical concentrations were determined twice weekly, alternating between the replicates.

Fathead Minnows. These tests were initiated with 30 embryos (24 hours old) per egg cup. Two egg cups were used per tank. The tests were generally terminated 30 days after initiation and, with the exception of the test on condensate, they were performed in duplicate. After hatching, the fry were counted and transferred into larval rearing chambers. During the post-hatch exposure period, the fry were fed brine shrimp nauplii three times daily. Excess food and waste materials were siphoned from the bottom of the tanks as necessary. Total fry length was determined photographically or by direct measurement at the end of the exposure period.

Chemical concentrations were routinely determined prior to initiating the test and weekly thereafter, alternating between replicates.

Rainbow Trout. These tests were initiated with 60 eggs per duplicate tank at each exposure level. The eggs were fertilized in the presence of the toxicant and allowed to water-harden before they were transported to the laboratory. In the first series of tests, a different female was used for each concentration; in the second test series, eggs from different females were randomized over the mixing containers before they were fertilized. Because a low overall fertility was observed in one of the tests in the second series, the test was restarted using eyed eggs obtained directly from the hatchery. The first series of tests were terminated 30 days after hatching was completed, and the second series were terminated after a 60-day post-hatch exposure period. Once the fry entered the swim-up stage, they were fed a combination of Artemia nauplii, dry trout food, and frozen adult brine shrimp three times per day. Throughout the tests, the tanks were inspected daily and dead eggs and fry were removed. At the end of the test, surviving fry were anesthetized with MS-222 and individually weighed and measured (total length).

Chemical concentrations were determined weekly, alternating between the replicates.

Chronic Studies

Fathead Minnows. These tests were initiated by randomly distributing a minimum of 40 eggs to each of two egg cups suspended in each tank. The duplicate series were started approximately 1 week apart. During the period of embryo development, the egg cups were inspected daily and dead eggs were removed. Once the fry began to hatch, the cups were not disturbed except for daily checks to determine whether hatching had been completed. If the hatching process took longer than 24 hours to complete, brine shrimp nauplii were added to the egg cup twice daily to ensure a food source for the hatched fry. When hatching was completed in all cups, deformed and normal fry were counted, and the normal fry were transferred to rearing chambers.

Fry were maintained in the rearing chambers for 90 days. During this period they were fed a mixture of brine shrimp nauplii, dry trout food, and

METHODS

Toxicity Testing

General

Tests were performed in duplicate with six treatment levels including the controls. Chronic tests were performed in 30.5 x 91.4 x 30.5 cm (H x L x W) glass aquaria containing approximately 40 L of water for the fathead minnow tests and 28 L of water for the daphnid tests. Early life stage studies were performed in 19-L aquaria containing 15 L of test solution. Fathead minnow and channel catfish eggs were exposed in eggs cups made from 5-cm diameter glass or PVC tubing with one end covered with 200-μ Nitex screen. Fathead minnow fry hatched in the egg cups were transferred to rearing chambers (30 x 30 x 5 cm, H x L x W) constructed from glass except for the front panels, which were made from Nitex screen (200 μ) to allow passage of water through the chambers. Channel catfish fry were transferred directly to the aquaria. Eggs from rainbow trout were simply placed on the bottoms of the aquaria during exposure.

The locations of the test aquaria were randomized within each replicate series. Nominal test temperatures were 12°C for the trout early life stage studies, 25°C for the catfish early life stage studies, 25°C for the fathead minnow early life stage studies, 25°C for the fathead minnow chronic studies, and 20°C for the daphnid chronic studies. Diluter flow rates were set to provide a minimum of four tank volumes per day; this rate was increased as necessary to maintain water quality and/or desired chemical concentrations. A photoperiod of 16 hours light and 8 hours dark was used for all tests except the fathead minnow chronic studies, which used an EPA-recommended variable photoperiod corresponding to that of Evansville, IN (EPA 1972). At the start of each series, this photoperiod corresponded to 1 December and was adjusted as appropriate at 2-week intervals.

Early Life Stage Studies

Channel Catfish. These tests were scheduled to be initiated with 30 eggs per treatment level in each of the duplicate test series. However, we encountered difficulties separating the eggs because they were past the initial hardening stage and were easily damaged when handled. Therefore, we cut off similar-sized clumps of eggs from the original egg masses, counted the eggs in each mass, weighed them, and transferred them into the egg cups.

A problem with using this approach was that, because the eggs were not separate, the fungus-affected eggs could not be removed to prevent the spread of disease to other eggs. To minimize problems with fungal infection, we flushed the eggs daily with malachite green up to the time of hatching (Leitritz and Lewis, 1980). After hatching, the fry were transferred from the egg cups into the aquaria for a 30-day post-hatch exposure period. During this period, the fry were fed brine shrimp nauplii (cysts obtained from San Francisco Bay Brand, Newark, CA), frozen adult brine shrimp, and dried trout

was delivered directly into the main mixing cell of the diluters used in the daphnid chronic studies by Mariotte bottles filled from the stock barrels or by metering pumps directly from the stock barrels. All Mount-Brungs diluters were equipped with counters to monitor the cycling rate and to aid in ensuring the proper function of the diluters.

other. The upper reservoir, measuring approximately 15 x 5 x 183 cm (H x W x L), was used to deliver stock solutions of the toxicant. The bottom reservoir, measuring approximately 22 x 15 x 365 cm (H x W x L), was used to deliver water.

The water reservoir was equipped with 24 adjustable flow meters capable of measuring flows up to 300 ml per minute. Water was pumped to this reservoir from a secondary reservoir equipped with temperature-control and aeration devices and connected to the laboratory water supply through a float valve. Excess water in the primary reservoir was returned to the secondary reservoir by gravity. The toxicant stock solution was recirculated by pump between the toxicant reservoir and a 55-gallon polyethylene-lined steel drum in which the toxicant stock was prepared. The toxicant was metered by Teflon capillary tubes, and the delivery rate was adjusted by increasing or decreasing the vertical distance of the distal end of the tube from the level of the liquid in the toxicant reservoir.

The toxicant stock solution and water were delivered to a mixing chamber that divided the total volume equally between two exposure chambers. After the flows of toxicant and water necessary to obtain each desired toxicant concentration were calculated, the flows were set, using a graduated cylinder and stopwatch.

Early life stage studies with fathead minnows and chronic studies with fathead minnows and D. magna were conducted using Mount-Brungs style diluters (Mount and Brungs, 1967). Each diluter delivered approximately 500 mL of exposure solution to each mixing cell, where it was split into two 250-mL volumes and delivered to each of two duplicate aquaria. During the early rearing stage, when the fathead minnow fry were being reared in duplicate rearing chambers, the 250-mL volume was split again so that each chamber received approximately 125 mL.

The delivery rates of the Mount-Brungs diluters were controlled by regulating the water flow into the diluter with a valve during the tests with fathead minnows. Because much lower flows were required for the daphnid studies, the cycling rate of each diluter was controlled by a capillary tube to drain the bucket that operated a microswitch controlling the incoming water. By slowing the flow rate from the bucket, the time interval between cycles was increased, thereby reducing the overall delivery rate.

The toxicant was usually delivered to the diluters from 55-gal polyethylene-lined steel drums. In the fathead minnow tests, a recirculating system incorporating a toxicant head tank behind and slightly above the main mixing cell was used. A capillary tube drained the toxicant from the headtank into a calibrated beaker or graduated cylinder containing a glass siphon connected to the water outflow from the W-1 cell. When the W-1 cell started to drain, a vacuum started the siphon from the beaker containing the toxicant, and both diluent and toxicant entered the mixing cell. The amount of material in the beaker could be varied by raising or lowering the beaker in relation to the toxicant headtank. In addition, the beaker contained a drain to prevent overfilling in case the diluter cycling rate slowed or stopped. The toxicant

Table 3. MEAN, RANGE, AND STANDARD DEVIATION OF WATER QUALITY PARAMETERS MEASURED ROUTINELY IN SRI'S DECHLORINATED TAP WATER

Period: June 1979 to February 1982

Parameter	Units	Mean	S.D.	Range	Number of Analyses
Hardness	mg/L $CaCO_3$	32.4	22.4	7-123	90
Alkalinity	mg/L $CaCO_3$	30.8	18.1	10-110	91
Acidity	mg/L $CaCO_3$	6.0	3.6	5-22.5	34[a]
pH	—	8.1	0.5	6.6-9.0	90
Conductivity	µmhos/cm	82.0	51.4	28-263	87
Total residual chlorine	µg/L	1.9	1.2	0.1-7.0	195[b]

[a] 57 values below detectable limits (< 5 mg/l).

[b] Chlorinity tester inoperative on 25 of the 91 days water quality was tested. Value was below detectable limits 4 times (<0.001 mg/l).

Table 2. CHEMICAL ANALYSIS OF SRI DECHLORINATED TAP WATER (1975)

Analysis	Concentration (mg/L)
Calcium (as Ca)	8.4
Magnesium (as Mg)	2.5
Potassium (as K)	0.40
Sulfate (as SO_4)	9.2
Nitrate (as NO_3-N)	<0.005
Nitrite (as NO_2-N)	0.001
Free ammonia	0.060
Organic ammonia	0.375
Phenol	<0.001
Residual chlorine	<0.003
Chloride	4.04
Fluoride	0.30
Cyanide	<0.01
Iron	0.08
Copper	0.0041
Zinc	0.0026
Cadmium	0.0012
Chromium	0.008
Nickel	<0.050
Lead	0.0007
Total alkalinity (as $CaCO_3$)	23.3
Total hardness (as $CaCO_3$)	31.2
Total dissolved solids	48.0

Table 1. WATER QUALITY CHARACTERISTICS OF WATER FROM HETCH HETCHY, SAN ANTONIO, AND CALAVERAS RESERVOIRS

Measured Parameter	Hetch Hetchy			San Antonio			Calaveras		
	Mean[a]	Range	N[b]	Mean[a]	Range	N[b]	Mean[a]	Range	N[b]
Calcium	1.08	0.3-1.6	7	31.7	23.4-50.5	4	29.3	25.7-34.5	3
Magnesium	0.42	0.0-1.8	7	12.0	8.6-15.1	4	10.4	7.3-13.1	3
Sodium	0.89	0.5-1.3	7	20.9	15.5-30.0	4	9.7	8.5-11.0	3
Potassium	0.34	0.2-0.6	7	2.2	2.0-2.4	4	1.6	0.9-2	3
Bicarbonate	6.0	2.6-9.2	7	131.6	106.6-146.4	4	114	89-146.4	3
Carbonate	0.0	0.0-<0.5	7	3.6	0-9.6	4	0.4	0-1.1	3
Carbonic Acid	2.25	1.9-2.6	2	43	0-86	2	72	-	1
Chloride	0.09	0.1-0.5	7	29.2	13.5-52.1	4	9.5	8-10.5	3
Sulfate	0.51	0.25-1.3	7	26.4	21.8-31.9	4	22.5	18.0-28.3	3
Fluoride	0.008	0.02-0.03	6	0.098	0-0.17	4	0.1	0.1-0.15	3
Aluminum	0.02	0.01-0.05	7	0.035	0.01-0.06	4	0.04	0.01-0.07	3
Arsenic	0.02	<0.001-0.037	2	<0.01	-	4	<0.01	-	3
Barium	<0.5	-	7	<0.5	-	4	<0.5	-	3
Cadmium	<0.002	-	7	<0.002	-	4	<0.002	-	3
Chromium	<0.01	-	7	<0.01	-	4	<0.005	-	3
Copper	0.005	0.0-0.01	4	0.5	<0.01-0.01	4	0.045	<0.01-0.06	2
Iron	0.06	0.005-0.12	5	0.08	<0.01-0.14	3	0.25	<0.01-0.25	1
Lead	<0.05	-	7	<0.05	-	4	<0.02	-	3
Manganese	<0.02	-	7	<0.02	-	4	<0.01	-	3
Selenium	<0.01	-	6	<0.01	-	4	<0.01	-	3
Silver	<0.03	-	7	<0.06	-	4	<0.005	-	3
Zinc	0.04	<0.01-0.06	3	0.016	0.009-0.03	3	0.012	0.003-0.02	2
Silica	3.5	0.2-6.4	7	3.7	0.2-7.0	4	7.9	3-10.5	3
Ammonia	0.03	0.02-0.05	5	0.15	0.02-0.214	2	0.115	0.1-0.13	2
Ammonia (free)	0.05	-	7	0.05	-	4	0.03	0.02-0.04	2
Boron	0.03	0.006-0.07	7	0.17	0.005-0.25	4	0.075	0.025-0.13	3
Cyanide	0.01	-	6	0.01	-	3	0.001	-	2
Nitrate	0.5	-	7	1.3	0.05-2.9	3	2.4	0.05-2.4	1
Nitrite	-	0.001-0.004	7	0.014	0.001-0.014	1	0.003	0.001-0.003	1
Phosphate	0.01	0-0.025	3	0.11		3	0.05	0.04-0.08	3
Tannins & Lignins	0.06	0.05-0.1	4	0.075	0.01-0.25	2	0.05	0.05-0.05	1
Dissolved oxygen	8.6	7.6-10.3	6	7.8	0.05-0.1	3	9.5	9.1-10.1	3
Total apparent ABS	0.05	-	7	0.05	5.9-9.0	4	0.05	-	3
Hardness (as $CaCO_3$)	3.6	3.0-4.6	7	128.4	-	4	116	99.0-130	3
Alkalinity (as $CaCO_3$)	5.1	2.6-7.5	7	107.5	94-163	4	98.8	87.3-120	3
Total solids	11.0	6.9-17	7	206.8	71-143	4	162.3	155-170	3
Conductivity (μmhos/cm)	14.3	8-20	7	334.8	196-216	4	262.3	252-283	3
pH (units)	7.1	7.0-7.2	7	8.1	259-368	4	8.1	7.9-8.2	3
Turbidity (units)	0.89	0.11-4	7	2.4	7.6-8.8	4	2.4	0.3-6.4	3
Color (units)	1.6	0-5	7	5.0	0.1-6	4	23.3	0-50	3
C chloroform extract (μg/L)	-	-	7	85.6	0-10	1	-	-	-
Radioactivity (μci/g)									
Alpha	0.43	0.3-0.6	3	0.6	-	1	-	-	-
Beta	1.8	0.6-3	2	7	-	1	-	-	-

[a] mg/L except where marked otherwise.
[b] Number of years the specified parameter was measured.

late spring through the fall, about 95% of the water comes from Hetch Hetchy Reservoir. During the winter and early spring before the snow begins to melt, the blend is composed primarily of water from the two low-elevation reservoirs.

The San Francisco Water Department (SFWD) annually analyzes the water from these reservoirs for various minerals and other constituents. Table 1 presents the average and range for each of the 43 parameters measured by the SFWD during the periods 1969 to 1971 and 1975 to 1978 in water samples from the three reservoirs.

We performed a less comprehensive analysis of our dechlorinated tap water in 1975. The results are presented in Table 2. In 1978, we began analyzing the dechlorinated tap water routinely for hardness, alkalinity, acidity, pH, conductivity, and residual chlorine. Table 3 presents the average, standard deviation, and range for each of these parameters during the study period.

Over the seven years that our aquatic toxicology facility has been in operation, our dechlorinated tap water has been satisfactory for rearing and maintaining a variety of aquatic animals. However, during Phases I to III of this study, we experienced intermittent problems with unacceptably high control mortality (>20%) during tests with daphnids. When this occurred, we repeated the tests until acceptable results were obtained. The problem was later determined to be caused by seasonal fluctuations in the hardness of the diluent water. Depending on the mixture of waters obtained from the three storage reservoirs, hardness dropped to levels as low as 15 mg/L (as $CaCO_3$). The periods in which the laboratory received very soft water were found to correspond to the periods in which we observed poor daphnid survival. To ensure that these fluctuations in the hardness of the diluent water did not adversely affect the results of the daphnid chronic studies, a solution of magnesium, calcium, and potassium salts (Marking and Dawson, 1973) was metered into each diluter at a rate sufficient to maintain a minimum hardness of 35 to 40 mg/L.

Temperature Control

To maintain the temperature of the exposure chambers at the desired level, both the water and room temperatures were controlled. Room temperatures were controlled by thermostatically controlled heat pumps set at the desired test temperature. In tests requiring heated diluent water, a thermostatically controlled 2000-W stainless-steel immersion heater was used to adjust the temperature of the incoming water in head tanks before the water entered the diluter. In tests requiring water at lower-than-ambient temperatures, chilled water was supplied to a head tank by a 9000-BTU water chiller at the approximate test temperature and was then maintained at the desired temperature by a thermostatically controlled 2000-W immersion heater.

Toxicant Dilution Equipment

In the early life stage studies on catfish and trout, we used a toxicant dilution system developed at SRI for conducting short-term tests. The system is composed of two constant-head reservoirs, one located above the

MATERIALS AND EQUIPMENT

Test Materials

The substances tested in this study were condensate water, 2,4-DNT, and photolyzed condensate water (Cond-Irrad). The sources and purities of the test materials and the methods used to prepare photolyzed and unphotolyzed condensate water are described in Volume II (Liu et al. 1984).

Test Organisms

The following species of fish and invertebrates were used:

Fathead minnow (Pimephales promelas)
Channel catfish (Ictalurus punctatus)
Rainbow trout (Salmo gairdnerii)
Water flea (Daphnia magna)

These species were selected because they represent a range of taxonomic groups that have different habitat requirements and also exhibit varying sensitivities to chemical toxicants. Consequently, it was felt that water quality criteria based on tests with these species should afford a reasonable degree of protection for most, if not all, species undergoing exposure to munitions wastewaters. In addition, test methods for the selected species were fairly well established which increased the probability of successfully conducting the laboratory exposures.

Fathead minnows and Daphnia magna were obtained from SRI's breeding colonies. The breeding stocks were reared under flow-through conditions at 20 and 25°C for the daphnids and minnows, respectively. The adult minnows were fed frozen adult brine shrimp (San Francisco Bay Brand, Newark, CA), live daphnids, and trout chow (Clark's Feed Company, Salt Lake City, UT). Adult daphnids were fed Selenastrum capricornutum alone or in conjunction with a vitamin supplement (Goulden et al., 1982). The photoperiod was set at 16 hours light (100 ft. candles) and 8 hours dark. Channel catfish eggs were obtained from Alex Fish Company, San Rafael, CA, and rainbow trout eggs were obtained from the Mt. Lassen Trout Farm, Red Bluff, CA.

Diluent Water

We used dechlorinated tap water to culture and maintain the test animals, to prepare the stock solutions, and as the diluent water for the flow-through tests. The water was dechlorinated by passing it through a series of columns, each containing 0.042 m^3 of activated carbon that was renewed every 3 months by a local water purification firm (Culligan, Santa Clara, CA).

The laboratory tap water is a blend from the Hetch Hetchy, Calaveras, and San Antonio Reservoirs. On the average, about 75% of the water originates from the Hetch Hetchy Reservoir, which is located in the Sierra Nevada. From

Note that the error bars depend only on the sample size and the number alive pooled over chambers. Consequently, in a plot of aquaria survival proportions, an aquarium with widely differing survival proportions in its two chambers can have error bars similar to another aquarium where the two chambers have similar proportions.

Error bars were not plotted for the global indices of total survivability and total productivity, two variables that are composites of several other variables. The total survivability index was defined as the product of the cumulative F_0 survival to 180 days, the average number of eggs per female, and the cumulative F_1 survival after 60 days of exposure. The total productivity index was defined as the product of the total survivability index and the average weight of the F_1 generation after 60 days of exposure. Because an estimate of the variability for each aquarium would have required the questionable assumption that the component variables were all mutually independent, it was decided that error bars in this case might be misleading. Note that the statistical tests were performed using the mean squared error from a one-way ANOVA, allowing sufficient degrees of freedom for these variables.

Global Indices

In addition to the two global indices defined above, five additional such indices were used in analyzing the data from the fathead minnow chronic studies. These indices were defined as follows:

F_0 90-day cumulative fry survival--fry survival at the end of 90 days as a proportion of the number of embryos exposed.

F_0 178-day cumulative fry survival--fry survival at the end of the juvenile growth phase (up to the point where the sexes were identified and breeding pairs established) as a proportion of the number of embryos exposed.

F_0 60-day biomass--the product of length at 60 days and 60-day fry survival.

F_1 60-day cumulative fry survival--fry survival at the end of 60 days as a proportion of the number of embryos exposed.

F_1 60-day standing crop--the product of weight at 60 days and 60 day fry survival.

RESULTS AND DISCUSSION

Early Life Stage Studies

Early life stage studies were conducted with channel catfish, rainbow trout, and fathead minnows.

Channel Catfish

Early life stage studies with channel catfish were not successful. The eggs could not be separated because of their advanced stage of development; heavy losses occurred due to fungal infection in spite of prophylactic treatment with malachite green. In addition, because we had to start the tests with clumps of eggs, the initial number of eggs varied markedly among the treatment groups. Nonetheless, data on egg hatchability and fry survival from tests performed on 2,4-DNT and condensate water are shown in Tables 4 and 5, respectively.

Table 4. EFFECT OF 30 DAYS OF EXPOSURE TO 2,4-DNT ON CHANNEL CATFISH EGGS AND FRY

Mean Measured Concentration (mg/L)	Test Series	Eggs		Fry	
		Initial Number	% Hatched	Number Hatched	% Survival
Control	A	19	52.6	10	90.0
	B	31	67.7	21	52.4
3.4	A	57	71.9	41	34.1
	B	75	40.0	30	16.7
6.2	A	31	19.4	6	16.7
	B	53	32.1	17	29.4
8.7	A	71	29.6	21	0
	B	36	16.7	6	0
23.7	A	49	4.1	2	0
	B	51	43.1	22	0
32.6	A	67	3.0	2	0
	B	38	0	0	0

Table 5. EFFECT OF 30 DAYS OF EXPOSURE TO CONDENSATE WATER ON CHANNEL CATFISH EGGS AND FRY

Mean Measured Concentration (mg/L)	Test Series	Eggs		Fry	
		Initial Number	% Hatch	Number Hatched	% Survival
Control	A	23/40	57.5	23	39.1
	B	26/51	51.0	26	69.2
Solvent control[a]	A	28/61	45.9	28	50.0
	B	17/60	28.3	17	47.0
0.55	A	40/59	67.8	40	80.0
	B	37/68	54.4	37	59.4
0.92	A	19/58	32.8	19	94.7
	B	35/59	59.3	35	82.8
1.72	A	38/55	60.0	33	97.0
	B	40/62	64.5	40	97.5
4.35	A	26/47	55.3	26	3.8
	B	26/60	43.3	26	11.6
9.60	A	33/60	55.0	33	0
	B	11/49	22.4	11	0

[a] 40 µL/L acetone.

Although the data are highly variable and permit only the grossest inferences to be made, 2,4-DNT appeared to reduce hatching success at concentrations of 6.2 to 32.6 mg/L and fry survival at concentrations as low as 3.4 mg/L. Condensate water did not have a demonstrable effect on egg survival within an exposure range of 0.55 to 9.60 mg/L but appeared to reduce fry survival at concentrations of 4.35 to 9.60 mg/L. In both of these tests, the eggs were exposed for approximately 10 days and the fry for the remainder of the 30-day exposure period.

Rainbow Trout

The effects of 2,4-DNT on egg hatchability and fry survival and growth are shown in Table 6. 2,4-DNT did not affect hatching success or fry survival within the range of concentrations tested (0.10 to 2.05 mg/L). However, the pattern of effects on growth suggests a concentration-related response, particularly at the three highest concentrations (0.49 to 2.05 mg/L). The only exception to the overall trend was the relatively good growth that occurred in fish exposed to 0.23 mg/L. However, this may have been due, in part, to the relatively few fish present at this concentration. If we consider only the three highest concentrations, length was reduced an average of 10.2, 20.0, and 23.2 percent, while weight was reduced by 26.6, 45.0, and 51.7 percent. Although the overall density of fry in each tank was rather variable, the overall trends in growth effects as well as the magnitude of these effects suggests that, at least within a concentration range of 0.49 to 2.05 mg/L 2,4-DNT, the observed reductions in growth were toxicant-related.

The effect of condensate water on egg hatchability and fry survival and growth are shown in Table 7. Condensate water did not appear to affect egg hatching success or fry survival within a concentration range of 0.10 to 1.96 mg/L. However, growth was significantly affected in both series at concentrations of 0.43 to 1.96 mg/L and in Series B at 0.22 mg/L. These effects were marked. Length was reduced an average of 10.1, 13.6, and 32.3 percent in the pooled series at concentrations of 0.43, 0.88, and 1.96 mg/L, respectively. Weight was similarly reduced at these concentrations by 37.8, 26.9, and 62.2 percent. In Series B at 0.22 mg/L, length and weight were reduced by 8.1 and 25.0 percent, respectively, compared with the control.

Table 6. EGG HATCHABILITY, FRY SURVIVAL, AND FRY GROWTH IN RAINBOW TROUT EXPOSED TO 2,4-DNT FOR 60 DAYS

Concentration (mg/L)		Test	Eggs		Fry Alive	Average Fry Length	Average Fry Weight
Mean	S.D.	Series	No. Exposed	No. Hatched	at 60 Days	(cm)	(g)
0.00	—	A	60	53	12	2.67	0.164
		B	60	54	16	2.72	0.167
0.10	0.03	A	60	59	23	2.57	0.128[a]
		B	60	59	35	2.53	0.132[a]
0.23	0.06	A	60	40	9	2.45[a]	0.169
		B	61	43	9	2.71	0.187
0.49	0.07	A	60	57	17	2.38[a]	0.124[a]
		B	60	57	21	2.46[a]	0.119[a]
1.08	0.15	A	60	52	37	2.07[a]	0.075[a]
		B	61	48	28	2.24[a]	0.107[a]
2.05	0.23	A	60	56	33	2.07[a]	0.078[a]
		B	60	60	31	2.07[a]	0.082[a]

[a] Statistically significant, $p < 0.05$.

Table 7. EGG HATCHABILITY, FRY SURVIVAL AND FRY GROWTH IN RAINBOW TROUT EXPOSED TO CONDENSATE WATER FOR 60 DAYS

Concentration (mg/L) Mean	S.D.	Test Series	Eggs No. Exposed	Eggs No. Hatched	Fry Alive at 60 Days	Average Fry Length (cm)	Average Fry Weight (g)
0.00	—	A	60	57	26	2.49	0.121
		B	61	60	18	2.48	0.128
0.10	0.02	A	62	51[a]	11	2.68	0.172
		B	60	49[a]	22	2.57	0.137
0.22	0.05	A	61	39[a]	15	2.41	0.137
		B	60	39[a]	32	2.28[a]	0.096[a]
0.43	0.06	A	61	54	30	2.26[a]	0.080[a]
		B	60	58	45	2.16[a]	0.075[a]
0.88	0.19	A	63	57	14	2.11[a]	0.090[a]
		B	61	60	15	2.14[a]	0.092[a]
1.96	0.42	A	62	55	24	1.66[a]	0.045[a]
		B	60	54	14	1.67[a]	0.049[a]

[a] Statistically significant, $p < 0.05$.

Although hatching success in these two tests was generally quite good, fry survival was relatively low, averaging 26 and 38%, respectively, for the 2,4-DNT and condensate controls over the 60-day exposure period. Because of this occurrence and also because of problems with nonrandomization of gametes and lack of any apparent toxicant-related effects on egg-hatching success and fry survival, another test was performed on 2,4-DNT using an extended concentration range and eggs that were randomized over the treatment levels. Data from this test are summarized in Table 8.

Table 8. EFFECT OF 2,4-DNT ON RAINBOW TROUT EGG HATCHING SUCCESS AND FRY SURVIVAL AND GROWTH DURING A 90-DAY EXPOSURE PERIOD

Concentration (mg/L)		Test	Eggs		Fry		Average Length	Average Weight
Mean	S.D.	Series	No. Exposed	No. Hatched	No. Deformed	No. Alive at 90 Days	(cm)	(g)
0.00	—	A	60	34	6	25	4.78	0.942
		B	60	41	1	34	4.50	0.815
0.05	0.03	A	60	46	0	32	4.54[a]	0.830[a]
		B	60	48	0	44	4.36	0.720
0.12	0.05	A	55	47	4	36	4.50[a]	0.775[a]
		B	55	47	6	32	4.66	0.880
0.27	0.11	A	55	32	1	28	4.56[a]	0.921
		B	55	34	1	30	4.60	0.960
0.56	0.24	A	60	41	0	30	4.37[a]	0.745[a]
		B	60	43	0	37	4.33	0.746
1.17	0.54	A	60	46	4	40	4.08[a]	0.665[a]
		B	60	45	3	42	4.24[a]	0.730
2.26	0.73	A	60	52	2	46	3.82[a]	0.492[a]
		B	60	47	2	35	3.97[a]	0.582[a]
4.02	1.30	A	60	52	3	26	3.14[a]	0.287[a]
		B	60	47	1	36	3.03[a]	0.302[a]

[a] Statistically significant, $p < 0.05$.

Although egg hatching success was appreciably lower than observed in the two previous tests, fry survival was much better. The lower hatching success was probably due to the fact that the eggs were obtained late in the spawning season which made their viability quite varible.

There were no statistically significant effects on egg survival, number of deformed fry, or fry survival within the range of concentrations of 2,4-DNT tested (0.05 to 4.02 mg/L). However, all of the fry in both series in the highest concentration (4.02 mg/L) were unable to swim and remained on their sides at the bottom of the aquaria. On the basis of this observation, we conclude that 2,4-DNT at a concentration of 4.02 mg/L would have affected fry survival under natural conditions.

Fry length 60 days after hatching was reduced in Series A at all concentrations (0.05 to 4.02 mg/L) and in Series B at 1.17 to 4.02 mg/L. For the pooled series, the reductions in length were approximately 10.3, 16.1, and 33.5 percent at concentrations of 1.17, 2.26, and 4.02 mg/L, respectively. In Series A, the effects at 0.05, 0.12, 0.27, and 0.56 mg/L amounted to reductions of approximately 5.0, 5.9, 4.6, and 8.6 percent, respectively, compared with the control.

Fry weight was similarly affected by 2,4-DNT in this test. Significant reductions occurred in Series A at 0.05, 0.12, 0.56, 1.17, 2.26, and 4.02 mg/L and in Series B at 2.26 and 4.02 mg/L. For the pooled series, the reductions were approximately 38.9 and 66.5 percent at 2.26 and 4.02 mg/L, respectively. In Series A, the reductions in weight were approximately 11.9, 17.7, 20.9, and 29.4 percent at concentrations of 0.05, 0.12, 0.56, and 1.17 mg/L. Weight was also reduced in Series A at 0.27 mg/L compared with the control but the effect was not significant.

Water-quality data associated with the trout early life stage studies on 2,4-DNT and condensate water are summarized in Tables 9 and 10.

Table 9. WATER-QUALITY DATA ASSOCIATED WITH THE RAINBOW TROUT EARLY LIFE STAGE STUDIES WITH 2,4-DNT

Test 1

Test Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n
Control	9.8	1.1	7.8-10.8	6	7.5	0.4	6.9-8.0	9	12.7	0.6	11.8-13.8	50	132	91	62-270	9	64	42	24-110	6	48	33	20-100	8
0.10	9.2	1.2	7.9-10.4	3	7.4	0.2	7.1-7.6	4			—		112	76	65-225	4	49	36	26-90	3	40	17	30-60	3
0.23	9.3	1.2	8.0-10.4	3	7.4	0.2	7.1-7.6	4			—		111	73	65-220	4	49	36	26-90	3	43	23	30-70	3
0.49	9.3	1.3	8.0-10.6	3	7.5	0.3	7.1-7.8	4			—		113	79	63-230	4	48	36	24-90	3	43	23	30-70	3
1.08	9.3	1.2	8.0-10.4	3	7.5	0.3	7.1-7.8	4			—		114	78	65-230	4	49	36	26-90	3	43	23	30-70	3
2.05	9.3	1.2	8.0-10.4	3	7.5	0.3	7.1-7.8	4			—		111	73	65-220	4	52	42	26-100	3	43	23	30-70	3

Test 2

Control	11.6	0.8	11.0-12.2	2[a]	7.0	0.2	6.8-7.4	14	11.3	1.0	10.0-13.0	50	96	46	35-160	12	33	18	10-60	14	32	15	16-58	14
0.05	9.6	0.8	9.0-10.2	2	6.9	0.2	6.8-7.2	3			—		40			1	16	0	—	2	16	0	—	2
0.12	9.7	0.7	9.2-10.2	2	7.0	0.2	6.8-7.3	3			—		39			1	14	3	12-16	2	16	1	15-16	2
0.23	9.5	0.7	9.0-10.0	2	7.0	0.3	6.7-7.3	3			—		40			1	16	2	14-17	2	16	1	15-16	2
0.56	9.3	0.7	8.8-9.8	2	7.0	0.4	6.7-7.4	3			—		40			1	16	1	15-16	2	16	1	15-16	2
1.17	9.2	0.6	8.8-9.6	2	7.0	0.4	6.7-7.4	3			—		35			1	15	3	13-17	2	15	1	14-16	2
2.26	9.3	0.4	9.0-9.6	2	7.0	0.4	6.7-7.4	3			—		35			1	14	4	12-17	2	16	1	15-16	2
4.02	9.3	0.4	9.0-9.6	2	7.0	0.4	6.7-7.4	3			—		40			1	15	1	14-16	2	16	0	—	2

[a]Dissolved oxygen meter broken during test.

Table 10. WATER-QUALITY DATA ASSOCIATED WITH THE RAINBOW TROUT EARLY LIFE STAGE STUDY WITH CONDENSATE WATER

Test Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n
Control	9.8	1.1	8.0-10.6	5	7.7	0.4	7.1-8.3	9	12.7	0.6	11.8-13.8	50	132	90	61-270	9	65	34	26-100	5	52	37	20-100	8
0.10	9.2	1.2	8.0-10.5	3	7.6	0.4	7.1-8.1	4			—		115	84	65-240	4	49	36	26-90	3	50	44	20-100	3
0.22	9.3	1.3	8.0-10.6	3	7.6	0.4	7.1-8.1	4			—		115	84	65-240	4	49	36	26-90	3	50	35	30-90	3
0.43	9.3	1.2	8.0-10.4	3	7.6	0.4	7.1-8.0	4			—		118	89	65-250	4	52	42	26-100	3	53	40	30-100	3
0.88	9.3	1.2	8.0-10.4	3	7.6	0.3	7.2-8.0	4			—		113	79	62-230	4	52	42	26-100	3	50	35	30-90	3
1.96	9.3	1.2	8.0-10.4	3	7.6	0.3	7.2-8.0	4			—		113	79	61-230	4	52	42	26-200	3	48	36	25-90	3

Fathead Minnows

The results of the 30-day early life stage study on fathead minnows exposed to 2,4-DNT are summarized in Table 11. The data from this test were not statistically analyzed since it was intended primarily as a range-finding test for the subsequent chronic study. In spite of the uniformly low hatching success (approximately 50 to 60 percent), 2,4-DNT had no appreciable effect on egg hatchability and fry growth and survival except at the highest concentration (6.8 mg/L), which markedly reduced all of these parameters compared with the controls.

Table 11. EFFECT OF 2,4-DNT ON FATHEAD MINNOW EGG HATCHABILITY AND FRY SURVIVAL AND GROWTH DURING A 30-DAY EARLY LIFE STAGE STUDY

Chemical Concentration (mg/L)		Test	No. of Eggs	Fry	Fry
$\bar{x}$	SD	Series	Hatched (out of 60)	Survival	Length (cm)
0	—	A	34	34	1.3
		B	33	32	1.1
1.0	0.23	A	36	35	1.3
		B	48	42	1.2
1.1	0.30	A	40	35	1.2
		B	37	34	1.3
2.0	0.44	A	31	29	1.3
		B	34	33	1.2
3.1	1.16	A	31	28	1.3
		B	28	24	1.2
6.8	1.55	A	18	9	0.8
		B	16	3	0.7

A similar test was performed on condensate water. Because of insufficient numbers of embryos, this test was not performed in duplicate. These data are shown in Table 12. As with the test on 2,4-DNT, the data were

Table 12. EFFECT OF CONDENSATE WATER ON FATHEAD MINNOW EGG HATCHABILITY AND FRY SURVIVAL AND GROWTH DURING A 30-DAY EARLY LIFE STAGE STUDY

Chemical Concentration (mg/L)		No. of Eggs	Fry	Fry	Fry
X	SD	Hatched (out of 60)	Survival	Length (cm)	Weight (gm)
0	—	57	31	0.98	0.013
0.6	0.3	60	32	0.95	0.008
1.4	0.7	60	22	1.12	0.015
2.0	0.9	60	7	1.17	0.022
3.1	1.4	60	8	1.14	0.019
6.9	3.8	60	1	1.10	0.010

not statistically analyzed. However, condensate water had no apparent effect on egg hatchability at a concenration range of 0.6 to 6.9 mg/L. Fry survival appeared reduced at 1.4 to 6.9 mg/L. There were no apparent effects on fry growth, but such effects could have been neutralized by the reduced density of fry in most of the treated aquaria. As evidenced by the large standard deviations associated with the mean chemical concentrations, the concentrations in the test tanks fluctuated. This was due primarily to problems with microbial growth in reducing the flow of toxicant in the capillary tube that connected the toxicant head tank to the toxicant metering device. This tended to reduce the toxicant flow rate and lower the test concentrations.

Water-quality data associated with these tests are summarized in Tables 13 and 14.

Table 13. WATER QUALITY DATA ASSOCIATED WITH THE FATHEAD MINNOW EARLY LIFE STAGE STUDY WITH 2,4-DNT

Test Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n
Control	7.6	0.15	7.3-7.8	10	7.6	0.17	7.4-7.8	4	26.3	0.58	25.4-27.4	25	37.2	4.4	33-43	4	14.5	1.91	12-16	4	23.1	3.75	20-28	4
1.0	7.6	0.15	7.4-7.8	10		--				--				--				--				--		
1.1	7.6	0.16	7.4-7.8	10		--				--				--				--				--		
2.0	7.6	0.14	7.4-7.9	10		--				--				--				--				--		
3.1	7.7	0.14	7.4-7.8	10		--				--				--				--						
6.8	7.5	0.27	6.9-7.8	10	7.4	0.08	7.3-7.5	4					25.5	14.4	31-40	4	15.3	2.99	12-19	4	19.4	7.18	15-30	4

Table 14. WATER QUALITY DATA ASSOCIATED WITH THE FATHEAD MINNOW EARLY LIFE STAGE STUDY WITH CONDENSATE WATER

Test Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n
Control	7.8	1.05	6.8-9.6	5	7.8	0.25	7.4-8.0	5	22.9	0.97	21.8-24.4	25	34.8	6.34	29-45	5	14.0	1.41	12-16	5	20.0	3.54	15-25	5
0.6	8.1	0.88	7.3-9.6	5		--				--				--				--				--		
1.4	7.9	0.93	7.2-9.5	5		--				--				--				--				--		
2.0	8.0	0.84	7.4-9.5	5		--				--				--				--				--		
3.1	8.1	0.73	7.7-9.4	5		--				--				--				--				--		
6.9	8.0	0.77	7.5-9.4	5	8.1	0.13	8.0-8.3	5					31.4	2.61	28-35	5	14.2	1.48	12-16	5	16.0	2.24	15.20	5

Chronic Studies

Chronic studies were conducted with fathead minnows and *Daphnia magna*.

Fathead Minnows

2,4-DNT. The effects of 2,4-DNT on egg hatching success and fry survival, deformities, and growth during a 179-day period are shown in Table 15. The number of eggs that survived to hatch was reduced at concentrations of 2.69 and 6.71 mg/L in series B compared with the controls. Fry survival was reduced in Series A at concentrations of 2.69 and 6.71 mg/L over an exposure period of 30 to 179 days. Fry survival was reduced in Series B at a concentration of 6.71 mg/L over an exposure period of 30 to 179 days and at a concentration of 2.69 mg/L after an exposure period of 179 days.

The effect of 2,4-DNT on fry length in Series A was as follows: the reduction at a concentration of 0.29 mg/L was significant over the first 60 days of exposure, but not after 90 days; the reduction at 2.69 mg/L was significant after the first 30 days of exposure, but not after 60 and 90 days; the reduction at 6.71 mg/L was significant over the first 60 days of exposure and there were no survivors thereafter. In Series B, the reduction in fry length was significant at concentrations of 2.69 and 6.71 mg/L over the first 30 days of exposure but not at 60 or 90 days; it was also significantly reduced at 1.31 mg/L after 30, 60, and 90 days of exposure. This reduction could have been concentration-related because the lack of effect of growth in series B at higher concentrations was associated with comparatively fewer fry in these tanks, which could have mitigated any small toxicant-related effect on growth.

The effect of 2,4-DNT on reproduction is shown in Table 16. These data indicate that although the survival of the spawning pairs was not significantly affected over the range of exposure, their ability to produce eggs was significantly reduced at concentrations as low as 1.31 mg/L. In the pooled series, eggs per spawn was significantly reduced at 0.62 mg/L. The data also suggest that the reduction in reproductive capability extended to the lowest concentration tested, 0.28 mg/L.

The survival of F_1 fry exposed to condensate water for 30 days was significantly reduced for the B series at a concentration of 0.19 mg/L and for the A series at 0.34 mg/L; at both of these concentrations, survival was significantly reduced when the series were pooled. However, survival in both series at a concentration of 0.75 mg/L was similar to the control value, leading one to believe that the results in intermediate concentrations may be related to factors other than dose level.

Survival of F_1 fry reared to 60 days was significantly reduced only in the A series at a concentration of 0.34 mg/L. Because it was not significantly reduced in the B or pooled series at this level or in either series at a concentration of 0.75 mg/L, there does not appear to be any concentration-related effect on survival.

The length and weight of F_1 fry reared to 30 days was significantly reduced in the A series at 0.19 mg/L. This does not appear to be a dose-related effect because there is no significant reduction in length or weight relative to controls in either series at concentrations of 0.34 and 0.75 mg/L.

The length of F_1 fry reared to 60 days was significantly reduced in the A series at concentrations of 0.19 and 0.75 mg/L, in the B series at 0.34 and 0.75 mg/L and in the pooled series at 0.19, 0.34, and 0.75 mg/L, which were the only concentrations tested. There was a definite trend toward shorter length with increase in concentration in the B series, but such a definite trend was not seen in the A series.

The weight of F_1 fry reared to 60 days was significantly reduced in the A series at a concentration of 0.19 mg/L, in the B series at 0.19, 0.34, and 0.75 mg/L (all the concentrations tested), and in the pooled series at 0.19 and 0.75 mg/L. The trends seen in length of F_1 fry raised to 60 days were repeated here, with the B series showing a definite downward trend in weight as concentration increased and the A series showing the most serious reduction at 0.19 mg/L, then reaching a value greater than that of the control at 0.34 mg/L, and again falling lower than the control at 0.75 mg/L. Although the effects on growth are suggestive of deleterious toxicant activity at the lower concentrations, interactions between growth and fry density could be responsible for at least part of the observed responses.

The effects of condensate on the global indices of total survivability and total productivity are shown in Figures 3 and 4 and in Table 24. The A series shows a general trend toward diminished survivability as the concentration levels increase, whereas the B series shows less effect. The same general trends are seen in the productivity index.

Table 23. EFFECT OF CHRONIC EXPOSURE OF CONDENSATE WATER ON F_1 FATHEAD MINNOWS

Mean Measured Concentration (mg/L)	Test Series	Eggs			Fry							
					30-Day				60-Day			
		No. Exposed	No. Hatched	No. Deformed	No. Transfer	No. Survive	Avg. Length (cm)	Avg. Weight (g)	No. Transfer	No. Survive	Avg. Length (cm)	Avg. Weight (g)
0.00	A	1550	1257	80	76	74	2.03	0.084	69	61	2.83	0.180
	B	1200	951	60	81	78	1.90	0.067	70	46	2.88	0.215
0.19	A	750	634	10	39	35[b]	1.80[a]	0.054[a]	90	86	2.57[a]	0.145[a]
	B	1518	1304	26	86	71[a]	2.01	0.066[b]	90	44	2.72[b]	0.153[a]
0.34	A	1550	1291	57	90	55[a]	2.22	0.100	87	47[a]	2.88[b]	0.219
	B	1200	1023	39	80	74[b]	1.93	0.060	88	75	2.57[a]	0.159[a]
0.75	A	1000	795[b]	21	74	69	2.07	0.087	89	73	2.67[a]	0.158[b]
	B	1253	905[a]	31	73	69	1.92	0.068	93	75	2.56[a]	0.141[a]
2.06	A	—										
	B	--										
4.73	A	--										
	B	--										

[a]Statistically significant, $p < 0.05$.

[b]Statistically significant, $p < 0.05$, if series are pooled.

Table 22. THE EFFECT OF CHRONIC EXPOSURE OF CONDENSATE WATER ON REPRODUCTIVE PARAMETERS IN FATHEAD MINNOWS

Mean Measured Concentration (mg/L)	Test Series	Spawning Pair Survival (Days)	Spawns/Pair	Eggs/Pair	Eggs/Spawn	Eggs/Pair/Day
0.00	A	131	22	4004	195	30
	B	130	17	2926	189	22
0.19	A	105	11	1819[a]	165	17
	B	130	24	3881	154	30
0.34	A	89	23	3488	163	39
	B	115	16	2918	170	25
0.75	A	120	16	2172	146	18
	B	130	18	2757	141	21
2.06	A	--	--	--	--	--
	B	130	0[a]	0[a]	0[a]	0[a]
4.73	A	--	--	--	--	--
	B	--	--	--	--	--

[a]Statistically significant, $p < 0.05$.

Table 21. THE EFFECT OF CHRONIC EXPOSURE OF CONDENSATE WATER ON EGG HATCHABILITY AND FRY SURVIVAL AND GROWTH IN FATHEAD MINNOWS

Mean Measured Concentration (mg/L)	Test Series	Eggs		Fry										
		No. Exposed	No. Hatched	No. Deformed	No. Transfer	Survival (days) 30	60	90	120	150	185	Length (cm) 30	60	90
0.00	A	120	113	2	90	72	70	70	70	69	64	2.06	3.09	3.68
	B	120	116	3	90	60	59	59	59	58	54	1.98	3.37	3.97
0.19	A	120	116	5	90	72	71	71	68	67	65	2.04	3.05	3.68
	B	120	117	2	90	57	55	55	55	55	54	2.14	3.46	4.01
0.34	A	120	107	2	90	73	73	73	72	72	70	1.95	3.00	3.64
	B	120	116	2	90	43	43	43	43	43	43	2.14	3.64	4.21
0.75	A	120	113	4	90	41[a]	41[a]	41[a]	40[a]	40[a]	36[a]	1.83[a]	3.34	3.93
	B	120	115	5	90	34[a]	34[a]	34[a]	33[a]	32[a]	30[a]	2.01[b]	3.67	4.31
2.06	A	120	106[b]	68[a]	38	2[a]	2[a]	2[a]	1[a]	1[a]	1[a]	2.45	3.55	4.60
	B	120	108[b]	75[a]	33	5[a]	3[a]	2[a]	2[a]	2[a]	2[a]	1.75	3.47	4.30
4.73	A	120	76[a]	63[a]	13	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]			
	B	120	85[a]	85[a]	0	0	0	0	0	0	0			

[a]Statistically significant, $p < 0.05$.

[b]Statistically significant, $p < 0.05$, if series are pooled.

Condensate water. The effects of condensate water on egg-hatching success and on fry survival, deformities, and growth of fathead minnows during a 185-day period are shown in Table 21. The number of eggs which survived to hatch was significantly reduced at a concentration of 4.73 mg/L in both series. The number of deformed F_0 fry was greatly increased in concentrations of 2.08 and 4.73 mg/L in both series. Survival of F_0 fry was significantly reduced in both series at concentrations from 0.75 to 4.73 mg/L at 30, 60, 90, 120, 150, and 185 days. Length was significantly reduced in F_0 fry after 30 days in the A series at a concentration of 0.75 mg/L; however, this may have been an artifact because no significant reduction was observed at any other concentration or time. In fact, at 60 and 90 days there is a trend toward an increase in length with increase in concentration. This may be a density dependent phenomenon; the number of surviving fry was reduced in the higher concentrations.

The effect of condensate on reproduction is shown in Table 22. Spawning could not be established in Series A at a concentration of 2.06 mg/L or in either series at a concentration of 4.73 mg/L due to high F_0 mortality at these concentrations. Only one pair was available for spawning in the B series at a concentration of 2.06 mg/L, and this pair produced no eggs. Consequently, there was a significant reduction, compared with controls, in productivity at this concentration as measured by all the reproductive parameters used except the number of days that the breeders survived. At concentrations of 0.19, 0.34, and 0.75 there was no significant reduction, compared with controls, in spawning pair survival, spawns per pair, eggs per spawn, or eggs per pair per day in either series. There was a significant reduction in eggs per pair at a concentration of 0.19 mg/L in the A series. However, this effect does not appear to be dose-related because there is no reduction in the B series at this concentration or in either series at concentrations of 0.34 and 0.75 mg/L.

The effect of condensate water on the F_1 generation is shown in Table 23. The number of eggs surviving to hatch was significantly reduced in the B series and the pooled series at a concentration of 0.75 mg/L. No significant increase in F_1 fry deformity is seen in any concentration in which eggs were produced. In fact, the controls had the highest rate of deformed fry, approximately 6 percent of those that hatched.

Table 20. WATER QUALITY DATA ASSOCIATED WITH THE FATHEAD MINNOW CHRONIC STUDY WITH 2,4-DNT

Mean Measured Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n
Control	6.8	1.2	2.3-8.7	43	7.7	0.2	7.2-8.2	41	25.3	0.5	24.5-26.0	32	105	65	39-290	41	32	16	14-70	34	30	15	13-75	38
0.28	6.5	1.2	2.2-8.7	43	7.6	0.3	7.1-8.1	41	25.3	0.5	24.0-26.0	29	106	67	33-300	41			--				--	
0.62	6.5	1.2	1.9-8.6	43	7.6	0.3	7.0-8.1	41	25.1	0.4	24.0-26.0	21	106	66	34-300	41			--				--	
1.31	6.2	1.2	2.0-8.9	43	7.5	0.3	7.0-8.1	41	25.1	0.4	24.0-26.0	21	106	65	34-295	41			--				--	
7.69	6.1	1.2	1.8-8.3	43	7.5	0.3	7.0-8.1	41	25.1	0.5	24.0-26.0	19	105	65	33-300	41			--				--	
6.71	7.1	1.2	3.2-8.9	43	7.6	0.3	6.8-8.3	41	25.0	0.4	24.0-25.5	20	103	64	32-295	41			--				--	

Table 19. MEASURED CHEMICAL CONCENTRATIONS ASSOCIATED WITH THE FATHEAD MINNOW CHRONIC STUDY WITH 2,4-DNT

Nominal Concentration (mg/L)	Measured Concentration			
	$\bar{x}$[a]	SD	Range	n
Control	0	--	--	93
0.44	0.28	0.10	0.11-0.54	93
0.88	0.62	0.16	0.25-0.90	93
1.75	1.31	0.39	0.25-2.17	94
3.50	2.69	0.63	0.76-3.89	94
7.00	6.71	1.30	3.97-9.64	160

[a]Does not include analyses associated with five diluter malfunctions.

Table 18. TOTAL SURVIVABILITY AND PRODUCTIVITY INDICES IN FATHEAD MINNOWS AFTER CHRONIC EXPOSURE TO 2,4-DNT

Mean Measured Concentration (mg/L)	Total Survivability		Total Productivity	
	Series A	Series B	Series A	Series B
0.00	1076	583	101	67
0.28	718	472	83	49
0.62	561	451	66	48
1.31	274	140	22	25
2.69	49	--	4	--
6.71	--	--	--	--

Measured chemical concentrations and water quality parameters associated with the fathead minnow chronic study with 2,4-DNT are shown in Tables 19 and 20, respectively. Measurements of chemical concentrations associated with diluter malfunctions were not included in the calculations because the problems were short-term ($\leq$ 12 hours) in duration and typically resulted in relatively brief reductions of the concentrations in the tanks. Because of the short-term nature of these occurrences, including them in the calculations would have given them appreciably greater weight than the samples taken regularly at weekly intervals.

2,4-DNT TØTAL PRØDUCTIVITY INDEX

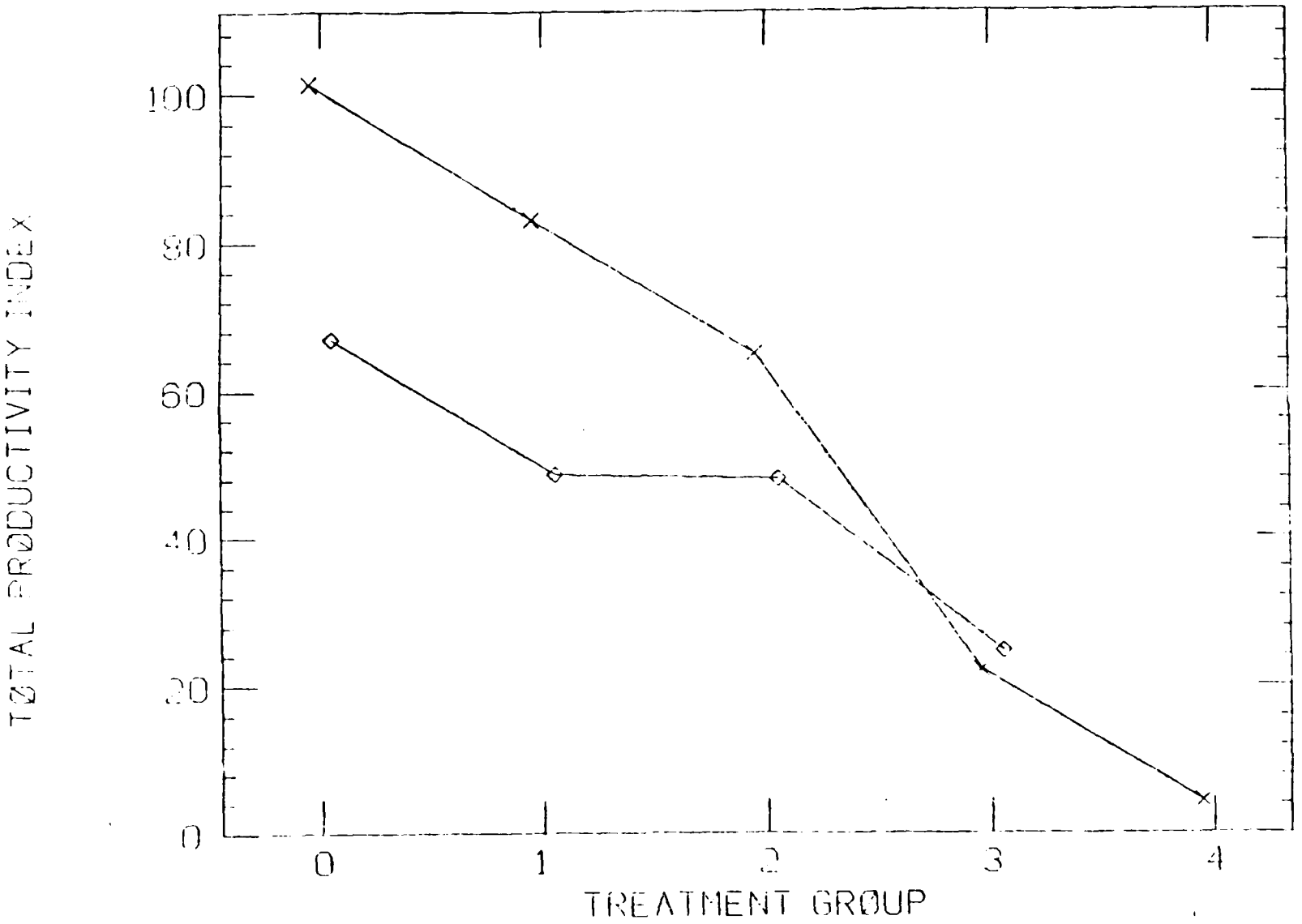

FIGURE 2

LEGEND: 0 = CONTROL 1 = 0.28 2 = 0.62 3 = 1.31 4 = 2.69 5 = 6.71 MG/LITER DNT
X = TEST SERIES:A DIAMOND = TEST SERIES B

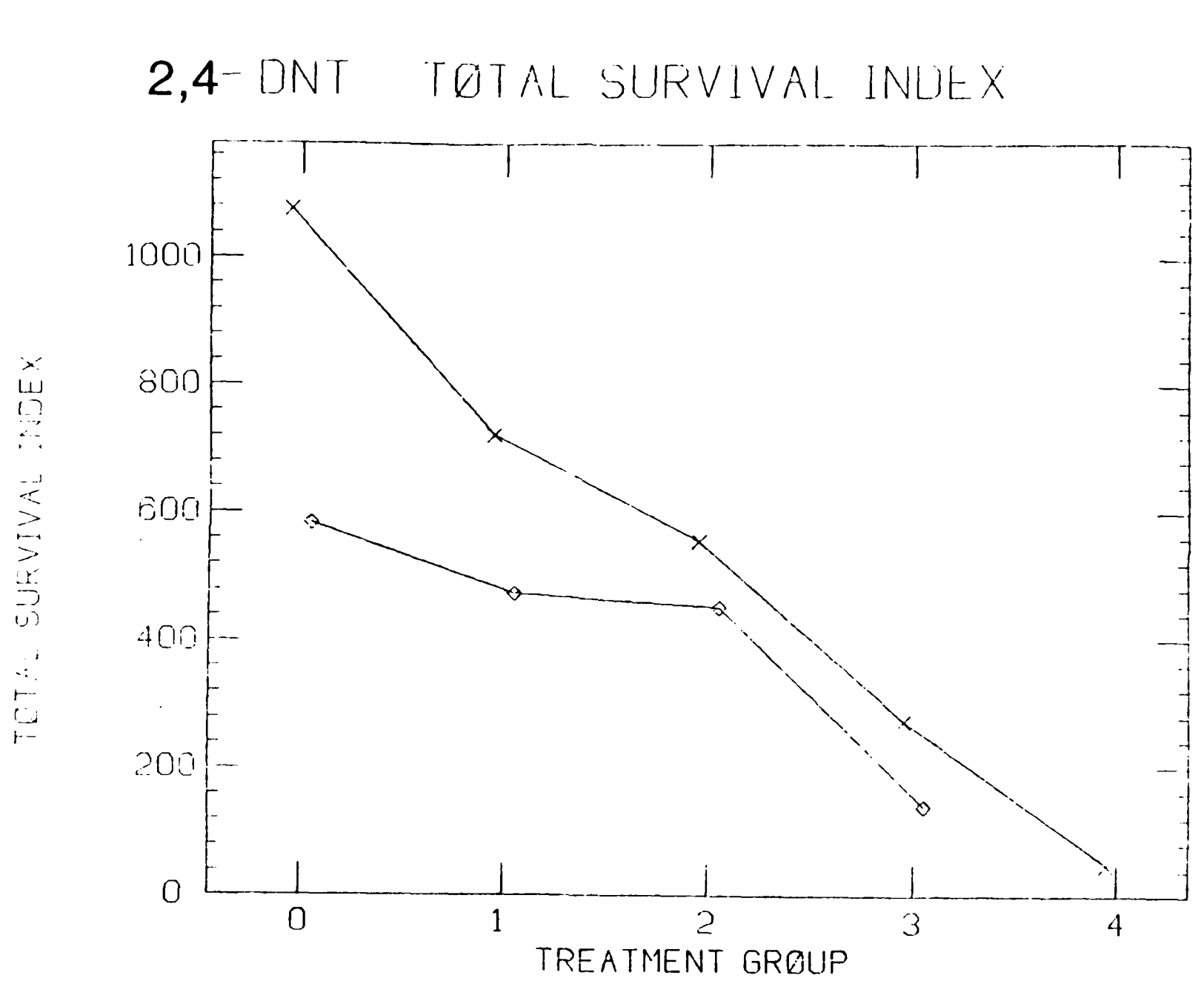

FIGURE 1

LEGEND: 0 = CONTROL 1 = 0.28 2 = 0.62 3 = 1.31 4 = 2.69 5 = 6.71 MG/LITER DNT
X = TEST SERIES:A DIAMOND = TEST SERIES B

Table 17. EFFECT OF CHRONIC EXPOSURE OF 2,4-DNT ON F_1 FATHEAD MINNOWS

Mean Measured Concentration (mg/L)	Test Series	Eggs			F1 Fry							
					30-Day				60-Day			
		No. Exposed	No. Hatched	No. Deformed	No. Transfer	No. Survive	Avg. Length (cm)	Avg. Weight (g)	No. Transfer	No. Survive	Avg. Length (cm)	Avg. Weight (g)
0.00	A	1550	836	6	43	43	1.70	0.066	81	81	2.30	0.094
	B	965	605	10	90	65	1.72	0.036	73	69	2.45	0.114
0.28	A	1350	873	25[a]	73	71	1.85	0.048[a]	78	65[a]	2.41	0.116
	B	1138	732	18	71	67	1.71	0.052	85	77[b]	2.29[a]	0.103
0.62	A	1250	859	22[a]	90	68[a]	1.71	0.047[a]	79	63[a]	2.33	0.117
	B	834	587	8	82	71	1.72	0.076	83	70[b]	2.32[a]	0.106
1.31	A	450	356	16[a]	39	34	1.70[b]	—	46	46[b]	2.19	0.081
	B	393	305	3	40	37	1.57[a]	0.032[b]	77	45[a]	2.64	0.176
2.69	A	388	143[a]	2	45	17[a]	1.56[a]	0.033[a]	83	78	2.23[b]	0.090
	B	167	55[a]	0	—	—	—	—	—	—	—	—
6.71	A	—	—	—	—	—	—	—	—	—	—	—
	B	—	—	—	—	—	—	—	—	—	—	—

[a]Statistically significant, $p < 0.05$.

[b]Statistically significant, $p < 0.05$, if series are pooled.

The effect of 2,4-DNT on the F_1 generation of fathead minnows is shown in Table 17. A statistically significant reduction in egg-hatching success occurred in both series at a concentration of 2.69 mg/L. A statistically significant increase in fry deformities occurred in series A at concentrations of 0.28, 0.62, and 1.31 mg/L and in the pooled series at concentrations of 0.28 and 1.31 mg/L. Although these increases were significant, the numbers were not large, constituting less than 5% of the total hatched fry.

Survival of F_1 fry reared to 30 days was significantly reduced in Series A at a concentration of 0.62 mg/L. This may be an artifact because there was no significant reduction in survival in the B series or in the the pooled series at this concentration and there was no effect in either series at a concentration of 1.31 mg/L. Survival of F_1 fry raised to 30 days was also significantly reduced in the A series at a concentration of 2.69 mg/L. (No fry were reared at this dose level in B series.) Survival of F_1 fry reared to 60 days was significantly reduced in the A series at concentrations of 0.28 and 0.62 mg/L, in the B series at a concentration of 1.31 mg/L, and in the pooled series at 0.28, 0.62 and 1.31 mg/L. Again, these results are difficult to interpret as being dose-related because there is no significant reduction in survival in the A series at concentrations of 1.31 and 2.69 mg/L but in the B series there is a steady trend toward reduced survival with increase in concentration.

Length of F_1 fry reared to 30 days was significantly reduced in the B and pooled series at 1.31 mg/L and in the A series and pooled series at 2.69 mg/L. Length of F_1 fry reared to 60 days showed a significant reduction in the B series at 0.28 and 0.62 mg/L and in the pooled series at 2.69 mg/L. There was no concurrent significant reduction in weight after 60 days of exposure at any concentration, although significant reductions in weight were seen after 30 days of exposure at concentrations of 0.28, 0.62, and 2.69 mg/L in the A series and at concentrations of 1.31 and 2.69 mg/L in the pooled series. Based on the number of fry present and the lack of effect on length, the weight reductions at 0.28 and 0.62 mg/L could be related to differences in fry density rather than to the toxicant.

The effects of 2,4-DNT on the global indices of total survivability and total productivity are graphically displayed in Figures 1 and 2, respectively, and also in Table 18. Both parameters show a trend of depression as toxicant concentration increases.

Table 16. EFFECT OF CHRONIC EXPOSURE OF 2,4-DNT ON REPRODUCTIVE PARAMETERS IN FATHEAD MINNOWS

Mean Measured Concentration (mg/L)	Test Series	Spawning Pair Survival (Days)	Spawns/Pair	Eggs/Pair	Eggs/Spawn	Eggs/Pair/Day
0.00	A	129	17	2870	150.6	22.2
	B	121	10	1477	141.0	12.4
0.28	A	129	18	2332	127.6	18.1
	B	131	13	1312	101.1	10.0
0.62	A	91	14	1583	101.3[b]	15.9
	B	99	11	1156	97.8[b]	16.8
1.31	A	91	4	487[a]	103.7[b]	7.2[b]
	B	68	8	469[b]	45.0[a]	4.3[b]
2.69	A	66	3[a]	206[a]	38.0[b]	1.6[a]
	B	91	2	56[a]	9.4[a]	0.4[a]
6.71	A	--	--	--	--	--
	B	38	0[a]	0[a]	0[a]	0[a]

[a]Statistically significant, $p < 0.05$.

[b]Statistically significant, $p < 0.05$, if series are pooled.

Table 15. EFFECT OF CHRONIC EXPOSURE OF 2,4-DNT ON EGG HATCHABILITY AND FRY SURVIVAL AND GROWTH IN FATHEAD MINNOWS

Mean Measured Concentration (mg/L)	Test Series	No. Eggs Hatched (n = 90)	No. Deformed	Survival (Days)						Length (cm)		
				30	60	90	120	150	179	30 d	60 d	90 d
0.00	A	71	2	65	64	63	62	62	62	2.02	2.92	3.81
	B	90	4	60	61	61	60	60	60	2.13	3.03	3.95
0.28	A	69	0	58	58	58	58	56	53[a]	1.73[a]	2.68[a]	3.77
	B	85	2	61	60	59	59	59	57	2.21	2.99	3.91
0.62	A	82	1	65	65	63	63	61	59[a]	1.97	2.97	3.79
	B	88	4	63	62	62	62	62	60	2.08	2.99	3.77
1.31	A	75	0	68	68	68	68	67	67	1.99	2.91	3.77
	B	87	3	71	69	67	67	66	60	1.96[a]	2.83[a]	3.73[a]
2.69	A	76	1	59[a]	58[a]	57[a]	56[a]	55[a]	47[a]	1.76[a]	2.92	3.73
	B	80[a]	1	59	55	45	44	43	38[a]	1.88[a]	2.98	3.84
6.71	A	75	0	22[a]	19[a]	--[b]	--	--	—	1.40[a]	2.59[a]	--
	B	59[a]	6	16[a]	11[a]	10[a]	6[a]	4[a]	4[a]	1.48[a]	2.87	3.78

[a]Statistically significant, $p < 0.05$.
[b]Fry lost due to diluter malfunction that resulted in no water being delivered to this series and a concomitant reduction in dissolved oxygen.

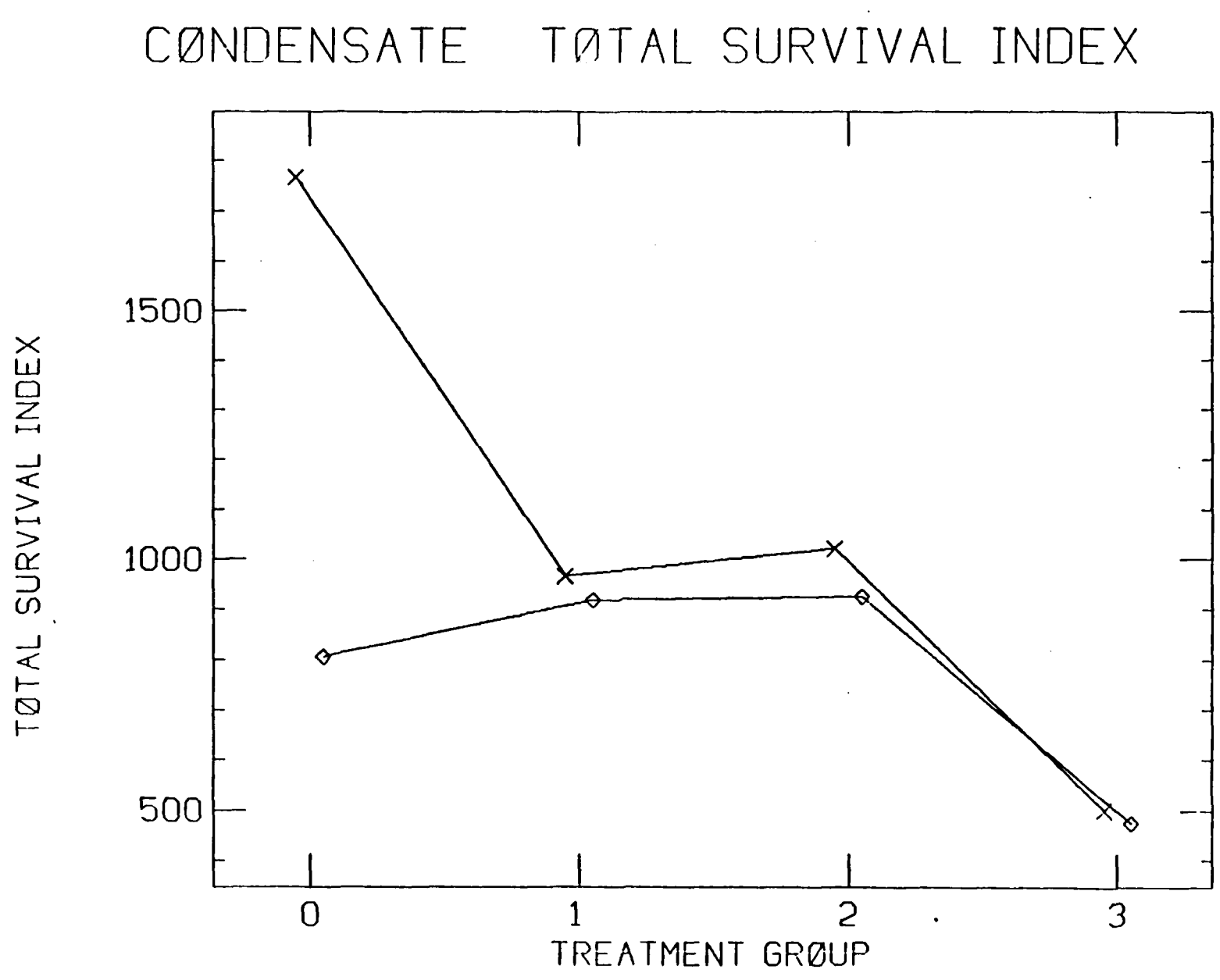

FIGURE 3

LEGEND: 0 - CØNTRØL 1 - 0.30 2 - 0.60 3 - 1.25 4 - 2.50 5 - 5.00 MG/LITER CØNDENSATE
X - TEST SERIES A DIAMØND - TEST SERIES B

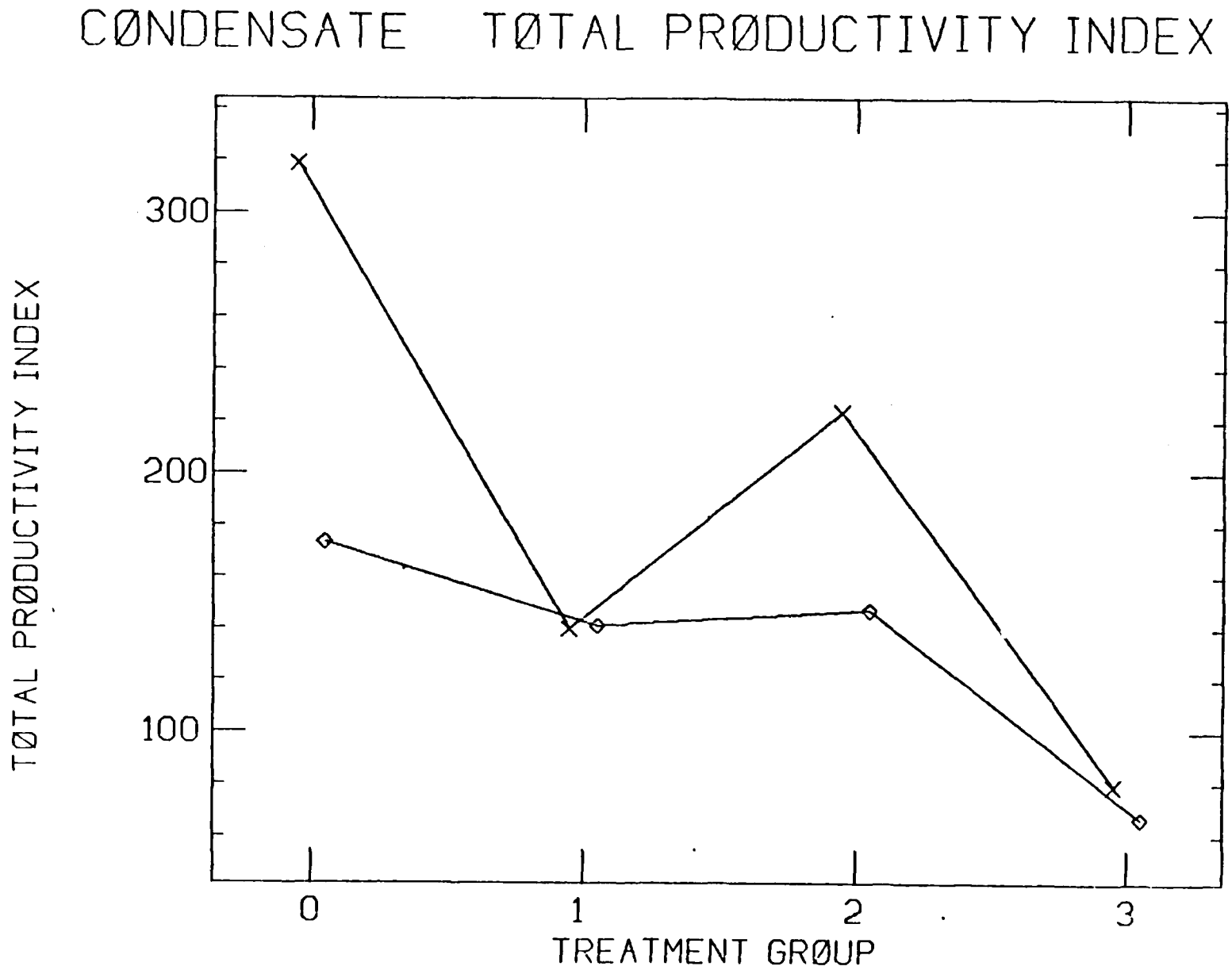

Figure 4

LEGEND: 0 - CØNTRØL 1 - 0.30 2 - 0.60 3 - 1.25 4 - 2.50 5 - 5.00 MG/LITER CØNDENSATE
X - TEST SERIES A DIAMØND - TEST SERIES B

Table 24. TOTAL SURVIVABILITY AND PRODUCTIVITY INDICES IN FATHEAD MINNOWS AFTER CHRONIC EXPOSURE TO CONDENSATE WATER

Average Actual Concentration (mg/L)	Total Survivability		Total Productivity	
	Series A	Series B	Series A	Series B
0.00	1768	807	319	173
0.19	966	918	140	141
0.34	1021	926	224	147
0.75	501	468	79	66
2.06	—	—	—	—
4.73	—	—	—	—

Measured chemical concentrations and water quality parameters associated with the fathead minnow chronic study on condensate water are shown in Table 25 and 26, respectively. As indicated earlier, measured concentrations associated with diluter malfunctions were not included in the analyses.

Table 25. MEASURED CHEMICAL CONCENTRATIONS ASSOCIATED WITH THE FATHEAD MINNOW CHRONIC STUDY WITH CONDENSATE WATER.

Nominal Concentration (mg/L)	Measured Concentration (mg/L)			
	$\bar{x}$*	SD	Range	n
Control	0.00	—	—	53
0.30	0.19	0.06	0.10 - 0.35	54
0 60	0.34	0.14	0.11 - 0.76	54
1.25	0.75	0.27	0.33 - 1.61	54
2.50	2.06	0.46	1.23 - 2.85	54
5.00	4.73	0.67	2.27 - 6.28	56

* Does not include analyses associated with two diluter malfunctions.

Table 26. WATER QUALITY DATA ASSOCIATED WITH THE FATHEAD MINNOW CHRONIC STUDY WITH CONDENSATE WATER

Test Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n
Control	6.6	0.8	3.8-8.0	47	7.5	0.3	6.5-8.2	44	24.9	1.2	21.0-26.0	16	129	57	42-282	43	44	24	14-129	42	39	18	12-100	44
0.19	6.6	0.8	4.6-8.0	47	7.4	0.4	6.5-8.2	44	25.0	1.2	21.0-26.0	17	131	58	42-299	43	24	8	18-29	2	25	7	20-20	2
0.34	6.6	0.8	3.8-8.0	47	7.4	0.4	6.5-8.1	44	25.2	0.7	23.5-26.0	14	131	58	40-300	43	24	11	17-32	2	28	11	20-35	2
0.75	6.4	0.8	4.2-7.8	47	7.4	0.3	6.5-8.0	44	25.2	0.7	23.5-26.0	13	131	59	40-299	43	24	10	17-31	2	25	7	20-30	2
2.06	7.4	0.6	6.2-8.4	44	7.6	0.3	6.6-8.2	44	25.2	0.8	23.5-26.0	12	127	57	40-299	43	22	8	17-28	2	28	4	25-30	2
4.73	7.6	0.6	6.1-8.4	43	7.8	0.3	7.3-8.7	44	25.1	0.9	23.5-26.0	11	127	57	40-287	43	23	8	17-29	2	25	7	20-30	2

Daphnia magna

2,4-DNT. The effect of 2,4-DNT on the survival of daphnids is shown in Table 27. The only statistically significant reduction in survival occurred at a concentration of 0.19 mg/L when the beakers and series were pooled. Because survival was not significantly reduced at the three higher levels, this does not appear to be a dose-related effect.

Table 27. CUMULATIVE MORTALITY OF DAPHNIDS DURING A 28-DAY EXPOSURE TO 2,4-DNT

Mean Measured Concentration	Number Dead (n = 22)					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
Control	0	0	0	0	0	0
0.07	0	0	0	0	0	0
0.19	0	0	4[a]	0	2	2[a]
0.40	0	0	0	0	1	1
1.02	0	0	1	0	0	1
1.78	0	0	1	2	3	3

[a] Statistically significant, $p < 0.05$, when series are pooled.

The effect of 2,4-DNT on the reproductive success of daphnids is shown in Table 28. The number of young produced per individual female was significantly reduced in the following groups: at a concentration of 0.40 mg/L in the B series and the pooled series after 21 and 28 days of exposure; at a concentration of 1.02 mg/L in the B series and pooled series after 21 and 28 days of exposure and in the A series after 28 days of exposure; and at a concentration of 1.78 mg/L in the A and B series after 21 and 28 days of exposure and in the pooled series after 14, 21, and 28 days of exposure.

Table 28. AVERAGE NUMBER OF YOUNG PRODUCED PER INDIVIDUAL FEMALE DAPHNID EXPOSED TO 2,4-DNT

Mean Measured Concentration (mg/L)	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
Control	3.3	28.8	53.1	3.4	51.0	59.1
0.07	2.0	34.0	48.7	4.6	43.1	53.1
0.19	5.6	40.6	59.4	8.4	48.0	69.2
0.40	2.3	24.7[b]	44.3[b]	7.6	18.4[a]	41.3[a]
1.02	1.8	16.4[b]	25.6[a]	3.3	18.6[a]	31.2[a]
1.78	0.1[b]	9.7[a]	20.7[a]	1.7[b]	16.8[a]	28.2[a]

[a] Statistically significant, $p < 0.05$.

[b] Statistically significant, $p < 0.05$, when series are pooled.

Data on the onset of reproduction, number of young produced per day during the reproductive period, and average length of surviving individual females exposed to 2,4-DNT are shown in Table 29. The time to onset of reproduction does not seem to have been affected by the presence of 2,4-DNT. The number of young produced per day was significantly reduced in the A series at concentrations of 1.02, and 1.78 mg/L and in the B series and pooled series at concentrations of 0.40, 1.02 and 1.78 mg/L. The average length of isolated daphnids surviving the 28-day exposure period was significantly reduced in the A, B, and pooled series at concentrations of 1.02 and 1.78 mg/L.

Table 29. TIME TO FIRST BROOD, NUMBER OF YOUNG PRODUCED PER REPRODUCTIVE DAY AND AVERAGE LENGTH OF SURVIVING FEMALE DAPHNIDS EXPOSED TO 2,4-DNT FOR 28 DAYS

Mean Measured Concentration (mg/L)	Test Series	Time to First Spawn (days)	No. of Young Produced/Day/Female	Average Length (mm)
0.00	A	15.2	4.2	3.5
	B	12.3	3.8	3.6
0.07	A	14.4	3.6	3.6
	B	12.6	3.4	3.9
0.19	A	12.0	3.7	3.5
	B	12.3	3.8	3.8
0.40	A	13.4	3.0[b]	3.5
	B	12.0	2.6[a]	3.5
1.02	A	13.7	1.8[a]	3.2[a]
	B	13.4	1.9[a]	3.3[a]
1.78	A	16.1	1.7[a]	3.2[a]
	B	15.9	2.3[a]	3.2[a]

[a] Statistically significant, $p < 0.05$.

[b] Statistically significant, $p < 0.05$, when series are pooled.

The chemical analyses associated with this test are summarized in Table 30. Traces of what appeared to be 2,4-DNT showed up in control samples, but no problem could be discovered in the diluter or delivery systems. This occurred intermittently and we were frustrated in our efforts to ascertain the origin of the peaks. Because these peaks also occurred in diluent water blanks, we considered them as background and subtracted them from the values obtained for the other samples in the same series, to arrive at the actual exposure concentrations. The three lowest concentrations were 42%, 24%, and 20% below their nominal values, respectively (low to high). This may have been caused by sorption of the toxicant or microbial or photolytic breakdown once it reached the test vessel.

Water quality data associated with this test are summarized in Table 31.

Table 30. ANALYSES OF 2,4-DNT IN A DAPHNIA CHRONIC STUDY

Nominal Concentration (mg/L)	Actual Concentration			
	Mean	S.D.	n	Range
0.00	0.00	--	9	--
0.12	0.07	0.04	7	0.0-0.12
0.25	0.19	0.08	8	0.10-0.27
0.50	0.40	0.17	8	0.16-0.57
1.00	1.02	0.33	8	0.60-1.37
2.00	1.78	0.62	13	0.90-2.73

Table 31. WATER QUALITY DATA ASSOCIATED WITH THE DAPHNIA MAGNA CHRONIC STUDY ON 2,4-DNT

Test Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n
Control	8.7	0.07	8.6-8.8	7	8.1	0.04	8.1-8.2	6	19.8	1.72	18-22	9	40	1.4	39-41	2	48	15	30-70	8	18	0	--	2
0.07	8.7	0.09	8.5-8.8	7	8.2	0.05	8.1-8.2	6	19.8	1.72	18-22	9			--				--					
0.19	8.8	0.09	8.6-8.9	7	8.2	0.05	8.1-8.2	6	19.8	1.72	18-22	9			--				--					
0.40	8.8	0.05	8.7-8.8	7	8.2	0.12	8.1-8.4	6	19.8	1.72	18-22	9			--				--					
1.02	8.8	0.13	8.5-8.9	7	8.2	0.12	8.1-8.4	6	19.8	1.72	18-22	9			--				--					
1.78	8.8	0.15	8.5-8.9	7	8.2	0.10	8.1-8.4	6	19.8	1.72	18-22	9			--				--					

Condensate water (nonirradiated). The effect of condensate water on the survival of daphnids is shown in Table 32. There was a statistically significant increase in mortality after 28 days of exposure at a concentration of 4.16 mg/L in the A series and when the series are pooled.

Table 32. CUMULATIVE MORTALITY OF DAPHNIDS DURING A 28-DAY EXPOSURE TO CONDENSATE WATER

Mean Measured Concentration (mg/L)	Number Dead (n = 22)					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Day
0.00	0	1	2	0	2	5
0.18	0	1	3	0	4	6
0.41	0	1	2	0	2	7
0.94	0	1	2	0	2	2
2.30	0	2	2	0	1	3
4.16	0	8	11[a]	0	4	10[b]

[a] Statistically significant, $p < 0.05$.

[b] Statistically significant, $p < 0.05$, when series are pooled.

The effect of condensate wastewater on the reproductive success of daphnids is shown in Table 33. The number of young produced was significantly reduced at a concentration of 4.16 mg/L after 21 days of exposure in the A series and when the series were pooled. This effect could possibly be considered transitory because there was no significant reduction at this concentration after 28 days of exposure when compared with control value.

Table 33. AVERAGE NUMBER OF YOUNG PRODUCED PER INDIVIDUAL FEMALE DAPHNID EXPOSED TO CONDENSATE WATER

Mean Measured Concentration (mg/L)	Young Produced					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
0.00	16.4	53.6	86.0	6.6	33.4	50.4
0.18	17.7	46.1	70.3	16.4	45.7	67.6
0.41	16.1	46.8	67.4	16.4	49.4	73.4
0.94	11.3	56.8.	88.3	15.1	54.4	106.6
2.30	11.8	38.0	61.4	7.7	31.1	60.7
4.16	11.5	34.8[a]	56.8	4.8	21.4[b]	36.3

[a] Statistically significant, $p < 0.05$.

[b] Statistically significant, $p < 0.05$, when series are pooled.

Data on the onset of reproduction, number of young produced per day during the reproductive period, and average length of surviving daphnids is shown in Table 34. Condensate water had no significant effect on the time to first brood over the exposure range tested. At a concentration of 4.16 mg/L, the number of young produced per day per female was reduced significantly when the series were pooled. The average length of the surviving daphnids was significantly reduced in the A series and the pooled series at a concentration of 0.41 mg/L and in the pooled series at 2.30 mg/L; because there was no significant effect at 0.94 or 4.16 mg/L, this reduction does not appear to be concentration-related.

Table 34. TIME TO FIRST BROOD, NUMBER OF YOUNG PRODUCED PER REPRODUCTIVE DAY AND AVERAGE LENGTH OF SURVIVING FEMALE DAPHNIDS EXPOSED TO CONDENSATE WATER FOR 28 DAYS

Actual Concentration (mg/L)	Test Series	Time to First Spawn (Days)	No. of Young Produced/Day/Female	Average Length (mm)
0.00	A	11.1	5.1	3.8
	B	12.1	3.6	3.6
0.18	A	11.1	4.6	3.7
	B	11.0	4.8	3.8
0.41	A	12.4	4.6	3.4[a]
	B	11.0	5.0	3.6[b]
0.94	A	11.6	5.6	3.7
	B	11.3	6.4	3.8
2.30	A	11.6	4.1	3.6[b]
	B	11.6	3.7	3.5[b]
4.16	A	11.8	4.6	3.6
	B	12.1	3.9	3.4

[a] Statistically significant, $p > 0.05$.

[b] Statistically significant, $p > 0.05$, when series are pooled.

The chemical analyses associated with this test are summarized in Table 35. Again, what appeared to be the test substance showed up sporadically in control samples. Because this also occurred in blank samples of diluent water, the problem may have been residual toxicant in the chromatography column or spurious peaks. They were considered as background and were subtracted from other values in each series as they occurred. The actual concentrations were up to 40% lower than the nominal concentrations. This was likely due to microbial degradation and photolytic breakdown. Water quality data associated with this test are summarized in Table 36.

Table 35. ANALYSES OF CONDENSATE WATER IN A DAPHNIA CHRONIC STUDY

Nominal Concentration (mg/L)	Mean[a]	S.D.	n	Range
0.00	0.00	--	6	--
0.30	0.18	0.09	6	0.08-0.17
0.60	0.41	0.15	6	0.23-0.61
1.25	0.94	0.38	6	0.48-1.54
2.50	2.30	0.64	6	1.54-3.26
5.00	4.16	0.97	6	2.98-5.80

[a]Does not include values associated with one diluter malfunction.

Table 36. WATER QUALITY DATA ASSOCIATED WITH DAPHNIA MAGNA CHRONIC STUDY ON CONDENSATE WATER

Mean Measured Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity (µmhos)				Hardness (mg/L $CaCO_3$)				Alkalinity (mg/L $CaCO_3$)			
	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n	$\bar{x}$	SD	Range	n
Control	8.6	0.26	8.2-8.8	6	8.0	0.41	7.6-8.5	5	21.0	0.71	20-22	5	176	107	120-300	3	52.3	31.8	33-89	3	37.0	16.5	27-56	3
0.18	8.6	0.23	8.3-8.8	6	8.0	0.39	7.6-8.5	5	21.0	0.71	20-22	5	175	98	110-288	3			--				--	
0.41	8.6	0.23	8.3-8.8	6	8.1	0.43	7.6-8.5	5	21.0	0.71	20-22	5	173	98	109-285	3			--				--	
0.94	8.6	0.23	8.3-8.8	6	8.0	0.40	7.6-8.5	5	21.0	0.71	20-22	5	163	93	100-270	3			--				--	
2.30	8.6	0.21	8.3-8.8	6	8.1	0.34	7.7-8.5	5	21.0	0.71	20-22	5	158	84	101-255	3			--				--	
4.16	8.5	0.32	8.3-8.8	6	8.1	0.37	7.7-8.6	5	21.0	0.71	20-22	5	109	53	75-170	3			--				--	

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CONCLUSIONS AND RECOMMENDATIONS

Conclusions

- A comparison of the results of the acute and chronic toxicity tests with D. magna and fathead minnows indicated that 2,4-DNT exhibited chronic toxicity to organisms at average concentrations 50 to nearly 100 times less than those that caused acute effects. Similarly, condensate water also showed evidence of cumulative toxicity with chronic effects occurring at concentrations 4 to 8 times lower than those that caused acute effects.

- Photolyzed condensate water appeared to be no more toxic to D. magna than condensate water under chronic exposure conditions. This result is in agreement with that of acute studies performed on irradiated and non-irradiated condensate water and indicates that a criterion based on non-irradiated material should provide sufficient protection against photolyzed solutions.

- Implementation of the criteria for average concentrations of 2,4-DNT and condensate water should be monitored carefully because results from long-term fish studies suggest that effects occurred below concentrations that produced statistically significant differences from control values.

Recommendations

- For 2,4-DNT, 8.1 mg/L should be considered the maximum allowable concentration and 0.12 mg/L should be considered the maximum allowable 24-hour average concentration.

- For condensate water, 2.3 mg/L should be considered the maximum allowable concentration and 0.14 mg/L should be considered the maximum allowable 24-hour average concentration.

- Given the suggestion of chronic effects below levels that produced statistically significant differences from the controls and the fact that the condensate standard is based on a synthetic condensate wastewater, additional work should be considered to assure the appropriateness of the criteria. Field monitoring at discharge sites would aid in determining the occurrence of effects on local populations of organisms. Given the observed effects on reproduction and fry survival, such monitoring should be on a long-term basis as effects may not be apparent for several generations. Additional bioassays that focus on concentrations at, and below, the criteria would also aid in determining the presence of effects at these levels.

Table 45. DATA BASE FOR FRESHWATER CHRONIC VALUE--CONDENSATE WATER

Species	Test Type	Response Parameter	MATC Limits (mg/L)	Geometric Mean (mg/L)
Rainbow trout	Early life stage	Fry growth	0.10-0.22	0.15
Channel catfish[a]	Early life stage	Fry survival	1.72-4.35	2.74
Fathead minnow[b]	Early life stage	Fry survival	0.6-1.4	0.92
	Chronic	Fry survival	0.34-0.75	0.50
Daphnia magna	Chronic	Survival	2.30-4.16	3.09

[a]Unacceptable test; included for comparison purposes only.

[b]Range-finding test; data not used in computing water quality criterion.

Table 44. SUMMARY OF ACUTE TOXICITY DATA--LC50 VALUES FOR CONDENSATE WATER

Bluegill	Fathead Minnow	Channel Catfish	Rainbow Trout	Daphnia magna	Lumbriculus variegatus	Hyalella azteca	Paratanytarsus dissimilis
7.1	22.0	17.5	7.1	23.7	24.6	22.8	37.9
2.7	3.7	8.0	7.3	11.0	18.9		
7.0	7.6			>17.9			
6.0	8.6						
6.3	8.6						
5.2	6.5						
5.5	9.3						
5.4							
7.4							
7.0							
5.7							
5.0							
7.7							
6.2							
2.6							
5.4							
4.1							
Geometric Mean							
5.4	8.3	11.8	7.2	16.1	21.6	22.8	37.9

Condensate Water

Final Acute Value. The data (Liu et al., 1984) used to derive the Final Acute Value for condensate water are summarized in Table 44. Application of the EPA procedure gives a value of 4.8 mg/L. This value is higher than the incipient LC50--2.3 mg/L--determined for rainbow trout during a two-week flow-through test (Liu et al., 1984). Consequently, we suggest that 2.3 mg/L be considered as the Final Acute Value.

Final Chronic Value. The data used to calculate the Final Chronic Value for condensate water are summarized in Table 45. Because the guidelines for calculating water quality criteria specify that chronic data from a minimum of three species must be used to determine the Final Chronic Value, we included the early life stage test on rainbow trout in the data base used for these calculations. While this test had some problems associated with it, as described earlier, the lack of any toxicant-related mortality within a concentration range of 0.10-1.96 mg/L and a consistent effect on growth within a range of 0.22-1.96 mg/L support the validity of the endpoints obtained from this test. Had these responses been random in nature, the validity of the effect and no-effect concentrations would have been more questionable. Acute/chronic toxicity ratios were calculated using the 96-hour LC50 estimates from flow-through tests on rainbow trout and fathead minnows and the 48-hour LC50 estimate from the flow-through tests on D. magna divided by the appropriate geometric means from Table 44. The geometric mean of these three values--16.1--was then divided into the final acute value--2.3 mg/L--to obtain a Final Chronic Value of 0.14 mg/L. As with 2,4-DNT, some caution should be used in applying this value because it is very close to the geometric mean obtained from the trout early life stage test. In addition, there was a suggestion of toxicant-related effects on F_1 fry growth at 0.19 mg/L in the fathead minnow chronic study.

Final Plant Value. Selenastrum capricornutum was the most sensitive algal species tested (Liu et al., 1984) The lowest concentration of condensate water that caused significant effects on growth was 4.8 mg/L, which is therefore the Final Plant Value.

Final Residue Value. A final Residue Value cannot be calculated for condensate water because insufficient data are available to define the maximum permissible tissue concentration.

Water Quality Criterion. The maximum allowable concentration of condensate water is the Final Acute Value--2.3 mg/L. The allowable 24-hour average concentration is the lowest value selected from the Final Chronic Value, the Final Plant Value, and the Final Residue Value. In this case, the Final Chronic Value--0.14 mg/L--is the lowest value and should be considered as the allowable 24-hour average concentration. In order for the average concentration to be met, condensate could only be discharged between one and two hours per day at 2.3 mg/L with no discharge taking place the rest of the day.

Table 43. DATA BASE FOR FRESHWATER CHRONIC VALUE--2,4-DNT

Species	Test Type	Response Parameter	MATC Limits (mg/L)	Geometric Mean (mg/L)
Rainbow trout	Early life stage	Fry growth	0.27-0.56[a]	0.39
Channel catfish	Early life stage[b]	Fry survival	<3.4	
Fathead minnow	Early life stage[c]	Hatching success, fry survival, fry growth	3.1-6.8	4.6
	Chronic	Reproduction	0.28-0.62	0.42
Daphnia magna	Chronic	Reproduction	0.19-0.40	0.27

[a]< 0.05 mg/L if growth effects of less than 5 percent are considered biologically significant.

[b]Unacceptable test; data included for comparison purposes only.

[c]Range-finding test; data not used in computing water quality criterion.

Table 42. SUMMARY OF ACUTE TOXICITY DATA--LC50 VALUES (MG/L) FOR 2,4-DNT

Bluegill	Fathead Minnow	Channel Catfish	Rainbow Trout	Daphnia magna	Lumbriculus variegatus	Hyalella azteca	Paratanytarsus dissimilis
13.5	31.4	24.8	13.6	47.5	>83.2	>83.2	22.5
24.0	32.8	32.0	13.9	38.3	80.9		
12.8	28.5			30.4			
7.8	36.1						
18.8							
16.4							
8.4							
9.4							
16.0							
Geometric Mean							
13.2	32.1	28.2	13.7	38.1	80.9	--	22.5

Calculation of Water-Quality Criteria

The primary purpose of developing an aquatic toxicity data base on 2,4-DNT and condensate water is to establish water-quality criteria for these materials. The following procedure is taken from EPA (1980). The criteria consists of two components. The first is the maximum allowable concentration, which is the Final Acute Value, as calculated from the results of 48- and 96-hour acute studies. The second criterion is the maximum 24-hour average concentration, which is the lowest of the following values: the Final Chronic Value, the Final Plant Value, and the Final Residue Value.

2,4-DNT

Final Acute Value--2,4-DNT. The data base (Liu et al., 1984) used to derive the Final Acute Value for 2,4-DNT is shown in Table 42. Application of the EPA procedure gives a Final Acute Value of 8.1 mg/L. This value is slightly lower than the lowest incipient lethal concentration (9.2 mg/L--bluegill) determined during 2-week flow-through tests on 2,4-DNT (Liu et al., 1984) and therefore appears to be a reasonable estimate of the maximum allowable concentration.

Final Chronic Value--2,4-DNT. The data used to calculate the Final Chronic Value for 2,4-DNT are summarized in Table 43. Acute/chronic toxicity ratios were calculated using the 96-hour LC50 estimates from flow-through tests on fathead minnows and rainbow trout and the 48-hour LC50 estimate from the flow-through tests on D. magna divided by the appropriate geometric means from Table 42. The geometric mean of these three values was then calculated. This average acute/chronic ratio--70--was then divided into the Final Acute Value--8.1 mg/L--to obtain a Final Chronic Value of 0.12 mg/L. This value should be adequate to protect aquatic organisms from appreciable effects of chronic exposure to 2,4-DNT. However, some caution is urged in its application because effects of 2,4-DNT on growth, although relatively small (< 5%), were seen in the early life stage test on rainbow trout at the lowest concentration tested, 0.05 mg/L, and data on egg production in the fathead minnow chronic study suggest an effect at the lowest concentration, 0.28 mg/L.

Final Plant Value. The final plant value for 2,4-DNT is the lowest concentration that significantly affected growth. Data from the 14-day algal assays presented in Volume II (Liu et al., 1984) indicate that Microcystis aeruginosa was the most sensitive algal species, with effects occurring at concentrations as low as 0.5 mg/L.

Final Residue Value. A Final Residue Value cannot be calculated for 2,4-DNT because insufficient data are available to define the maximum permissible tissue concentration.

Water Quality Criteria. The maximum allowable concentration of 2,4-DNT is the Final Acute Value--8.1 mg/L. The 24-hour average concentration is the lowest value selected from the Final Chronic Value, the Final Plant Value, and the Final Residue Value. In this case, the Final Chronic Value--0.12 mg/L--is the lowest value and is therefore the allowable 24-hour average concentration. This is actually a fairly rigorous standard as 2,4-DNT could only be discharged at the 8.1 mg/L level for 21 minutes per day followed by zero discharge, in order to meet the 24-hour average criteria.

Table 41. WATER QUALITY DATA ASSOCIATED WITH THE DAPHNIA MAGNA CHRONIC STUDY ON IRRADIATED CONDENSATE WATER

Test Concentration (mg/L)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity (mhos)				Hardness (mg/L CaCO)				Alkalinity (mg/L CaCO)			
	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n
Control	8.6	0.4	8.2-9.1	5	8.4	0.3	7.9-8.7	5	20.1	0.7	20-21	6	240	57	200-280	2	45	17	22-62	4	32	9	19-38	4
0.44	8.7	0.4	8.2-9.1	5	8.4	0.3	8.0-8.8	5	20.1	0.7	20-21	6	238	60	195-280	2			--				--	
0.88	8.7	0.4	8.2-9.2	5	8.4	0.3	8.0-8.8	5	20.1	0.7	20-21	6	235	57	195-275	2			--				--	
1.75	8.5	0.4	8.1-9.1	5	8.4	0.3	8.0-8.9	5	20.1	0.7	20-21	6	185	--	--	1			--				--	
3.50	8.4	0.5	7.8-9.0	5	8.4	0.3	8.0-8.8	5	20.1	0.7	20-21	6	185	--	--	1			--				--	
7.00	8.3	0.6	7.6-9.0	5	8.2	0.2	8.0-8.4	5	20.1	0.7	20-21	6	125	--	--	1			--				--	

Table 40. CHEMICAL ANALYSES OF IRRADIATED CONDENSATE WATER IN A DAPHNIA CHRONIC STUDY

Nominal Concentration (mg/L)	Actual Concentration (mg/L)			
	Mean	S.D.	n	Range
0.00	0.00	0.04	6	--
0.44	0.03	0.02	5	0.01-0.06
0.88	0.07	0.05	6	0.02-0.14
1.75	0.17	0.08	6	0.08-0.30
3.50	0.44	0.14	6	0.30-0.68
7.00	0.90	0.24	6	0.58-1.18

Based on the results of the daphnid chronic studies, it appears that the toxicity of irradiated condensate water is similar to condensate water on an equivalent (before photolysis) basis of condensate water. This suggests that allowable discharge concentrations for condensate water will provide sufficient protection from the effects of photolyzed condensate water. This conclusion appears to be justified on the basis of results obtained from the mortality, reproduction, and growth parameters. Specifically, the effect/no effect concentrations for mortality were 2.09-3.68 and 3.5-7.0 mg/L for condensate water and Cond-Irrad, respectively. Length was not affected in the highest concentrations tested (3.68 and 7.00 mg/L) for either of the two mixtures. Finally, the effect/no-effect concentrations for condensate water, based on reproduction, were 2.09-3.68 mg/L compared to the effect/no effect concentrations for Cond-Irrad which, while not as definitive, probably were between 3.50 and 7.00 mg/L.

Table 39. TIME TO FIRST BROOD, NUMBER OF YOUNG PRODUCED PER REPRODUCTIVE DAY AND AVERAGE LENGTH OF SURVIVING FEMALE DAPHNIDS EXPOSED TO IRRADIATED CONDENSATE WATER FOR 28 DAYS

Concentration (mg/L)	Test Series	Time to First Brood (days)	No. of Young Produced/Day/Female	Average Length (mm)
0.00	A	10.6	6.8	3.6
	B	14.0	12.2	4.2
0.44	A	10.0	6.6	3.9
	B	13.0	9.2	4.3
0.88	A	10.3	4.8[b]	3.5
	B	14.6	7.9[b]	4.0
1.75	A	11.6	5.1	3.5
	B	12.0	10.8	4.2
3.50	A	10.6	4.2[b]	3.7
	B	11.8	10.2[b]	4.2
7.00	A	10.0	6.4[b]	3.6
	B	12.5	6.9[a]	4.3

[a] Statistically significant, $p < 0.05$.

[b] Statistically significant, $p < 0.05$, when series are pooled.

The chemical analyses associated with this test are summarized in Table 40. The actual concentrations are low because the condensate water was irradiated. The ratio of each concentration to the next higher one (~ 1:2) shows the diluter was performing well. What appeared to be toxicant showed up in the controls but also showed up in blank diluent water samples, indicating that the problem may have been contamination of the chromatography column or spurious peaks. Again, these values were considered as background and were subtracted from other values in the same series of concentrations.

Water quality data associated with this test are summarized in Table 41.

Table 38. AVERAGE NUMBER OF YOUNG PRODUCED PER INDIVIDUAL FEMALE DAPHNID EXPOSED TO IRRADIATED CONDENSATE WATER

Nominal Concentration (mg/L)	Young Produced					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
0.00	28.7	53.0	111.3	7.4	63.3	171.3
0.44	40.4	78.1	120.0	6.3	48.6	137.7
0.88	25.6	41.0[b]	61.0[b]	5.0	23.0[a]	98.6[a]
1.75	26.3	47.4	83.1	8.8	88.0	172.4
3.50	13.3	35.8	72.3	13.1	62.3	163.6
7.00	38.1	75.0	93.1[b]	7.0	44.7	95.7[a]

[a] Statistically significant, $p > 0.05$.

[b] Statistically significant, $p > 0.05$, when series are pooled.

The effects of Cond-Irrad on the onset of reproduction, number of young produced per day during the reproductive period, and average length of surviving daphnids are shown in Table 39. Length of surviving daphnids and time to first brood were not significantly different from control values over the range of concentrations tested (0.44 to 7.00 mg/L). The number of young produced per reproductive day per isolated female was significantly reduced at concentrations of 0.88 and 3.50 mg/L when the series are pooled and at 7.00 mg/L in the B series and in the pooled series. This response, especially at 0.88 mg/L, does not appear to be concentration-related because there was no significant effect at 1.75 mg/L. In addition, the significant pooled effect appears largely due to relatively low values in Series A (compared to the control) at concentrations of 0.88-3.50 mg/L. Since the young/day value at 7.00 mg/L nearly approaches the control value in Series A, it would appear that the lower values seen in 0.88-3.50 mg/L were not toxicant-related.

Irradiated condensate water. The effect of irradiated condensate water is shown in Table 37. The only significant increase in mortality was seen at a nominal concentration (based on the concentration of condensate water before photolysis) of 7.00 mg/L after 28 days of exposure when the series are pooled.

Table 37. CUMULATIVE MORTALITY OF DAPHNIDS DURING A 28-DAY EXPOSURE TO IRRADIATED CONDENSATE WATER

Nominal Concentration (mg/L)	Number Dead (n = 22)					
	Series A			Series B		
	14 Days	21 Days	28 Days	14 Days	21 Days	28 Days
0.00	2	5	5	0	0	0
0.44	0	4	5	0	0	0
0.88	1	4	8	1	2	2
1.75	0	4	7	0	0	0
3.50	0	3	3	0	0	2
7.00	2	9	10[a]	2	2	6[a]

[a] Statistically significant, $p < 0.05$, for the pooled series only.

The effect of Cond-Irrad on the reproductive success of daphnids is shown in Table 38. Production of young in the B series and in the pooled series was significantly reduced at a nominal concentration of 0.88 mg/L after 21 and 28 days of exposure and at a nominal concentration of 7.00 mg/L after 28 days of exposure. These responses do not appear to be concentration-related because there were no significant reductions at concentrations of 1.75 and 3.50 mg/L, particularly in Series B.

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