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Acute Toxicity and Bioaccumulation of Chloroform to Four Species of Freshwater Fish

Salmo gairdneri, Rainbow Trout
Lepomis macrochirus, Bluegill
Micropterus salmoides, Largemouth Bass
Ictalurus punctatus, Channel Catfish

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ABSTRACT

Acute toxicity of chloroform to four species of freshwater fish was studied in flow-through 96-hr toxicity tests. Chloroform is toxic to fish in the tens of parts per million, a concentration well above that which would be expected to be produced under normal power plant chlorination conditions. Investigations of acute toxicity of chloroform and the bioaccumulation of chlorinated compounds in tissues of fish revealed differences in tolerance levels and tissue accumulations. Mean 96-hr LC_{50}s for chloroform were 18 ppm for rainbow trout and bluegill, 51 ppm for largemouth bass and 75 ppm for channel catfish. Mortalities of bluegill and largemouth bass occurred during the first 4 hr of exposure while rainbow trout and channel catfish showed initial tolerance and mortalities occurred during the latter half of the 96-hr exposure. Rainbow trout had the highest level of chloroform tissue accumulation, 7 µg/g tissue, catfish the second highest, 4 µg/g tissue, followed by bluegill and largemouth bass which each accumulated about 3 µg/g tissue. Accumulation of chloroform was less than one order of magnitude above water concentrations for all species.

SUMMARY

The acute toxicity of chloroform ($CHCl_3$) to four species of freshwater fish was studied in 28 flow-through 96-hr toxicity tests. Mean LC_{50} for each species is: rainbow trout (Salmo gairdneri) 18 ppm, bluegill (Lepomis macrochirus) 18 ppm, largemouth bass (Micropterus salmoides) 51 ppm and channel catfish (Ictalurus punctatus) 75 ppm. Mortality of bluegill and largemouth bass occurred during the first 4 hr of exposure while rainbow trout and channel catfish exhibited an initial tolerance to chloroform with mortality occurring during the latter half of the 96-hr test. Behavioral responses to chloroform were noted in rainbow trout and largemouth bass.

Chloroform accumulation/depuration studies consisting of 24-hr exposures to 1.0-1.5 ppm $CHCl_3$ followed by 24 hr of depuration were conducted on the same four species of fish. Of the four species of fish tested, rainbow trout accumulated the highest level of chloroform in the tissues, 7 μg $CHCl_3$/g tissue. Catfish accumulated the next highest tissue concentration of chloroform at 4 μg $CHCl_3$/g tissue, followed by bluegill and bass which each accumulated approximately 3 μg $CHCl_3$/g tissue. Bioaccumulation of chloroform in these fish above the water concentration was less than one order of magnitude.

Bluegill apparently had higher tissue levels of chloroform after 4-hr exposure than after 24 hr exposure to 1 ppm $CHCl_3$. Rainbow trout and bass apparently reached maximum accumulation levels after 4 hr of exposure. Catfish was the only species tested that had an apparent increase in tissue concentration of chloroform between the 4-hr and 24-hr sampling times. The bioconcentration factor in catfish cannot be determined from our data since tissue concentrations did not reach equilibrium within 24 hr.

The 96-hr LC_{50}s for each species are several orders of magnitude above the chloroform levels found in previous studies of chlorinated natural waters simulating power plant chlorination conditions across the U.S. Therefore, although chloroform is toxic to fish in the range of tens of parts per million, this amount is well above that which could be expected to be produced under normal power plant chlorination conditions.

Since chloroform has been classified by the Environmental Protection Agency as a carcinogen, further studies beyond 96-hr acute toxicity studies are needed to determine the environmental impact of chloroform entering the aquatic environment. Acute toxicity testing is a first step in determining the hazard of chloroform to fish. Evaluation of the environmental impact of chloroform should take into consideration the widespread and long-term use of chlorine and thus long-term production of chloroform by steam generating power plants across the United States. Other studies of long-term effects or effects of chloroform on reproduction, carcinogenicity, teratogenicity and mutagenicity are warrented given existing information in the literature.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PREFACE

This report includes data and analysis for the Freshwater Biology Task of the program on Biocide By-Products in Aquatic Environments.

Reports prepared for the entire program are:

Title	Author
• Investigation of Halogenated Components Formed from Chlorination of Natural Waters: Preliminary Studies, NUREG/CR-1299	Roger M. Bean Robert G. Riley
• Acute Toxicity and Bioaccumulation of Chloroform to Four Species of Fresh Water Fish *Salmo gairdneri*, Rainbow Trout *Lepomis macrochirus*, Bluegill *Micropterus salmoides*, Largemouth Bass *Ictalurus punctatus*, Channel Catfish, NUREG/CR-0893	David R. Anderson E. William Lusty
• Chronic Effects of Chlorination By-Products on Rainbow Trout, *Salmo gairdneri*, NUREG/CR-0892	David R. Anderson Roger M. Bean Roger E. Schirmer
• Toxicity, Bioaccumulation and Depuration of Bromoform in Five Marine Species *Protothaca staminea*, Littleneck Clam *Mercenaria mercenaria*, Eastern Hard Clam, Quahog *Crassostrea virginica*, Eastern oyster *Penaeus aztecus*, Brown Shrimp *Brevoortia tyrannus*, Atlantic Menhaden, NUREG/CR-1297	Charles I. Gibson Fredrick C. Tone Peter Wilkinson J. W. Blaylock Roger E. Schirmer
• Growth and Histological Effects to *Protothaca staminea*, (Littleneck Clam) of Long-Term Exposure to Chlorinated Sea Water, NUREG/CR-1298	Charles I. Gibson Robert E. Hillman Peter Wilkinson Dana L. Woodruff
• Analysis of Organohalogen Products from Chlorination of Natural Waters Under Simulated Biofouling Control Conditions, NUREG/CR-1301	Roger M. Bean Dale C. Mann Robert G. Riley
• Biocide By-Products in Aquatic Environments, Final Report Covering Period September 10, 1976 through September 30, 1979, NUREG/CR-1300	Roger M. Bean Charles I. Gibson David R. Anderson

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INTRODUCTION

A study on effects of chlorination by-products was undertaken on selected aquatic biota in freshwater and marine environments. Objectives of one phase of the study were to determine the acute toxicity and bioaccumulation of chloroform, a major freshwater chlorination by-product, to four species of freshwater fishes. Choice of chloroform as a toxicant was based on results of the analytical phase of the Chlorination By-Products Program. Studies conducted to determine the biologically available chlorinated organics produced from chlorination of freshwater across the United States indicated that chloroform was the major chlorination by-product produced in freshwater. Therefore we conducted acute (96-hr) toxicity studies with rainbow trout (Salmo gairdneri), bluegill (Lepomis macrochirus), channel catfish (Ictalurus punctatus) and largemouth bass (Micropterus salmoides). The species were selected because of their economic and ecological importance in aquatic ecosystems near power plants using freshwater as a secondary coolant. A major objective of the acute toxicity tests was to provide data for the evaluation of potential toxicity resulting from chloroform produced during power plant chlorination. Acute 96-hr LC_{50}s were determined and compared to expected levels of chloroform produced during power plant chlorination.

Objectives of the bioaccumulation studies were: (1) to determine if chloroform bioaccumulates (2) to determine the level of bioaccumulation during shortterm exposures of fish to chloroform. One to two year old fish were used for bioaccumulation tests.

Previous experiments with marine species indicated that bromoform rapidly accumulated in animal tissues. This, along with the acute toxicity tests, suggested short term accumulation/depuration tests be conducted. A 24-hr exposure to chloroform followed by 24-hr depuration was selected.

METHODS AND MATERIALS

ACUTE TOXICITY STUDIES

Chloroform saturated water cannot be effectively prepared by simply stirring the two together due to the slow rate of solution, so a flow-through toxicant delivery system was constructed (Figure 1) that produced a continuous supply of stock solution saturated with chloroform at 8000 ppm. A chloroform saturation column was developed to increase the water-chloroform contact. The saturation column is a 0.61 m glass column filled with 3 mm glass beads, with bands of glass wool at 15 mm intervals. A peristaltic pump with Viton tubing, which is resistant to the solvent properties of chloroform, metered chloroform into the saturation column at a flow rate of ∿1.0 mℓ/min. Deionized water was added to maintain a constant head in the column at an average flow rate of 90 mℓ/min. The solution flowed into a magnetically stirred 4-ℓ amber-glass carboy; then into a second 4-ℓ amber-glass carboy which was not stirred. This permitted settling of fine chloroform droplets from the saturated solution prior to pumping it to the toxicant manifold.

A stainless steel, positive displacement pump with a ceramic head was used to pump the chloroform-saturated water to the toxicant manifold. The saturated solution was metered into funnels in dilution cells where it mixed with Columbia River water prior to flowing to the aquaria. The toxicant delivery system was designed to minimize turbulent mixing and bubbling which could reduce the chloroform concentration in water. Variability of chloroform concentration with time in each aquarium was determined by daily chloroform measurement. Daily variations between samples from different aquaria were no larger than the variance between multiple samples from a single aquarium. Because of day-to-day variability, chloroform samples were collected twice during each bioassay, at the beginning and near the end of the 96-hr test. Chloroform samples were analyzed on a Hewlett Packard-5830 gas chromatograph with an 18" Porapak Q column operated at 185°C and using a ^{63}Ni electron capture detector.

Water flow rates to each 50-ℓ aquaria were equal during each test. At the beginning of a test, water flow in each aquarium was adjusted to a level ranging from 1.0 to 1.5 ℓ/min depending on fish oxygen requirements and the ASTM recommended flow rate (Sprague, 1973). The diurnal light cycle was automatically controlled to a 12-hr light/dark cycle. Aerated Columbia River water was maintained at a constant temperature within 0.1°C during each test using a Research Incorporated Temperature Controller with an Esterline Angus PD2064 microprocessor to monitor and record the temperature each half hour. Acclimation and test temperatures used were near the middle of the tolerance range for each species to avoid combined stresses of temperature and toxicant. Fish were held in the test temperature and light regime at least two weeks prior to testing and were not fed for two days prior to the testing.

Bluegill, catfish and largemouth bass were purchased from the Osage Catfish Fishery in Osage Beach, Missouri. Rainbow trout were purchased from the Soap Lake Hatchery in Soap Lake, Washington. Each species was held for at least

two months to insure that stocks were in good condition prior to testing. Fish were fed Silvercup fish food with the exception of largemouth bass which were fed live juvenile rainbow trout. In previous toxicity tests at our laboratory, and in tests by Mehrle et al. (1977), this diet was shown to provide adequate nutritional requirements.

In order to minimize effects of fish growth on the test results, the complete series of toxicity tests for each species were completed before testing another species. This was not possible with bluegill, in which an outbreak of columnaris disease occurred during the series of tests. Testing was suspended for bluegill until the columnaris infection was controlled. Tests for other species were conducted during the interim. Length and weight measurements of the test fish were made following each test (Table 1).

The experimental design of the toxicity tests is in accordance with that recommended by Sprague (1973). Chloroform concentration sequence in the test system is random. The sequence of loading fish into the aquaria for each test is performed following a random numbers table. This randomization minimizes the possibility of the testing system arrangement and fish loading selectivity on the outcome of the toxicity test.

Statistical analyses of the toxicity test results were analyzed on a PDP 1170 computer. Stephan's (1977) program was used to compute the LC_{50}s and 95% confidence intervals. The program computes LC_{50}s by probit, moving average and binomial computational procedures.

BIOACCUMULATION STUDIES

Fish used for the bioaccumulation studies were from the same stock purchased for use in the acute toxicity tests except for catfish. Juvenile catfish were required in the acute toxicity testing because fighting occurred when three or more adult fish were placed in the same aquarium. Both test stocks of catfish were obtained from the same vendor although the fish used in the bioaccumulation study were about one year older than those used in the acute toxicity tests. Size of the fish stocks is shown in Tables 2-5. The treatment and feeding of fish stocks is the same as discussed in the acute toxicity section. No disease or mortality of control fish was observed in any bioaccumulation studies.

The toxicant delivery system was modified to eliminate the contact column (Figure 1). This reduced the concentration of the stock solution permiting establishment of 1 to 2 ppm $CHCl_3$ in the test aquaria. Flow rates through each 50-ℓ aquarium were 1 to 2 liters per minute depending on the tests. Tests were conducted in paired aquaria. The toxicant delivery system was operated at test levels for 24 hr prior to start of the bioaccumulation tests so that chloroform levels could stabilize. Concentrations of chloroform were measured 1 to 2 hr prior to testing, at the time that the fish were added to the water and two to three times throughout the 24-hr testing period.

During the depuration phase of the tests, fish were transferred en masse to an aquarium without chloroform and the water monitored at 15 min and 1 hr to determine chloroform concentrations in the depuration tank. In all cases, <0.01 ppm $CHCl_3$ was found at 15 min. No chloroform was detected after 1 hr. In the bluegill tests, samples of water for chloroform analysis were taken after 24 hr of depuration. This further substantiated the rapid loss and low chloroform levels in the depuration aquaria. The light/dark diurnal period was regulated to 12 hr of light and 12 hr of darkness. Temperature was held constant at the acclimation temperature for each species. In all cases, filtered Columbia River water was used. Test temperature was controlled using a Research Incorporated Temperature Controller with an Esterline Angus PD2064 microprocessor to monitor and record the temperature for each half hour of the test.

At the beginning of each test the fish were transferred into test aquaria following a sequence from a random numbers table. The sequence of removal of fish at each sampling was also done randomly. Care was taken to minimize stress in handling when fish were added to the aquaria and during transfer to the depuration aquaria. At each sampling, fish were removed sequentially by a random sampling sequence, cudgeled to minimize activity; weighed, measured and placed on ice to await sectioning within the next few minutes. Sequence of sectioning was the same as removal from test aquaria. Single fish were sectioned vertically into 1 cm pieces and placed in glass jars and mascerated to facilitate chloroform extraction by methanol. Fish were covered with a known volume of methanol (Burdick and Jackson distilled in glass methanol). Extraction of chloroform occurred for 24 to 48 hr prior to a second methanol extraction. During the methanol extractions, tissues were held at 4°C to minimize chloroform loss due to volatilization. Methanol extracts were measured and a 50-mℓ aliquot from each jar retained for further analysis. A third volume of methanol was replaced, covering the fish if further extraction was found to be necessary.

Chloroform concentrations in water and methanol extracts were determined by gas chromatography. One microliter samples of water, methanol or water diluted with methanol were injected directly onto an 18 inch Porapak Q column operated isothermally at 130°C and the chloroform content was measured with a ^{63}Ni electron capture detector. Standards were run with each set of samples. The method is not linear over the entire calibration range, so sample values had to be calculated using standards of similar concentration. Analysis of spiked samples showed no bias in the method when aqueous samples were kept cold and tightly sealed to prevent loss of chloroform. The limit of detection of this method is approximately 5×10^{-10} g $CHCl_3$/mℓ water and the coefficient of variation has been estimated at 3% over the entire calibration range.

Preliminary studies were conducted with trout tissues in order to determine the number of methanol extractions required to remove chloroform from fish tissue. These preliminary studies indicated that 97.5% of the chloroform was removed during the first two chloroform extractions of fish tissue. Only in very few instances was any chloroform found in the third extraction. The samples were analyzed in a random order to minimize analytical bias.

RESULTS

ACUTE TOXICITY TESTS

Test fish can be placed in two groups by sensitivity to chloroform. The first group, trout and bluegill, with 96-hr LC_{50}'s less than 25 ppm chloroform and the second group, largemouth bass and catfish, with LC_{50}'s greater than 25 ppm (Tables 6-9). Mortality rate for bluegill and largemouth bass in response to toxic chloroform levels was high during the first day with little mortality occurring later in the test (Figures 2 and 3). Trout and catfish, (Figures 4 and 5), tended to exhibit an initial tolerance to chloroform with mortality increasing later in the toxicity test except at chloroform levels well above the LC_{50} where no tolerance was evident.

Largemouth Bass

Six 96-hr flow-through chloroform toxicity tests were conducted with largemouth bass. The 96-hr LC_{50}'s for largemouth bass range from 45 to 56 ppm chloroform (Table 6). Mortality rate during each test was very high during the first 12 hr with no further mortalities occurring between 12-96 hr (Table 10, Figure 2). Highest mortality rate was recorded during the first 4 hr where 90-100% of the mortalities during the 96-hr test occurred.

Distinct behavioral differences were noted between largemouth bass exposed to toxic levels of chloroform and controls. Exposed fish exhibited color changes, and "head down" position while resting. Occasionally the fish in the "head down" posture would swim energetically and erratically colliding with the top and sides of the aquarium. The energetic swimming was intermittent and occasionally concomitant with a sudden noise in the room. The behavioral response may be described as an excessive startle response.

Channel Catfish

In the initial toxicity tests adult channel catfish (one to two years old), exhibited aggressive behavior with two or more fish per aquarium. Mortality of all but one dominant fish occurred as a result of aggression. Since the aggressive behavior was not apparent in the fish holding tank, water siphoned from the tank was used to supply test aquaria. Results of behavioral studies by Todd (1971) suggested that "conditioned" water from areas of high catfish densities may contain a compound promoting survival under crowded conditions by reducing aggressive behavior. Although Todd's work explained why we were not finding excessive aggressive behavior in the fish in the holding tank, transfer of the water to a test aquarium with three fish and no chloroform did not reduce aggressive behavior in the test aquarium.

Yearling channel catfish were used to replace adult catfish used in early toxicity tests. The adult catfish (1-2 years old) became so aggressive in the confinement of the test aquarium that the dominant fish killed the others in

the aquarium. Studies in the literature suggested that juvenile catfish do not behave similarly (Roseboom and Richey, 1977). Because of the behavioral difference between juvenile and adult catfish, juvenile catfish were found to be excellent test fish for toxicity studies.

While awaiting arrival of the juvenile catfish, a toxicity test with one adult catfish per aquarium was conducted to estimate the range at which mortality occurred. No mortalities occurred in any aquarium up to the maximum concentration of 68 ppm $CHCl_3$.

Five toxicity tests were conducted with juvenile catfish. Survival of adult catfish during initial toxicity testing with one fish per aquarium at high chloroform levels indicated the LC_{50} was high. Mortality or lack of mortality during early testing (Table 11, Figure 3) provided little information to determine the range of the LC_{50}. Several tests were required to first determine the approximate LC_{50} range prior to testing with concentrations near the resultant LC_{50} 75 ppm.

Mortality resulting from chloroform exposure shows a threshold resulting in a high incidence of 100% mortality or 100% survival among experimental organisms over a narrow concentration range. Partial test group mortalities rarely occurred. These bioassay results are difficult to interpret with probit analysis, which requires several partial test group mortalities to maximize the accuracy of the LC_{50} and minimize the confidence intervals. Thus the binomial method and the moving average method were also used to compute 96-hr LC_{50}s (Stephan, 1977).

Rainbow Trout

Acute toxicity tests with juvenile rainbow trout produced a range of 96-hr LC_{50}s between 15-22 ppm $CHCl_3$ with a mean of 18.2 ppm $CHCl_3$ (Table 9). There appears to be a threshold effect of chloroform with rainbow trout as well as with bluegill. There was either nearly 100% mortality or 100% survival in concentrations differing less than 2-3 ppm during each toxicity test (Table 12, Figure 4).

There was an obvious behavioral difference between controls and fish in high chloroform concentrations. In concentrations of chloroform near 20 ppm or greater, rainbow trout stratified near the surface exhibiting what we describe as "escape behavior". Fish swim with their noses out of the water. Fish stratification, evident during the day in the high chloroform levels, did not occur at night. Frequently a fish would swim quickly in random directions hitting the tank cover and walls. This intense activity was followed by loss of equilibrium. At levels greater than 13 ppm, fish were excitable and easily startled and sudden illumination of the aquaria at night caused fish to swim in an erratic manner. Fish in lesser concentrations of chloroform did not exhibit the same startle response and responded more slowly to the light stimulation. Exhibiting loss of equilibrium, slow opercular movement and apparently near death on the tank bottom, these fish often revived after a few minutes, although loss of equilibrium sometimes lasted for as long as an hour. Fish near death often exhibited stress marks or patches of dark and light coloring over their bodies.

Bluegill

In six acute toxicity tests of chloroform with bluegill the 96-hr LC_{50}s ranged from 13.3-22.3 ppm with a mean LC_{50} of 18.2 ppm (Table 8). The mortality rate was highest during the first 12 hr with little further mortality occurring during the ensuing 84 hr of the test (Table 13, Figure 5). Color changes exhibiting stress were evident prior to mortality in some fish. No other behavioral differences were evident between control and exposed fish.

A prophylactic effect of chloroform on columnaris disease (Flexibacter columnaris) was demonstrated during a toxicity test with bluegill. The stock of test fish exhibited no obvious signs of columnaris within the two weeks prior to the initiation of the 96-hr toxicity test. During the second day of the test, the mortality rate in the control aquarium was extremely high, with complete mortality occurring within 48 hr. Each mortality was examined for evidence of columnaris disease. Columnaris was found in the control and in the two low chloroform concentrations of 12.0 and 13.6 ppm. In the two highest chloroform concentrations, 17.1 and 14.9 ppm, no mortalities occurred during the first three days when the test was terminated. Chloroform toxicity in earlier bioassays with bluegill occurred primarily during the third and fourth days. Thus, mortality early in the test and at relatively low chloroform concentrations can be attributed to columnaris infection of the gills in two test concentrations of 17.1 and 14.9 ppm. An examination of the unexposed bluegill holding stock indicated a low-level columnaris infection. Apparently the stress of handling fish prior to the outset of the bioassay was sufficient to cause an outbreak of the disease. Chloroform concentrations greater than 14.9 ppm were sufficient to inhibit or reduce the columnaris infection.

BIOACCUMULATION STUDIES

Rainbow Trout

Rainbow trout exhibited the highest bioaccumulation factor of any of the species tested (Table 2, Figure 6). Within 1 hr of exposure, the tissue level of chloroform in trout was 4 μg of chloroform per gram of fish tissue. This is the approximate tissue level achieved in other test species at 4 hr of exposure. Chloroform level in trout tissue then nearly doubled to 7 μg per gram of tissue at 4 hr. At the 24 hr sampling, the mean chloroform concentration of 5.5 μg $CHCl_3$/g tissue, was lower than at 4 hr. Preliminary tests were conducted with a much higher level of chloroform than the one per million level finally used to determine the period of exposure and depuration. They showed that chloroform levels did not increase from 4 to 8 hr. The 8-hr sampling time was eliminated and replaced by a 24-hr sampling to provide time for an equilibrium between fish and water concentrations to occur.

The mean chloroform concentration in rainbow trout decreased by 50% within 15 min of the start of depuration and within 4 hr, the levels were below 1 μg $CHCl_3$/g tissue. By 48 hr the levels in the fish had decreased to below the level of detection. No behavioral changes were noted in the trout during exposure to 1.0 ppm $CHCl_3$. The fish were evenly distributed throughout the aquaria during both accumulation and depuration phases of the test.

Bluegill

Bioaccumulations of chloroform in bluegill was similar to rainbow trout in that an initial high level of chloroform observed at 4 hr decreased at 24 hr (Table 3 and Figure 7). Although the maximum level of $CHCl_3$ in bluegill is less than half that of rainbow trout, a detectable level of chloroform was extracted from bluegill tissue following a day of depuration (Figure 7).

Largemouth Bass

Accumulation of chloroform in largemouth bass differs from trout and bluegill in that tissue levels do not decrease after 4-hr (Table 4). The 4-hr and 24-hr exposures are similar. Rapid depuration is shown by the decrease in concentration to below detection limits within 4 hr of removal from chloroform exposure (Figure 8). Sampling after 4-hr of depuration did not reveal a detectable level of chloroform in the tissues nor was there any following 24 hr of depuration.

The bioaccumulation of chloroform in bass was about half that of trout and approximately equal to the tissue levels of bluegill and catfish.

Channel Catfish

Channel catfish did not exhibit the rapid bioaccumulation of chloroform as did the other fish species during the first 4 hr of exposure (Figure 9). Peak concentration of chloroform occurred at 24 hr (Table 5). These results differ from those on trout or bluegill in which concentrations at 24 hr were less than at 4 hr. Concentrations of chloroform in catfish at 4 hr were approximately equal to trout tissue levels at 15 min of exposure. Chloroform levels in catfish tissue at 24 hr of exposure were approximately equal to levels in bass and bluegill at the same sampling time, and were about 2/3 of the level found in trout tissue at 24 hr. Since the shape of the accumulation curve does not exhibit a plateau or decrease from a peak in tissue level of chloroform, and catfish show the slowest rate of accumulation in the species tested, the maximum tissue level of chloroform may not have been reached. Further studies should be conducted with catfish to determine at what exposure period the tissue concentration of chloroform begins to stabilize or decrease.

Depuration of chloroform was rapid, decreasing to less than 0.1 ppm $CHCl_3$/g tissue following 24-hr depuration. The rapid depuration of chloroform from catfish was similar to that of trout and bass, but was more rapid than bluegill.

DISCUSSION

Fish sensitivity to chloroform as indicated by 96-hr LC_{50}s ranges from 18 ppm for rainbow trout and bluegill to 51 and 75 ppm for largemouth bass and channel catfish, respectively. Although the 96-hr LC_{50}s provide an indication of the sensitivity of fish to chloroform, examination of the mortality rates during each toxicity test provides additional information. Mortality rates for bluegill and largemouth bass were high during the first day of exposure with little further mortality occurring later in the toxicity test. Trout and catfish, which had the lowest and highest 96-hr LC_{50}s respectively, demonstrated similar mortality patterns at test concentrations. They tended to exhibit an initial tolerance and low mortality with increasing mortality later in the toxicity test. Although with relatively high chloroform concentrations in regard to the LC_{50}, mortality was high during the initial portion of the test.

In the four species tested, a threshold effect was evident. A high incidence of 100% mortality or 100% survival occurred over a narrow chloroform concentration range of 2 to 3 ppm $CHCl_3$. Partial test-group mortality rarely occurred. Although chloroform is acutely toxic to fish from 18-75 ppm, depending on the species, this is orders of magnitude higher than the levels of chloroform expected to be produced during chlorination at power plants. Thus given the expected levels of chloroform production from chlorination, acute chloroform toxicity to freshwater fish does not appear to be a problem.

A prophylactic effect of chloroform on columnaris disease was evident during a toxicity test with bluegill that was terminated. Although the stock of test fish exhibited no obvious signs of columnaris within two weeks prior to testing, the stress of handling apparently was sufficient to cause an outbreak of the disease. Chloroform concentrations of 15-17 ppm were found to be sufficient to inhibit or reduce the columnaris infection.

Chloroform was found to alter the behavior of rainbow trout and largemouth bass. Both species were observed to swim erratically and energetically during chloroform exposures. Intense activity was followed by a loss of equilibrium with slow opercular movement. Loss of equilibrium occurred for periods of a few minutes or as long as an hour. Although slow opercular movement was apparent prior to mortality, it is not clear if retardation of the respiratory process results in oxygen deprivation. Trout also exhibit a definite change in social structure or behavior in test aquaria at chloroform concentrations greater than 10 ppm. Trout stratify near the surface of test aquaria, whereas in the control aquarium fish are evenly distributed. There is an increased sensitivity to light or noise disturbances in fish exposed to chloroform. Sudden illumination of the room at night sends rainbow trout, bluegill and largemouth bass into frenzied swimming activity for several minutes. This heightened startle-response was not noted in channel catfish.

Of the four species of fish tested, rainbow trout accumulated the highest level of chloroform in the tissues, 7 μg $CHCl_3$/g tissue. Catfish accumulated the next highest tissue concentration at 4.3 μg $CHCl_3$/g tissue,

followed by bluegill and largemouth bass which accumulated approximately 3 μg $CHCl_3$/g tissue. In all species tested, the bioaccumulation of chloroform from the concentration in water was less than one order of magnitude. All species were exposed to levels of chloroform ranging from 1.0 to 1.5 ppm $CHCl_3$, which is equivalent to a tissue concentration of 1.0-1.5 μg $CHCl_3$/g tissue with a concentration factor of one.

Those species which were most sensitive to chloroform during the acute toxicity tests, exhibited similar patterns of accumulation or depuration during the chloroform bioaccumulation studies. Trout and bluegill both had their highest tissue levels of chloroform at 4 hr of exposure with a reduced chloroform level at 24 hr of exposure. Bass reached peak concentrations in about 4 hr. These were similar to the 24 hr concentrations.

Catfish was the only species tested that had a substantial increase in tissue concentration of chloroform between the 4-hr and 24-hr sampling times. Due to the nature of this pattern, e.g., the lack of decrease or plateau in the accumulation pattern, the bioconcentration factor cannot be determined. Catfish was the most tolerant to chloroform of species tested with 96-hr LC_{50} of 75 ppm $CHCl_3$. Also the pattern of mortality during the toxicity tests indicated an initial tolerance to chloroform levels near the LC_{50} with mortality increasing during the latter half of the toxicity tests. Trout also followed this pattern of an initial tolerance to chloroform levels, although the pattern of bioaccumulation of chloroform was substantially different than that of catfish.

The ability of fish to accumulate chloroform rapidly is shown in trout's accumulation of 3 μg $CHCl_3$/g tissue in one hour and 7 μg $CHCl_3$/g tissue at 4 hr of exposure to 1 ppm $CHCl_3$. Rapid depuration rates also occurred. Although power plants using fresh water normally chlorinate intermittently rather than continuously, the chloroform produced may be accumulated during the short-term exposures. Chloroform concentrations can be expected to be highest in tissues with high lipid content such as developing gonads and fish eggs due to chloroform solubility in lipid.

CONCLUSIONS AND RECOMMENDATIONS

- The 96-hr LC_{50}s were 18.2 ppm for rainbow trout and bluegill, 51.2 ppm for largemouth bass and 75 ppm for channel catfish.
- Although chloroform was observed to be lethal to these fish, lethal levels are several orders of magnitude above that expected to be produced under normal power plant operating conditions.
- Observed changes in fish behavior also occurred at chloroform threshold levels above that expected to be discharged from power plants.
- Chloroform accumulated in fish tissues at three to seven times the water concentration during exposures of up to 4 hr.
- Rapid depuration of tissue chloroform levels was observed in most species.
- Although most power plants sited on freshwater normally chlorinate intermittently, accumulation of chloroform in fish tissues can be expected during these short term exposures.
- Future research should examine the long term effects of repeated accumulation and depuration of chloroform that fish may experience as a result of power plant chlorination.
- Given the lipophilic nature of chloroform and the high lipid content of fish eggs relatively high chloroform concentrations may occur in the developing gonads of female fish. The implications of this to fish reproduction are unknown.
- Studies of the combined effects of chlorine and chlorination by-products should be conducted since these will occur simultaneously in power plant discharges and the demonstrated affects of chlorine on membrane permeability may exacerbate the effects of chlorination by-products.
- Studies are also recommended in the long term effects of chloroform on reproduction, carcinogenicity, teratogenicity and mutagenicity in freshwater biota.

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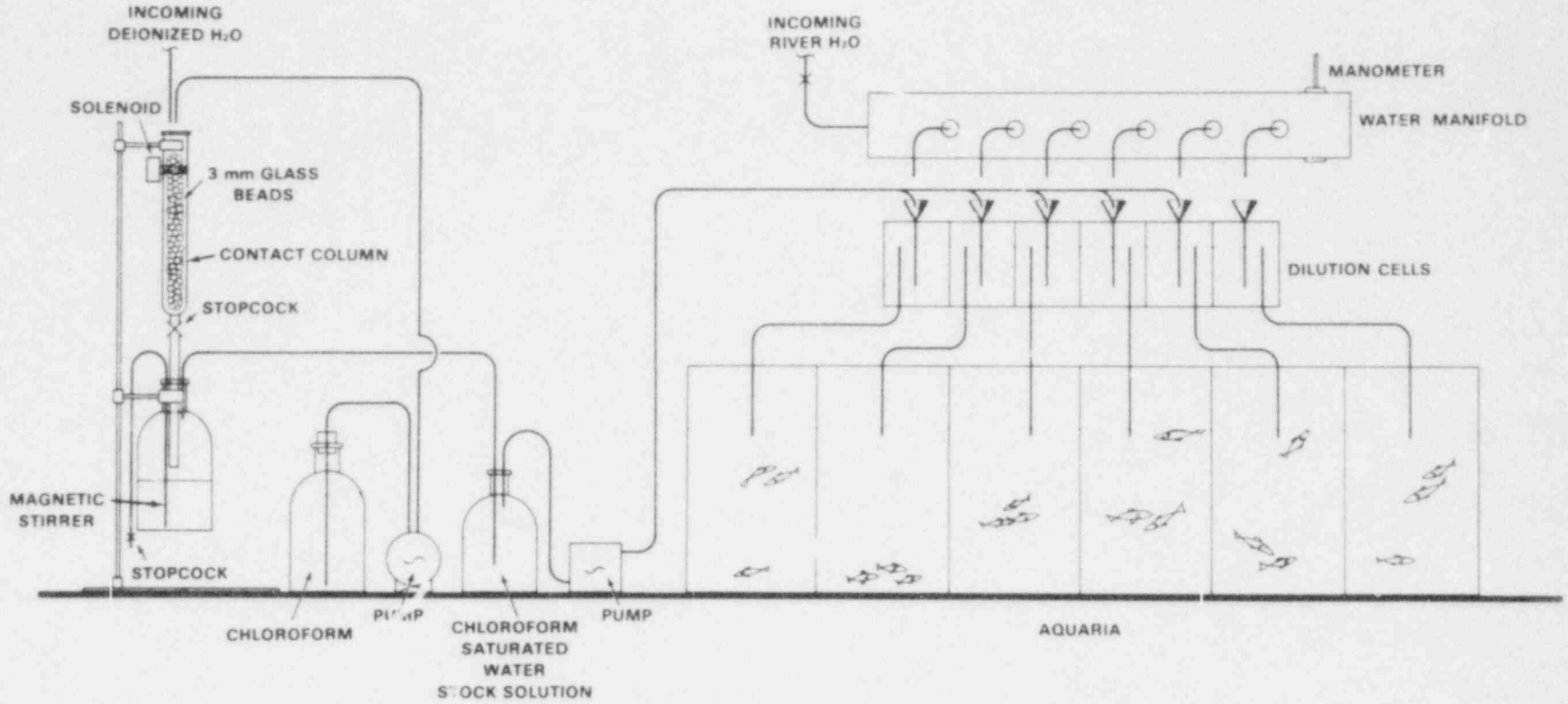

FIGURE 1. Delivery System Used for Acute Chloroform Tests of Freshwater Species.

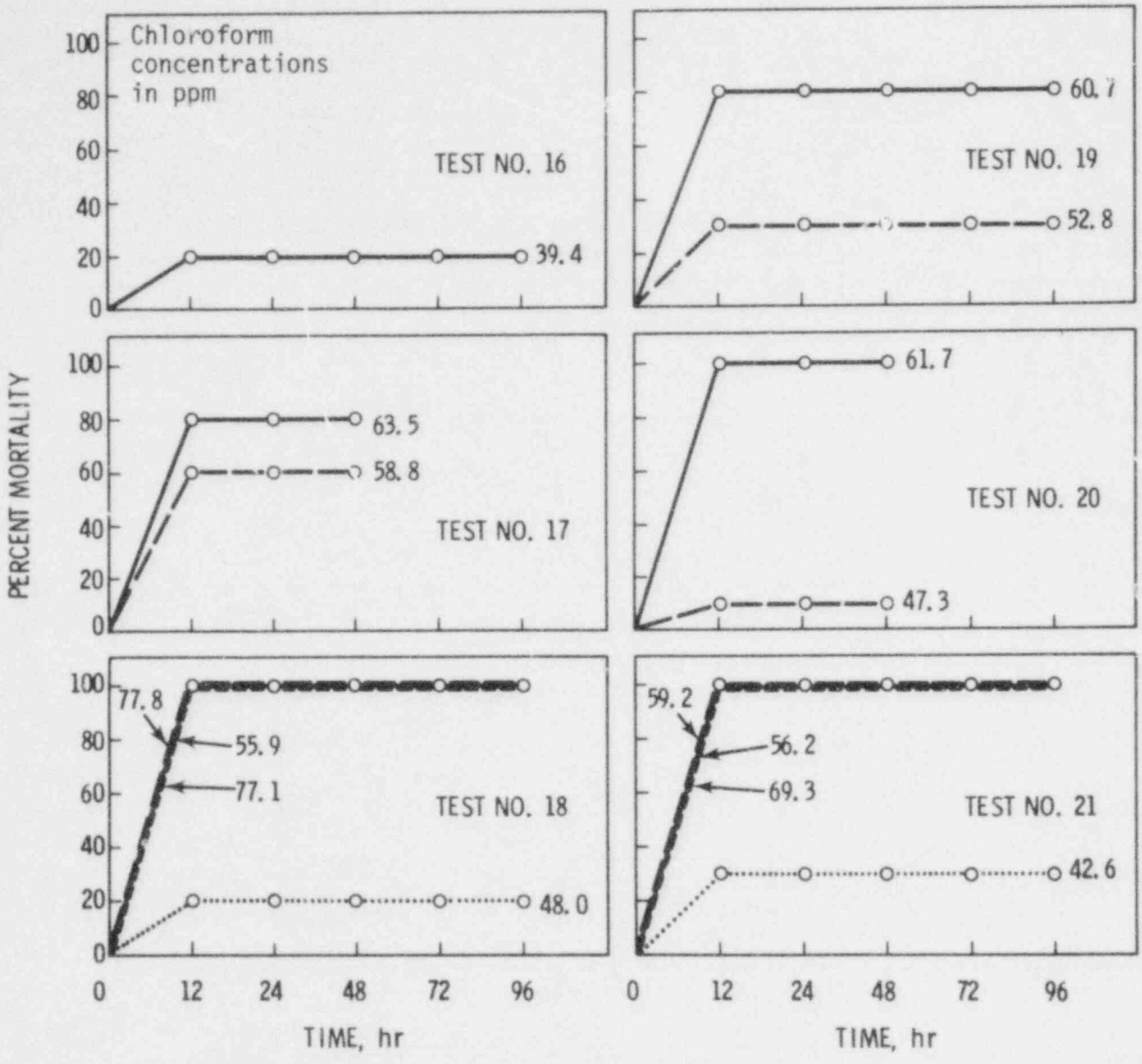

FIGURE 2. Mortalities of Largemouth Bass During 96-hr Toxicity Tests at 19°C with Chloroform. Concentrations that did not cause mortality are given in Table 10.

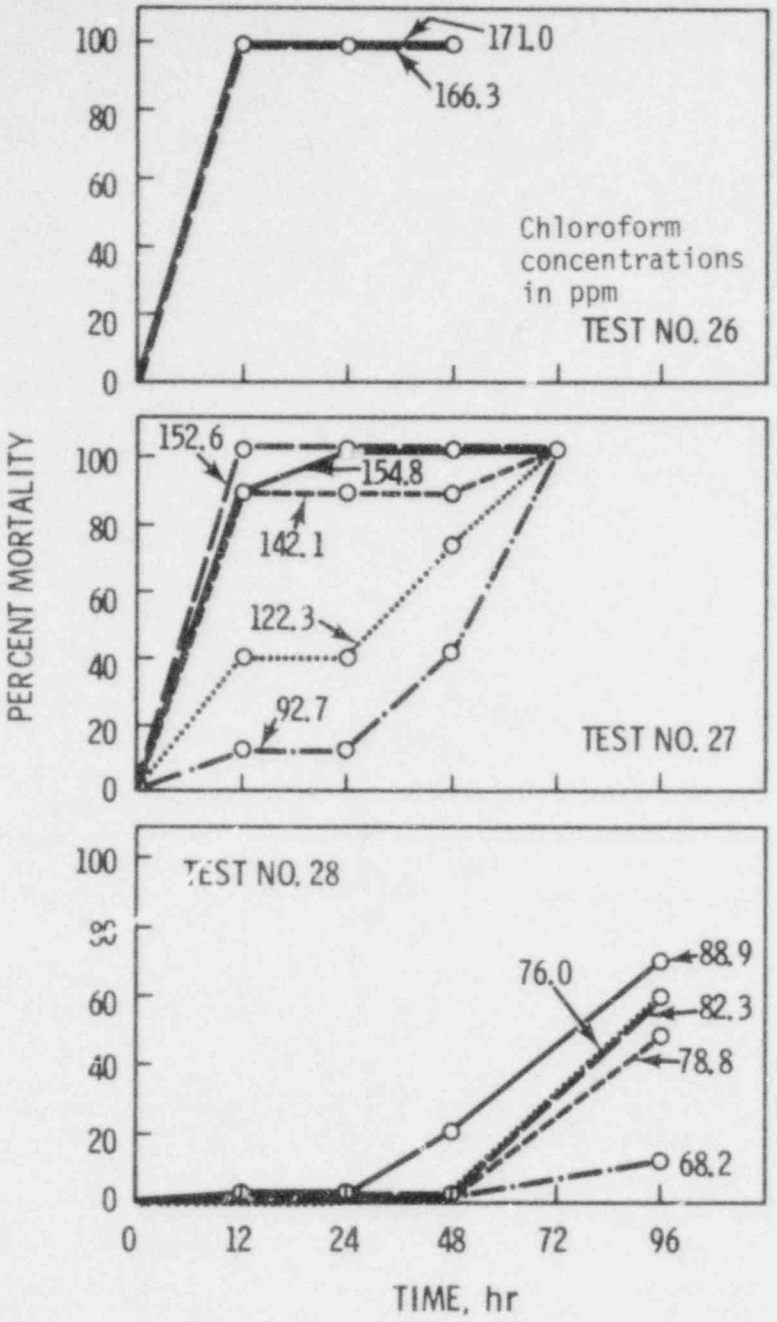

FIGURE 3. Mortalities of Channel Catfish During 96-hr Toxicity Tests at 19°C with Chloroform. Concentrations that did not cause mortality are given in Table 11.

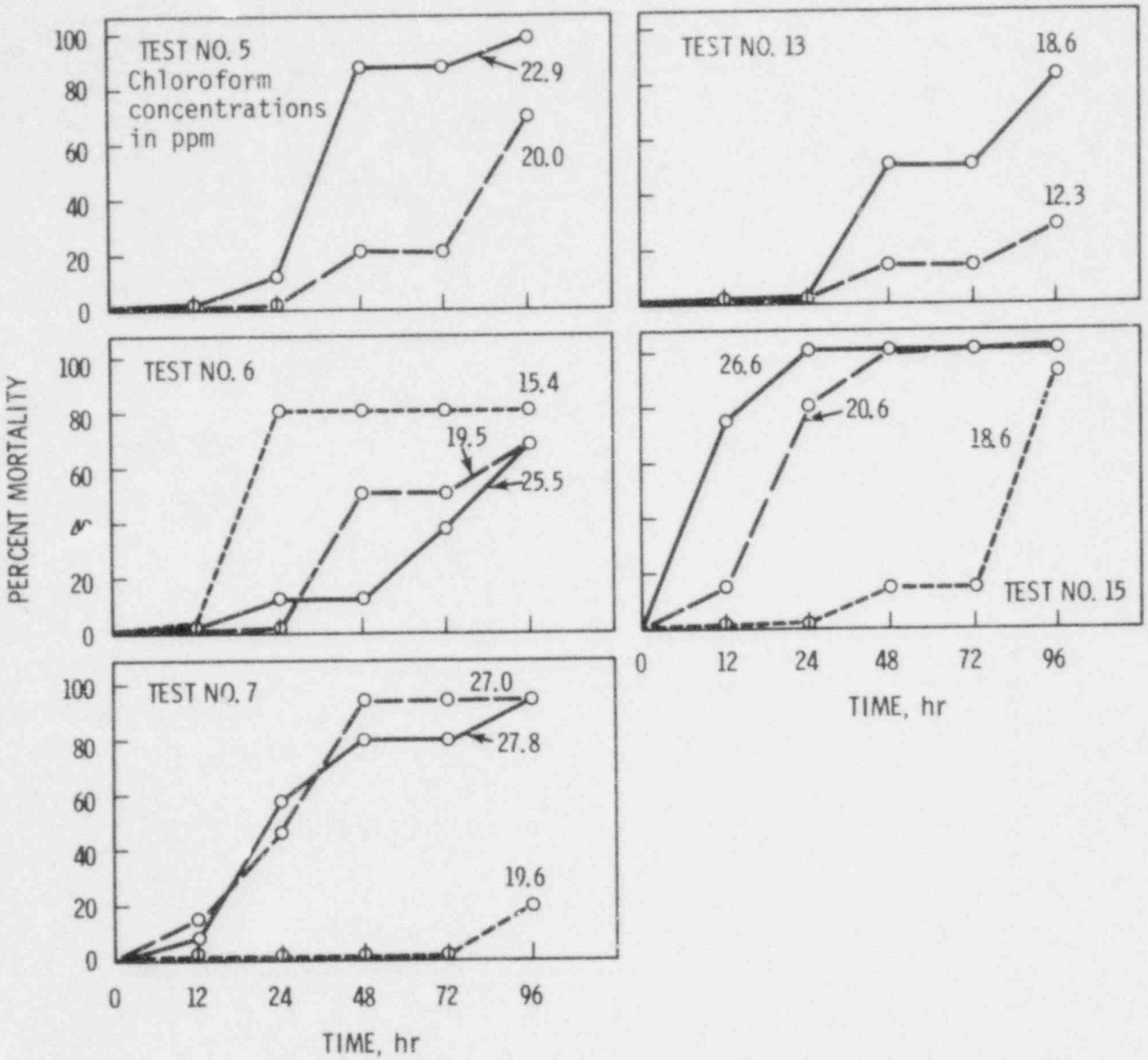

FIGURE 4. Mortalities of Rainbow Trout During 96-hr Toxicity Tests at 19°C with Chloroform. Concentrations that did not cause mortality are given in Table 12.

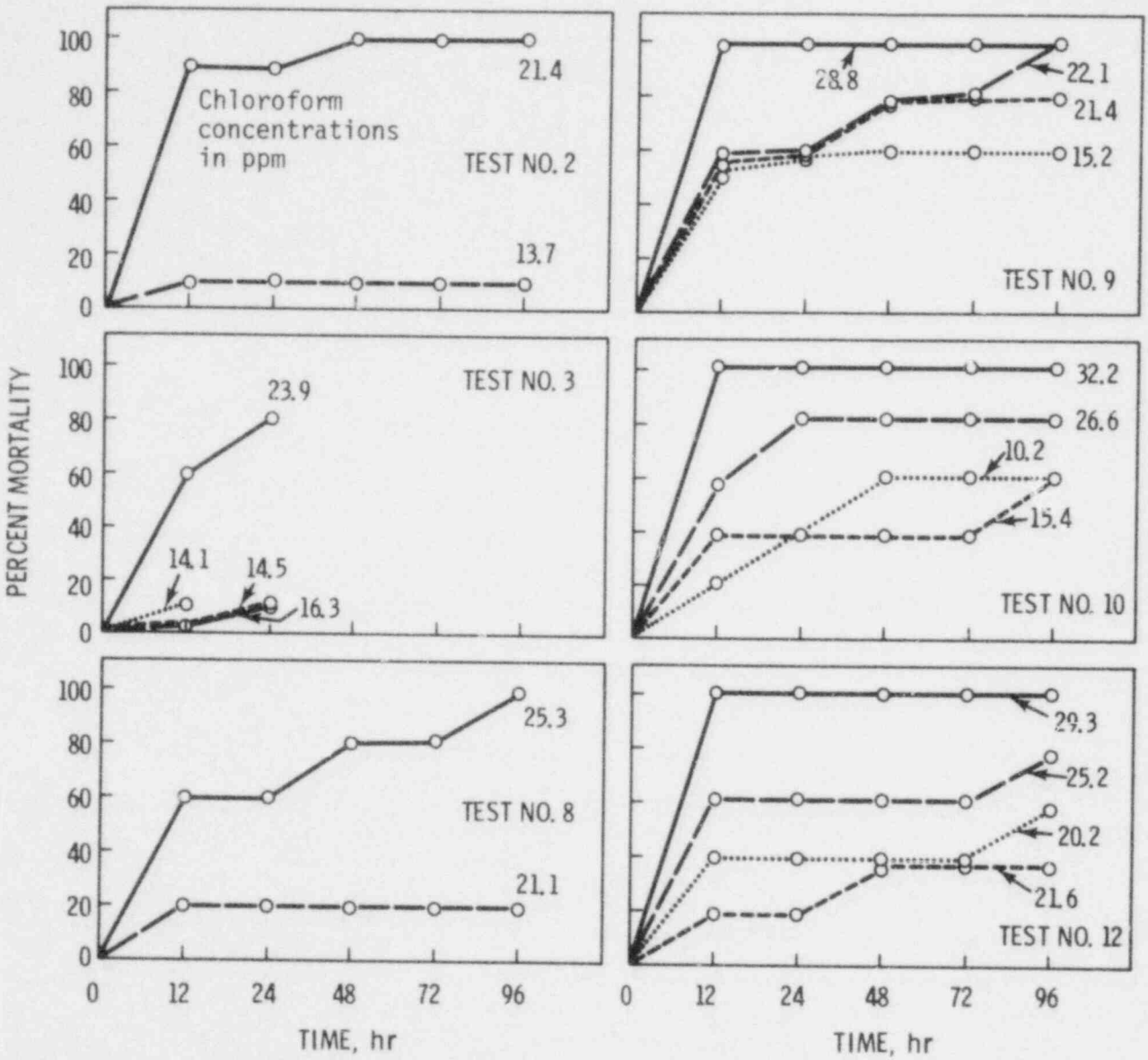

FIGURE 5. Mortalities of Bluegill During 96-hr Toxicity Tests at 19°C with Chloroform. Concentrations that did not cause mortality are given in Table 13.

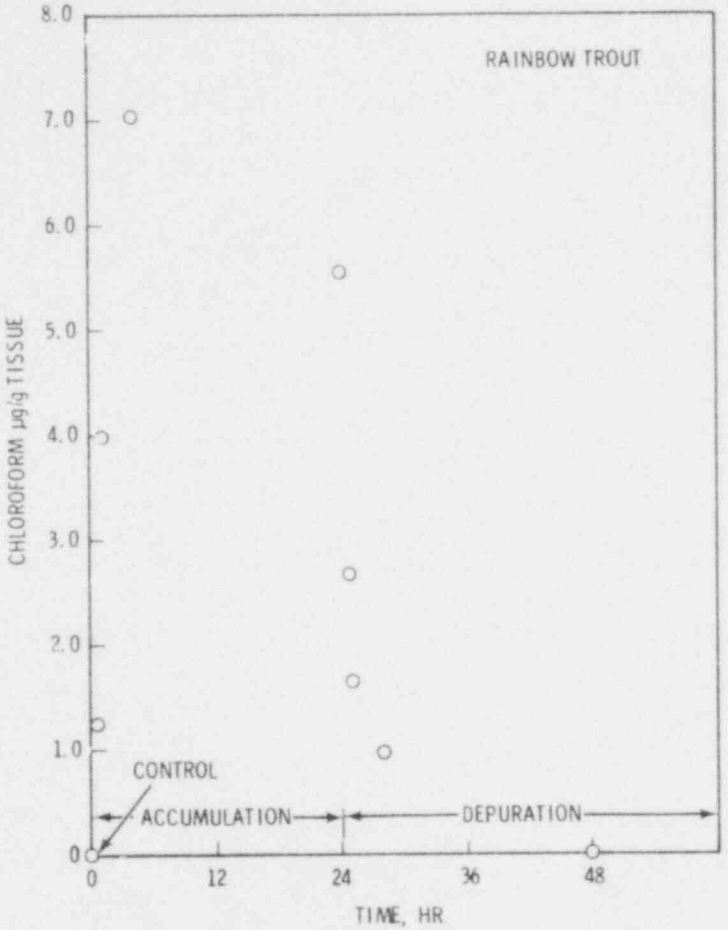

FIGURE 6. Chloroform Concentrations in Rainbow Trout Exposed to 1.0 ppm $CHCl_3$ for 24 hr (mean of two fish represented per point).

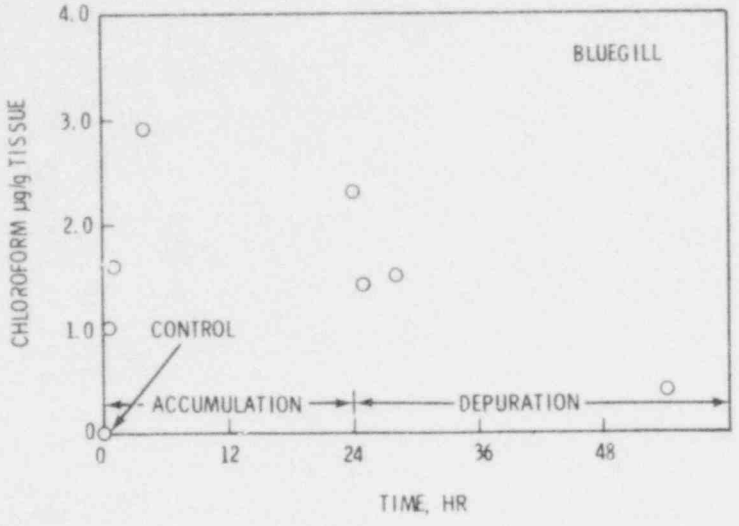

FIGURE 7. Chloroform Concentrations in Bluegill Exposed to 1.0 ppm $CHCl_3$ for 24 hr (mean of three fish represented per point).

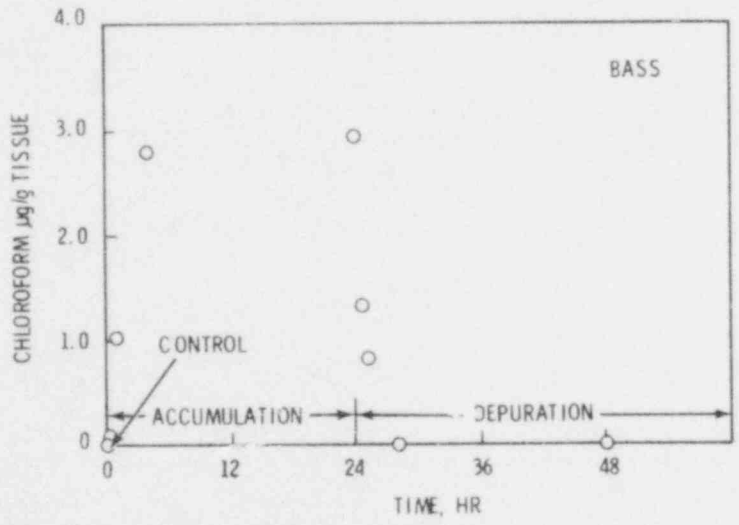

FIGURE 8. Chloroform Concentrations in Largemouth Bass Exposed to 1.0 ppm $CHCl_3$ for 24 hr (mean of three fish represented per point).

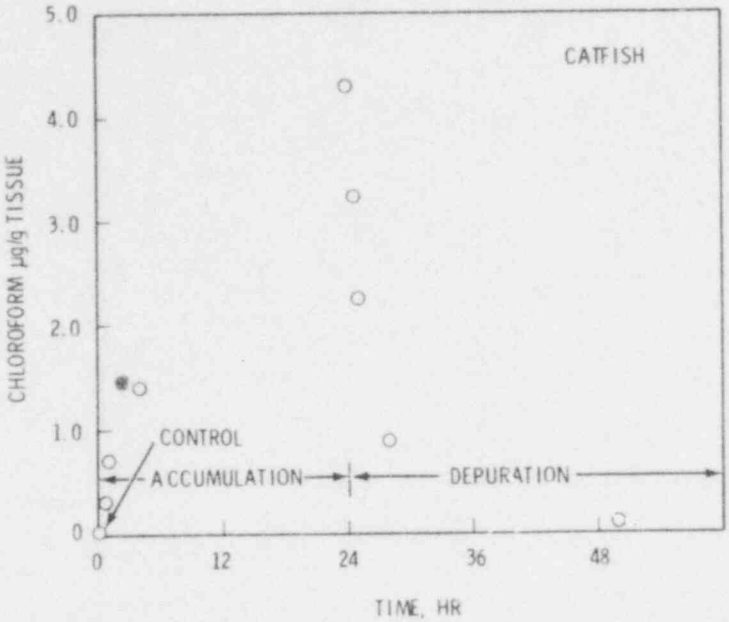

FIGURE 9. Chloroform Concentrations in Channel Catfish Exposed to 1.0 ppm $CHCl_3$ for 24 hr (mean of three fish represented per point).

TABLE 1. Mean and Standard Deviation for Length and Weight of Fish Used in 96-Hr Chloroform Toxicity Tests.

Test No.	Length (cm)		Weight (g)	
	$\bar{x}$	s	$\bar{x}$	s
Bluegill				
2	16.9	1.6	129.9	43.1
3	16.9	1.3	125.4	40.0
8	16.2	1.0	103.9	27.8
9	17.1	1.2	126.4	35.8
10	16.7	1.3	119.6	34.2
12	16.5	1.2	106.5	31.5
Rainbow Trout				
5	7.9	0.8	5.2	1.6
6	8.1	0.8	5.8	1.7
7	8.4	0.8	6.6	1.8
13	11.5	0.9	16.8	3.7
15	8.8	1.0	7.6	2.2
Channel Catfish				
11	23.6	1.4	152.5	28.2
22	26.4	2.1	239.2	68.1
25	11.9	1.5	16.8	7.6
26	12.0	1.2	20.3	8.0
27	11.9	1.8	21.6	9.8
28	12.1	1.5	21.4	9.2
Largemouth Bass				
16	16.0	1.2	62.8	18.8
17	16.1	1.2	63.8	20.0
18	13.0	0.8	30.5	5.8
19	12.9	0.8	27.9	5.0
20	12.7	1.0	26.3	7.3
21	13.1	1.1	30.9	8.5

TABLE 2. Chloroform Accumulation/Depuration in Rainbow Trout During a 24-hr Exposure to 1.0 ppm $CHCl_3$ Followed by 24 hr of Depuration at 20.2°C.

Sampling time (hr)	Fish length (cm)	Fish weight (g)[a]	$CHCl_3$ µg/g tissue[a]
		Exposure	
0	14.9	38.1	0
0	16.0	45.4	0
0.25	13.5	28.1	0.7
0.25	16.3	50.8	1.10
1	12.8	25.8	4.0
1	15.1	44.3	4.90
4	14.1	36.5	6.6
4	14.5	39.4	10.2
24	12.7	22.7	3.34
24	14.4	39.3	10.35
		Depuration	
24.25	13.0	26.4	3.3
24.25	15.0	39.6	3.31
25	14.2	35.0	1.7
25	16.0	48.7	2.4
28	14.4	33.6	1.1
28	15.8	49.7	1.2
48	13.6	30.0	0.0
48	16.3	48.2	0.0

a Fish weight is based on live wet weight at sampling time.

TABLE 3. Chloroform Accumulation/Depuration in Bluegill During a 24-hr Exposure to 1.0 ppm $CHCl_3$ Followed by 3.0 hr of Depuration at 26.3°C.

Sampling time (hr)	Fish length (cm)	Fish weight (g)[a]	$CHCl_3$ µg/g tissue[a]
		Exposure	
0	18.0	140	0
0	19.5	221	0
0.25	16.3	90	1.3
0.25	16.7	121	0.9
1	15.0	81	1.9
1	18.1	166	1.6
4	17.4	149	3.0
4	19.5	243	3.1
24	15.5	73	1.6
24	17.4	[illegible]0	2.5
		Depuration	
24.25	20.4	227	b
24.25	18.5	142	b
25	14.6	61	1.2
25	15.6	83	1.9
28	17.3	115	1.6
28	17.1	121	1.7
54	16.9	106	0.0
54	19.2	182	0.0

a Fish weight is based on live wet weight at sampling time.
b Samples contaminated during analysis.

TABLE 4. Chloroform Accumulation/Depuration in Largemouth Bass During a 24-hr Exposure to 1.4 ppm $CHCl_3$ Followed by 24 hr of Depuration at 23.4°C.

Sampling time (hr)	Fish length (cm)	Fish weight (g)[a]	$CHCl_3$ µg/g tissue[a]
	Accumulation		
0	18.0	83.0	0
0	14.3	35.4	0
0.25	16.0	46.1	0
0.25	15.1	62.7	0
1	13.2	36.1	2.0
1	16.4	47.5	0.1
4	15.9	49.5	2.2
4	14.4	38.0	3.0
24	24.6	40.0	3.1
24	14.6	42.4	2.9
	Depuration		
24.25	15.9	41.1	0.1
24.25	13.6	27.2	1.4
25	16.1	67.4	2.5
25	13.6	28.5	0.0
28	13.5	32.9	0.0
28	13.2	21.9	0.0
48	14.6	39.6	0.0
48	16.0	54.9	0.0

a Fish weight is based on live wet weight at sampling time.

TABLE 5. Chloroform Accumulation/Depuration in Catfish During a 24-hr Exposure to 1.1 ppm $CHCl_3$ Followed by 26 hr of Depuration at 24.3°C.

Sampling time (hr)	Fish length (cm)	Fish weight (g)[a]	$CHCl_3$ µg/g tissue[a]
	Accumulation		
0	38.4	588	0
0	39.0	698	0
0.25	33.5	409	0.3
0.25	36.4	528	0.3
1	39.6	640	0.5
1	37.7	641	0.8
4	42.0	750	0.7
4	38.5	630	1.5
24	37.2	562	3.3
24	33.1	422	3.7
	Depuration		
24.25	38.4	641	2.8
24.25	36.7	517	2.7
25	35.4	482	1.7
25	40.2	660	1.1
28	39.3	689	1.3
28	38.3	610	0.4
50	33.5	371	0.23

a Fish weight is based on live wet weight at sampling time.

TABLE 6. Acute Chloroform LC_{50}s (ppm) With Largemouth Bass at 19°C in Columbia River Water.

Census Times	12 hr	24 hr	48 hr	96 hr
Toxicity Test No.				
16	a	a	a	a
17	56.2	56.2	b	b
18	50.4	50.4	50.4	c
19	55.8	55.8	55.8	55.8
20	52.5	52.5	52.5	52.5
21	45.4	45.4	45.4	45.4

a No mortalities occurred in all test groups.
b Only one mortality occurred, insufficient data for LC_{50} calculation.
c Test terminated prior to the 48-hr census time.

TABLE 7. Acute Chloroform LC_{50}s (ppm) With Channel Catfish at 19°C in Columbia River Water.

Census Times	12 hr	24 hr	48 hr	96 hr
Toxicity Test No.				
11	a	a	b	b
22	a	a	a	a
25	a	a	a	a
26	135[c]	135[c]	b	b
27	126[d]	126[d]	101[e]	b
28	a	a	a	75[d]

a Insufficient mortality to compute an LC_{50}.
b Test terminated.
c Binomial method.
d Moving average method.
e Probit method.

TABLE 8. Acute Chloroform LC_{50}s (ppm) With Bluegill at 25°C in Columbia River Water.

Census Times	12 hr	24 hr	48 hr	96 hr
Toxicity Test No.				
2	17.1	17.1	16.3	16.2
3	23.9	21.8	a	a
8	24.2	24.2	23.1	22.3
9	16.2	16.2	14.6	13.3
10	24.4	20.2	19.4	18.3
12	24.1	24.1	22.3	20.8

a Test terminated prior to 48-hr census time due to disease mortality in the control aquarium.

TABLE 9. Acute Chloroform LC_{50}s (ppm) With Rainbow Trout at 13°C in Columbia River Water.

Census Times	12 hr	24 hr	48 hr	96 hr
Toxicity Text No.				
5	-	-	21.4	18.2
6	-	-	-	18.4
7	37.1	26.1	23.6	22.1
13	-	-	18.6	15.1
15	24.5	20.0	19.3	17.1

TABLE 10. Largemouth Bass Mortalities During 96-hr Acute Toxicity Tests at 19°C with Chloroform.

Test #14 20 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
15.2	0	0	0	0
8.3	0	0	0	0
0.0	0	0	0	0
8.6	0	0	0	0
1.1	0	0	0	0
16.7	0	0	0	0

Test #16 5 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
39.4	1	1	1	1
18.6	0	0	0	0
21.9	0	0	0	0
25.7	0	0	0	0
0.0	0	0	0	0
35.0	0	0	0	0

Test #17 5 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
63.5	4	4	a	a
27.0	0	0	a	a
42.7	0	0	a	a
25.2	0	0	a	a
0.0	0	0	a	a
58.8	3	3	a	a

Test #18 5 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
36.7	0	0	0	0
55.9	5	5	5	5
48.0	1	1	1	1
77.8	5	5	5	5
0.0	0	0	0	0
77.1	5	5	5	5

a Test terminated due to chloroform pump failure.

TABLE 10. (contd)

Test #19 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
30.3	0	0	0	0
47.0	0	0	0	0
42.1	0	0	0	0
52.8	3	3	3	3
0.0	0	0	0	0
60.7	8	8	8	8

Test #20 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
20.0	0	0	0	b
39.0	0	0	0	b
37.2	0	0	0	b
47.3	1	1	1	b
0.0	0	0	0	b
61.7	10	10	10	b

Test #21 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
42.6	3	3	3	3
56.2	10	10	10	10
31.0	0	0	0	0
59.2	10	10	10	10
0.0	0	0	0	0
69.3	10	10	10	10

b Bioassay terminated prior to 72 hrs due to disease mortality in the control aquarium.

TABLE 11. Channel Catfish Mortalities During 96-hr Acute Toxicity Tests with Chloroform.

Test #11 3 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
12.3	0	0	a	
12.1	0	0	a	
0	0	0	a	
6.1	0	0	a	
3.7	0	0	a	
14.4	0	0	a	

Test #22 (1 fish per aquarium)

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
39.8	0	0	0	0
41.2	0	0	0	0
28.1	0	0	0	0
67.4	0	0	0	0
0	0	0	0	0
67.8	0	0	0	0

Test #25 5 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
42.1	0	0	0	0
69.7	0	0	0	0
0	0	0	0	0
31.7	0	0	0	0
49.4	0	0	0	0
42.4	0	0	0	0

Test #26 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
92.3	0	0	b	
171.0	10	10	b	
0	0	0	b	
166.3	10	10	b	
108.9	0	0	b	
65.0	0	0	b	

Test #27 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
92.7	1	1	4	10c
152.6	10	10	10	10c
0	0	0	0	0c
154.8	9	10	10	10c
122.3	4	4	7	10c
142.1	9	9	9	10c

Test #28 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$(ppm)				
68.2	0	0	0	1
88.9	0	0	2	7
0	0	0	0	0
78.8	0	0	0	5
76.0	0	0	0	6
82.3	0	0	0	6

a Test terminated at 48 hr due to agressive fish behavior.
b Test terminated due to malfunction in toxicant delivery.
c Test terminated at 72-hr census time.

TABLE 12. Rainbow Trout Mortalities During 96-hr Acute Toxicity Tests with Chloroform.

Test #5 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
20.0	0	0	1	7
11.3	0	0	0	0
12.0	0	0	0	0
13.2	0	0	0	0
0.0	0	0	0	0
22.9	0	1	9	10

Test #6 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
19.5	0	0	5	7
15.4	0	8	8	8
15.0	0	0	0	0
16.6	0	0	0	0
0.0	0	0	0	0
25.5	0	1	1	7

Test #7 20 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
27.0	3	13	19	19
19.6	0	0	0	4
15.1	0	0	0	0
10.6	0	0	0	0
0.0	0	0	0	0
27.8	1	11	16	20

Test #13 20 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
18.6	0	0	10	17
8.4	0	0	0	0
3.3	0	0	0	0
12.3	0	0	3	6
0.0	0	0	0	0
12.1	0	0	0	0

Test #15 20 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
26.6	15	20	20	20
9.8	0	0	0	0
15.3	0	0	0	0
18.6	0	0	3	19
0.0	0	0	0	0
20.6	3	16	20	20

TABLE 13. Bluegill Mortalities During 96-hr Acute Toxicity Tests with Chloroform.

Test #2 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
2.4	0	0	0	0
0.4	0	0	0	0
3.0	0	0	0	0
13.7	1	1	1	1
0.0	0	0	0	0
21.4	9	9	10	10

Test #3 10 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
18.7	0	0	a	a
14.1	1	1	a	a
16.3	1	1	a	a
14.5	0	1	a	a
0.0	0	0	a	a
23.9	6	8	a	a

Test #8 5 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
25.3	3	3	4	5
18.7	0	0	0	0
12.7	0	0	0	0
14.7	0	0	0	0
0.0	0	0	0	0
21.1	1	1	1	1

Test #9 5 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
28.8	5	5	5	5
22.1	3	3	4	5
4.9	0	0	0	0
21.4	3	3	4	4
0.0	0	0	0	0
15.2	3	3	3	3

Test #10 5 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
26.6	3	4	4	4
15.4	2	2	2	3
15.4	0	0	0	0
10.2	1	2	3	3
0.0	0	0	0	0
32.2	5	5	5	5

Test #12 5 fish per aquarium

Census Times	12 hr	24 hr	48 hr	96 hr
$CHCl_3$ (ppm)				
29.3	5	5	5	5
21.6	1	1	2	2
5.7	0	0	0	0
20.2	2	2	2	3
0.0	0	0	0	0
25.2	3	3	3	4

a Test terminated prior to 48-hr census time due to mortality from columnaris disease in the control aquarium.

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RC FORM 335 (7 77)

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Acute Toxicity and Bioaccumulation of Chloroform to Four Species of Freshwater Fish

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ABSTRACT (200 words or less)

Acute toxicity of chloroform to four species of freshwater fish was studied in flow-through 96-hr toxicity tests. Chloroform is toxic to fish in the tens of parts per million, a concentration well above that which would be expected to be produced under normal power plant chlorination conditions. Investigations of acute toxicity of chloroform and the bioaccumulation of chlorinated compounds in tissues of fish revealed differences in tolerance levels and tissue accumulations. Mean 96-hr LC_{50}s for chloroform were 18 ppm for rainbow trout and bluegill, 51 ppm for largemouth bass and 75 ppm for channel catfish. Mortalities of bluegill and largemouth bass occurred during the first 4 hr of exposure while rainbow trout and channel catfish showed initial tolerance and mortalities occurred during the latter half of the 96-hr exposure. Rainbow trout had the highest level of chloroform tissue accumulation, 7 µg/g tissue, catfish the second highest, 4 µg/g tissue, followed by bluegill and largemouth bass which each accumulated about 3 µg/g tissue. Accumulation of chloroform was less than one order of magnitude above water concentrations for all species.

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