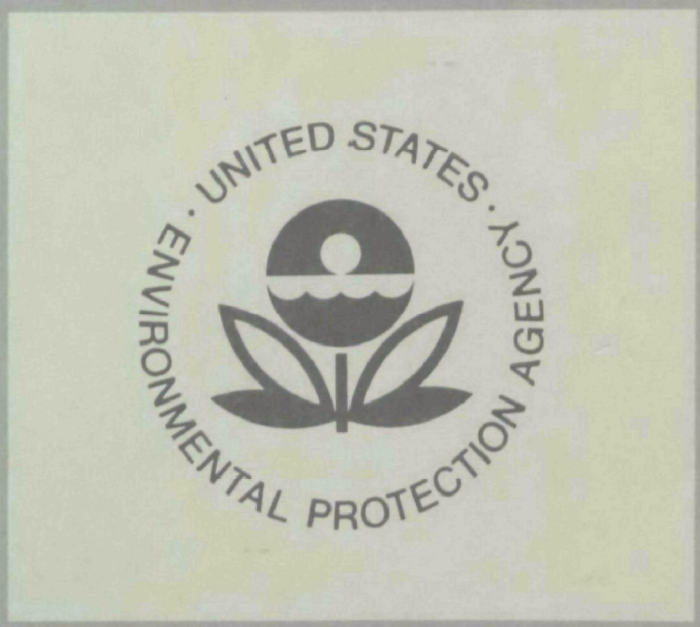
EPA-600/3-76-047
May 1976

Ecological Research Series

CHRONIC TOXICITY OF ATRAZINE TO SELECTED AQUATIC INVERTEBRATES AND FISHES



Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/3-76-047
May 1976

CHRONIC TOXICITY OF ATRAZINE TO SELECTED AQUATIC INVERTEBRATES AND FISHES

by

Kenneth J. Macek
Kenneth S. Buxton
Scott Sauter
Sarah Gnilka
Jerry W. Dean

Bionomics, EG&G Inc.
Wareham, Massachusetts 02571

Contract No. 68-01-0092
68-01-1844

Project Officer

John Eaton
Environmental Research Laboratory
Duluth, Minnesota 55804

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORY
DULUTH, MINNESOTA 55804

DISCLAIMER

This report has been reviewed by the Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

Representatives of the aquatic invertebrate species of water flea (Daphnia magna), midge (Chironomus tentans), and scud (Gammarus fasciatus); and the fish species bluegill (Lepomis macrochirus), fathead minnow (Pimephales promelas), and brook trout (Salvelinus fontinalis) were chronically exposed to various concentrations of atrazine in separate flowing-water systems.

Maximum acceptable toxicant concentrations (MATC) of atrazine for the selected species in soft water were estimated using survival, growth, and reproduction as indicators of toxic effects. The MATC was estimated to be between 0.11 and 0.23 mg/l for midges, between 0.14 and 0.25 mg/l for water fleas, and between 0.06 and 0.14 for the scud. For fishes the MATC was estimated to be between 0.09 and 0.50 mg/l for bluegills, between 0.21 and 0.52 mg/l for fathead minnows, and between 0.06 and 0.12 mg/l for brook trout. The incipient-LC50 for fishes and the 48-hour LC50 for invertebrates was estimated from acute exposures and was used to calculate application factors (MATC limits/LC50). For aquatic invertebrates and atrazine the estimated application factors were between 0.15 and 0.32 for midges, between 0.02 and 0.04 for water flea, and between 0.01 and 0.02 for scud. Application factors were estimated between 0.01 and 0.07 for bluegills, between 0.01 and 0.03 for fathead minnows, and between 0.01 and 0.02 for brook trout.

This report was submitted in fulfillment of Project Number 18050 HQH, Contracts 68-01-0092 and 68-01-1844, under the sponsorship of the Water Quality Office, Environmental Protection Agency.

CONTENTS

TABLES

PAGE

ACKNOWLEDGEMENTS

During the period of time devoted to this research effort, we were fortunate to have laboratory assistance from Mark Lindberg and Gerald LeBlanc, who maintained exposure systems and were significantly involved with routine aspects of the fish chronics. Gratitude is extended to Krogh Derr, Pat Costa and Karen Frey for their involvement and expertise in conducting the invertebrate chronics. Also, our appreciation is directed to Mr. Curtis Hutchinson for construction of all diluter systems utilized throughout this study, and to Rod Parrish and Dr. Sam Petrocelli for constructively reviewing the final report.

We are grateful to Mr. Richard Griffith, Northeast Regional Director, U.S. Bureau of Sport Fisheries, Boston, Mass. for his assistance in obtaining disease-free brook trout used in the chronic exposure and to Mr. Quentin Pickering, Newtown Fish Toxicology Station, EPA, Cincinnati, Ohio, for providing fathead minnow eggs used in the study.

Finally, our sincere appreciation is extended to Mr. John Eaton, Project Officer, Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, Minnesota, for his guidance and constructive advice during the performance of these studies.

SECTION I

CONCLUSIONS

Estimates (LC50) of the acute toxicity of atrazine generally ranged from 4.9-15.0 mg/l for all the species tested except the midges for which the estimated 48 hour LC50 was 0.72 mg/l.

All six species were similar in their susceptibility to chronic exposure to atrazine with the lower limit of the estimated MATC concentrations for all species ranging from 0.06-0.21 mg/l.

Continuous exposure of chironomids through two successive generations to a mean measured concentration of 0.23 mg/l atrazine resulted in reduced hatching success, larval mortality, developmental retardation, and a reduction in the number of organisms pupating and emerging.

Continuous exposure of daphnids to atrazine through three successive generations indicates that reproduction is a much more sensitive measure of species susceptibility than is survival during acute or chronic exposures. Although continuous exposure to a mean measured atrazine concentration of 1.15 mg/l had no significant effect on survival, continuous exposure to 0.25 mg/l significantly reduced production of progeny.

Morphological development of F_1 gammarids is a more sensitive indication of species susceptibility to chronic exposure to atrazine than is survival. Although continuous exposure of parental gammarids to 0.49 mg/l atrazine for 119 days had no effect on survival, and continuous exposure to 0.14 mg/l for 30 days had no effect on survival of F_1 gammarids, the number of F_1 gammarids exposed to 0.14 mg/l atrazine which successfully developed to the seventh instar form was reduced 25% when compared to lower treatments and controls.

Growth of brook trout is the most sensitive indication of species susceptibility to chronic exposure to atrazine. Although continuous exposure to a mean measured concentration of 0.72 mg/l atrazine for 44 weeks had no effect on survival of parental fish, continuous exposure during the period to 0.12 mg/l atrazine significantly reduced growth of fry when compared to lower treatments and controls.

The data indicate the tendency for all three fishes to bioconcentrate atrazine from water is very low when compared to other pesticides. Chemical analysis of fish tissue samples indicated that residue concentrations after prolonged exposure were below minimum detectable limits for all the fishes analyzed.

The similarity of the limits of the application factors for each of the fishes, and two of the three invertebrates, tested supports the general validity of the application factor concept.

SECTION II

RECOMMENDATIONS

Midges (*Chironomus tentans*) were more susceptible to atrazine than any of the other species studied. A variety of sublethal effects were observed. These observations, combined with the relatively short life history of midges, and the adaptability of these organisms to the laboratory, suggests the species as a desirable bioassay organism for evaluating the chronic toxicity of chemicals to aquatic organisms.

Concerning chronic toxicity studies with bluegill, we feel that more information must be developed on the laboratory conditions necessary to induce and maintain spawning activity and on the procedures necessary to handle and feed newly hatched bluegill fry, in order to allow successful completion of bluegill chronic toxicity studies.

Based on the lack of an acceptable level of spawning activity among several groups of fathead minnows in which the number of males was essentially equal to or greater than the number of females, we suggest that care be taken throughout a fathead minnow chronic to insure that during the spawning period the ratio of females to males is greater than 1.5:1.

We recommend that the fathead minnow be considered the fish species of choice, among those tested, for chronic toxicity bioassays. This recommendation is based on the fact that an adequate level of spawning activity can be induced under laboratory conditions, successful handling of eggs and larvae is possible, and excellent survival of fry is obtained. The combination of these factors provides opportunities for statistical and biological evaluation of possible toxic effects due to chronic exposure to chemicals. Finally, studies with this species represent the only "true chronic" (at least one complete life history) of the fishes studied.

SECTION III

INTRODUCTION

The current concern regarding the protection of aquatic life in surface waters has prompted evaluation of the effects of chemicals on aquatic invertebrates and fishes. Much of the toxicological research with aquatic biota has been limited to the development of acute toxicity values as a measure of the effect of chemicals on the biota. More recently, utilization of the chronic exposure of fishes and aquatic invertebrates to chemicals has received particular attention due to the numerous parameters that can be evaluated as indices of toxic effects (Mount, 1968; Eaton, 1970; Arthur, 1970; McKim and Benoit, 1971; Arthur et al., 1973; Macek et al., 1975) The "Laboratory Fish Production Index (LFPI)" as defined by Mount and Stephan (1967) reflects toxic effects on reproduction, growth, spawning behavior, egg hatchability, and fry survival. Somewhere between the highest observed toxicant concentration that has no effect on these parameters during continuous chronic exposure and the lowest effect concentration is a theoretical value termed the maximum acceptable toxicant concentration (MATC).

The occurrence and persistence of chlorinated hydrocarbon insecticides and heavy metals in surface waters has resulted in widespread interest in the effect of these chemicals in aquatic ecosystems. As a result, much information on the effect of these materials on aquatic organisms has been generated (Cope, 1966; Johnson, 1968; Eisler, 1973). However, much less is known of the effects of less persistent chemicals despite the fact that many of these are utilized extensively in agriculture. One of the most widely utilized of these non-persistent chemicals is the herbicide atrazine (2-chloro-4-ethlyamino-6-isopropylamino-s-triazine) with more than 100 million pounds being applied annually to agricultural lands in the United States (Hall et al., 1972).

Past studies with atrazine have been few and consisted of short-term aqueous exposures which generated acute toxicity values (Walker, 1964; FWPCA, 1968; Sanders, 1969). Due to the extensive use of this chemical and lack of information concerning its long-term, subtle effects on aquatic organisms, this study was undertaken to determine the MATC of atrazine for selected aquatic invertebrates and fishes based on continuous chronic exposure. The organisms selected for this research effort were:

INVERTEBRATES

1. Chironomus tentans (Chironomidae, midge)
2. Daphnia magna (Cladocera, water flea)
3. Gammarus fasciatus (Gammaridae, scud)

FISHES

1. Lepomis macrochirus (Centrarchidae, bluegill)
2. Pimephales promelas (Cyprinidae, fathead minnow)
3. Salvelinus fontinalis (Salmonidae, brook trout)

A reason for selecting both invertebrate and fishes is that the susceptibility of fishes to a chemical should be compared to the susceptibility of fish-food organisms to that same chemical. An understanding of both these phenomena will be invaluable in establishing realistic and meaningful water quality criteria and standards.

SECTION IV

MATERIALS AND METHODS

The methodology for chronic testing of fishes generally followed the recommended bioassay procedures issued by the Environmental Protection Agency's National Water Quality Laboratory, Duluth, Minnesota (Bioassay Committee 1971a, 1971b, 1971c). Acute bioassay procedures were bascially those recommended in Standard Methods for the Examination of Water and Wastewater (APHA, 1971). Chronic testing procedures for invertebrates were determined through communication of Bionomics staff members with personnel at the National Water Quality Laboratory.

CHRONIC EXPOSURE SYSTEMS

Proportional diluters (Mount and Brungs, 1967), with a dilution factor of 0.5 and a syringe injector, delivered the test water and toxicant, dissolved in dimethyl sulfoxide, to the mixing chamber, mixing cells, and ultimately to the test chambers. Five atrazine concentrations and a control flowed to mixing containers and into separate glass delivery tubes leading to the replicate test chambers. In the test system for Daphnia magna, baffles were inserted in each of the quadruplicate test chambers to minimize turbulence of influent water. The diluter was also modified to include food cells which delivered a measured amount of food along with the toxicant and diluent water. All other exposure systems utilized duplicate test chambers of varying construction and flow rates as summarized in Table 1.

Two growth chambers 21 x 20 x 15 cm (height x length x width) with a water depth of 16 cm were provided for the young Gammarus and received test water at a flow rate equal to that in the adult test chambers. Fathead minnow test chambers were subdivided to provide space for two growth chambers 25 x 20.5 x 12.5 cm, and test chambers for brook trout contained a shelf which supported two growth chambers 25 x 25 x 12.5 cm with a water depth of 12.5 cm. Water was delivered directly to both the adult test chamber and growth chambers through a glass, flow-splitting chamber calibrated to provide equal flow rate to all chambers. The bluegill test chambers supported two 18 x 28 x 12 cm growth chambers with a water depth of 10.5 cm, and air was utilized to pump water from the test chamber to the growth chambers at a rate of 6 chamber volumes every 24 hours. All fish growth chambers had 40-mesh stainless steel screen affixed to one end to allow water to flow out while retaining the young fish. Bluegill and brook trout test chambers were aerated with oil-free air in order to maintain dissolved oxygen levels above 60 percent saturation.

TABLE 1. PHYSICAL CHARACTERISTICS OF TEST CHAMBERS UTILIZED TO EVALUATE THE CHRONIC TOXICITY OF ATRAZINE TO FISHES AND FISH FOOD ORGANISMS

Species	Material (shape)	Dimensions[a] (cm)	Water Depth (cm)	Volume (liters)	Flow Rate (tank vol./ 24 hours)
Chironomus tentans	glass (rectangular)	21x26x18	16.0	7.5	6
Daphnia magna	glass (cylindrical)	17x13.5	14.0	1.8	2
Gammarus fasciatus	glass (rectangular)	25x40x21	19.0	16.0	3
Lepomis macrochirus	stainless steel (rectangular)	40x180x30.5	30.5	168.0	7
Pimephales promelas	glass (rectangular)	30.5x90x30.5	15.0	41.0	7
Salvelinus fontinalis	stainless steel (rectangular)	40x90x30.5	30.5	84.0	7

[a]Dimensions are height x length x width for rectangular chambers and height x diameter for cylindrical chambers.

Diluent water was pumped from a 400 foot subterranean well to a cement holding tank. Results of the chemical analysis of the diluent water are summarized in Table 2.

TABLE 2. CHEMICAL ANALYSIS OF THE DILUENT WATER UTILIZED DURING CHRONIC AQUEOUS EXPOSURE OF FISHES AND AQUATIC INVERTEBRATES TO ATRAZINE

Parameter	mg/liter	Parameter	mg/liter
Calcium	6.0	Chloride	17.6
Magnesium	2.1	Fluoride	0.5
Potassium	1.1	Cyanide	<0.005
Sulfate	11.6	Iron	<0.01
Nitrate	<0.05	Copper	0.004
Nitrite	<0.05	Zinc	0.01
Ammonia	<0.1	Cadimum	0.001
Phenol	<0.001	Chromium	<0.001
Chlorine	<0.01	Lead	<0.01

Diluent water was delivered through PVC pipes to the exposure systems. Unheated well water was mixed with heated well water from a glass-lined heater to provide a constant temperature of 28±1°C before delivery to the bluegill experimental unit. Test chambers in the fathead minnow and brook trout units were maintained in circulating water baths at the desired test temperatures. Water circulating in the water baths of the fathead minnow unit was heated by two immersion coil heaters controlled by a mercury column thermoregulator which maintained a temperature of 25±1°C. Water in the baths of the brook trout unit was cooled by recirculating through a mechanical chiller to temperatures of 15±1°C from May through August, 12±1°C during September, and 9±1°C from October through the end of juvenile exposure. Prior to entering experimental units for the invertebrate studies, diluent water was delivered to a stainless steel headbox where immersion coil heaters and thermoregulators maintained a temperature of 19±1°C for Daphnia and Gammarus and 23±1°C for

Chironomus. In addition, ultra-violet lights (24 watt) were placed over the headbox and water cells of each invertebrate diluter to minimize the introduction of fungus and pathogens into the test system.

Illumination was provided by a combination of Duro-test (Optima FS) and wide-spectrum Grow-Lux fluorescent lights fixed centrally above the test chambers in all experimental units. Incandescent bulbs (100 watt) simulated a 15-minute dawn or dusk light intensity change (Drummond and Dawson, 1970). A constant 16-hour light, 8-hour dark photoperiod was controlled by an automatic timer in the Chironomus and Daphnia experiments.

The photoperiod for Gammarus, bluegills, and brook trout followed the normal daylight hours of Evansville, Indiana (representative of U.S. daylength), and was adjusted the first and fifteenth of each month. The photoperiod for fathead minnows followed Evansville daylengths but was started with the daylength for December 1st on day one of the experiment (Nov. 11). All experimental units were screened with black polyethylene curtains to prevent unnecessary disturbance of test organisms and the influence of extraneous lighting on the intended photoperiod.

ACUTE TOXICITY PROCEDURES

Static acute toxicity bioassays were conducted with invertebrates and atrazine to estimate the 48-hour LC50 and its 95% confidence interval. Five organisms in three or four replicate containers at each concentration were exposed at 20 ± 1°C. First instar Chironomus tentans, Gammarus fasciatus, and <24-hour old Daphnia magna were the organisms exposed. A linear regression equation was calculated after converting test concentrations and corresponding percent mortalities to logarithms and probits, respectively, and this equation was utilized to estimate the 48-hour LC50's and confidence intervals.

Acute toxicity studies with trout and bluegill were conducted in flow-through systems using proportional diluters (Mount and Brungs, 1967). The incipient LC50 was estimated when no additional significant (>10%) mortality of the test organisms was observed for 48 hours among fishes exposed to any concentration. At this time, the exposure was terminated and a linear regression equation was calculated by converting atrazine concentrations and corresponding mortalities into logarithms and probits, respectively. This equation was utilized to estimate the incipient LC50 and 95% confidence interval. Due to the solubility of atrazine in water, a static bioassay was used to estimate the acute toxicity of atrazine to fathead minnows.

The chronic exposure concentrations for both invertebrates and fishes were selected by evaluating observed mortality and no-effect concentrations from acute studies. The 48-hour LC50 values for invertebrates and the incipient LC50 values for fishes (except minnows) were used to estimate application factors describing the relationship between acute and chronic toxicity.

CHEMICAL METHODS

Toxicant concentrations and basic water quality characteristics were initially monitored in each aquarium each week to establish that the toxicant concentrations and water quality characteristics were constant with minimum variability. After the determination of these concentrations and parameters in the experimental systems, a minimum monitoring effort was conducted to measure variability and detect changes from the established means. Generally, atrazine concentrations were determined once each week during each of the chronic exposures by taking 500 ml water samples from each aquarium. Stock solutions of atrazine (94% a.i., Ciba-Geigy Chemical Co.) in nanograde dimethyl sulfoxide (DMSO) were delivered to the dilution water from 50 ml glass syringes through stainless steel needles. The solvent (DMSO) was not added to the control water in any of the chronic exposures and the amount added to the highest atrazine concentration of each chronic test ranged from 12 µg/l during the brook trout chronic to 65 µg/l during the Daphnia chronic. In each case, these concentrations were determined to be less than 1/500 of the 48-hour or 96-hour LC50 of the solvent for each species.

Water samples were taken in amber-glass bottles with teflon-lined caps. The volume of water was accurately measured and transferred to a separatory funnel. The sample bottle was rinsed with 50 ml of methylene chloride to remove any adsorbed atrazine and this solvent was added to the separatory funnel containing the water sample. The separatory funnel was shaken for two minutes to extract atrazine from the water, allowed to stand until phase separation occurred, and the solvent drained into a beaker. The extraction was repeated three times using 25 ml of methylene chloride per extraction and the extracts combined in the beaker.

The solvent extract was passed through a 20 x 50 mm column of sodium sulfate and the column was rinsed with an additional 50 ml portion of methylene chloride. The solvent was evaporated to approximately 5 ml on a stream bath, quantita-

tively transferred to 15 ml centrifuge tubes with methylene chloride, and evaporated to dryness using a gentle stream of clean, dry air at 40°C. The extract was immediately dissolved in an accurately measured volume of hexane and a portion was withdrawn and analyzed by gas/liquid chromatography under the following conditions:

Instrument: Beckman Model 45 GC gas/liquid chromatograph

Detector: Helium-arc electron capture

Column: 183 x 2 mm I.D. glass containing 5% Dexsil on 80/100 mesh Supelcoport

Carrier gas: High purity helium at 55cc/min.

Detector gas: High purity helium at 110cc/min. and carbon dioxide at 3cc/min.

Inlet: 225°C, Column: 200°C, Outlet: 240°C, Detector: 260°C

Recorder: 1mv full-scale, chart speed 1.3 cm/min.

Atrazine was eluted in 4.2 minutes, and 10 ng gave full-scale pen deflection at an electrometer attenuation of 8×10^{-10} amperes. Linearity of response was not assumed during the study, rather a graph of peak height versus ng of atrazine injected was constructed prior to analyzing each batch of atrazine samples. The amount of atrazine in each sample was then determined by graphical interpolation. The recovery of atrazine from spiked water samples using the above methods was essentially quantitative and no adjustments were necessary for recovery (Table 3).

Fish tissue was extracted by the column method of Hesselberg and Johnson (1972). The extraction solvent was diethyl ether. The extract was analyzed by gas/ liquid chromatography using conditions previously described for water analysis. No clean-up of the fish extract was performed prior to analysis. The chromatographic column (5% Dexsil 300 GC) was cleansed of contamination by fish oils and other extractable interferences by heating overnight at 325°C. The next morning the temperature was reduced to 250°C, and injection of atrazine was made to condition the column, and finally the oven temperature was lowered to 200°C and atrazine analyses were resumed with no change in efficiency. The recovery of atrazine from spiked tissue samples was again essentially quantitative (Table 3).

TABLE 3. PERCENT RECOVERY OF ADDED ATRAZINE FROM WATER (µg/l) AND WHOLE FISH (µg/g)

Sample Type	Added Conc. (ppm)	Measured Conc. (ppm)	Recovery (%)
Water	0.05	0.052	104
	0.05	0.049	98
	0.05	0.050	100
	0.50	0.46	92
	0.50	0.51	102
	0.50	0.50	100
	5.0	4.6	92
	5.0	4.9	98
	5.0	5.0	100
			98.4 ± 4.1[a]
Whole Fish	0.380	0.374	98
	0.494	0.488	99
	0.392	0.384	98
	3.82	3.94	101
	3.52	3.44	98
	3.49	3.46	99
			98.8 ± 1.1[a]

[a]Standard deviation.

During the chronic exposures, total hardness, alkalinity, pH and acidity were generally measured bi-weekly in the control and one atrazine concentration according to Standard Methods for the Examination of Water and Wastewater (APHA, 1971). Temperature and dissolved oxygen concentrations were measured in selected tanks each day using a YSI dissolved oxygen meter with a combined oxygen-temperature probe.

STATISTICS

Means of the biological parameters measured in each duplicate container during chronic exposure were subjected to analysis of variance according to Steel and Torrie (1960). When treatment effects were indicated by analysis of variance, the means of these effects were subjected to Duncan's Multiple Range Test (1955) to determine which treatments were different from the controls. Statistical significance was accepted at P=.05.

CHRONIC EXPOSURE

Chironomus tentans

Stock cultures of Chironomus tentans were obtained from Michigan State University, East Lansing, Michigan. Cultures were maintained in 60 l glass aquaria utilizing a substrate prepared by placing 50 grams of paper hand towel (Nibroc, Brown Co.) in a blender with 5 grams of high protein chicken feed and 1 liter of water to cover the mixture. The mixture was homogenized for three to five minutes. This substrate was used to cover the bottom of each aquarium to a depth of 1.5 cm. Emergent adults were allowed to mate within the screened aquaria and provided a ready supply of egg masses.

Egg masses were selected from the stock cultures according to uniformity of age, and one egg cup of 100 eggs was suspended in each of the replicate experimental aquaria. Egg cups were made from 20 ml glass vials with the bottoms removed and replaced with 40 mesh nylon screen. After hatching was completed in all treatments (3-5 days), the number of surviving first instar larvae were counted and carefully placed on the substrate.

After 26 to 29 days, the number of emergent adults, pupal exuviae, and dead pupae were observed and recorded. Mating of adults was observed, and egg masses produced by mating pairs in each replicate aquaria were collected and utilized to conduct second generation exposures according to the above methods. At the initiation of the second generation exposure, new substrate was placed in the experimental aquaria which would receive first instar larvae. The number of emergent adults, pupal exuviae, and dead pupae was observed in each replicate concentration and the experiment terminated.

Daphnia magna

Laboratory stocks of Daphnia magna were obtained from the University of New Hampshire, Durham, New Hampshire, and successfully cultured in the laboratory according to the methods of Biesinger and Christensen (1972).

Typically, 10 daphnids (<24 hours old) were placed into each of four replicate experimental units, resulting in a total of 40 animals per concentration. A food supply consisting of trout starter and dry-powdered Cerophyl (2:1) was prepared in an aqueous suspension (12.5 mg/ml) and delivered from a Mariotte bottle via a volumetric delivery system to a mixing chamber each diluter cycle. The diluted food suspension was subsequently transferred to the food cells from which 25 ml (0.1 mg/ml) were delivered to each test container during each diluter cycle.

Survival and reproduction of daphnids were recorded after one, two and three weeks. Reproduction was measured by recording the number of young in each experimental chamber weekly and discarding the progeny after weeks one and two. At the end of the third week, the number of original animals remaining was recorded, the specimens discarded, and 10 daphnids (<24 hours old) were randomly selected from each chamber to begin the second generation exposure. The above procedures were followed for the second and third generation, after which the experiment was terminated.

Gammarus fasciatus

The test organisms were collected in October 1972 from the raceway outlets at the National Fish Hatchery, North Attleboro, Massachusetts. Sexually mature adults were acclimated at 17 ± 1°C in the laboratory for a period of three weeks. After the acclimation period, females were isolated and their progeny collected. When sufficient 1 to 22-day old gammarids were available, the chronic exposure to atrazine was initiated by placing 30 specimens into each replicate aquarium. During the exposure, the gammarids were fed pre-soaked maple leaves, water cress, and Elodea. Brine shrimp (Artemia) nauplii and Daphnia were also fed to the gammarids weekly.

Survival was recorded once each month by siphoning contents of each test chamber into a pan and counting the gammarids. At the onset of reproduction, all adult chambers were checked daily for gravid females which were then

isolated individually in 400 ml beakers containing the corresponding test solutions. Some females were maintained for up to 28 days in beakers with the test solution being replaced every 48 hours with fresh test solution from the appropriate experimental aquarium. Twenty to fifty young collected from isolated females in the same adult chamber on the same day were placed in the respective larval growth chamber and exposure continued for 30 days. At the end of 30 days, survival and growth were recorded by the procedure of Clemens (1950). After a period of 17 weeks, the exposure of the original gammarids to atrazine was terminated.

Lepomis macrochirus

In December 1971, chronic exposure of bluegills to atrazine was initiated using 7 to 10 cm fish obtained from a commercial hatchery in Wisconsin. Bluegills were acclimated to the test water for three months, after which twenty fish were randomly distributed to each test chamber. Bluegills were fed ad libitum the largest commercially prepared trout pellet which they would take twice daily. All tanks were siphoned twice weekly to remove fecal material, excess food, and detritus, and were brushed when algal growth became excessive. Total length and weight of each individual fish was measured at the initiation of exposure, after 90 days, and at thinning (185 days) using 100 mg/l of tricaine methanesulfonate (MS-222) to lightly anesthetize the fish.

During the first year, when secondary sex characteristics were well developed, fish in each tank were separated into groups of males, females and undeveloped fish, using the shape of the urogenital opening as the main criterion for sexual differentiation (McComish, 1968). Sexually mature fish were randomly reduced to 3 males and 7 females per duplicate tank and all other fish were discarded after examination of gonadal development and measurement of total length and weight. At this time, two spawning substrates similar to those described by Eaton (1970), were placed into each duplicate tank. Substrates were 30.5 x 40 x 5 cm with a bowl-shaped depression 25 cm in diameter and 4 cm deep. Although bluegills developed secondary sex characteristics and exhibited territorial behavior characteristic of spawning bluegill populations under natural conditions, no spawning occurred among either exposed or control fish during the first year. Consequently, substrates were removed and the exposure of bluegills to atrazine was continued a second year, repeating the photoperiod schedule.

During the second year of bluegill exposure, when males demonstrated territorial behavior, the substrates were replaced in the tanks and total length and weight was determined for each fish. Substrates were checked for eggs after 1:00 p.m. each day and eggs were brushed loose from the substrate under a stream of test water. Eggs were also siphoned from the tank bottom after each spawn. Two hundred eggs were randomly selected from each spawn and placed in an egg cup which oscillated in the respective test water by means of a rocker arm apparatus (Mount, 1968). Eggs from each spawn were counted volumetrically by first counting a subsample of exactly 1 ml and multiplying that number by the total measured volume (ml) of spawned eggs. Eggs were allowed to settle for two minutes in the graduated cylinder before measuring. Hatchability was expressed as the percent of live fry hatching out of 200 eggs.

Thirty bluegill fry from the earliest two spawnings in each tank with at least 25 percent live hatch were placed in the growth chambers. Fry from all other spawns were discarded after hatchability was determined. Fry in the growth chambers were fed live zooplankton from mixed laboratory cultures of copepods, rotifers, protozoans, and brine shrimp ad libitum three times daily. Cumulative mortality and total lengths of fry were determined after 30, 60 and 90 days using the photographic method of McKim and Benoit (1971). Parental bluegills were sacrificed after spawning had ceased in all tanks for three weeks. Total length, total weight, sex, and gonadal condition were determined for each fish and samples of muscle were retained for residue analysis.

Pimephales promelas

Chronic exposure of fathead minnows to atrazine began in September 1971 with 18-day old fish obtained as eggs from the Newtown Fish Toxicology Station, Newtown, Ohio. Forty fish were randomly distributed to each test chamber. Cumulative mortality and total length of live fish were determined after 30 and 60 days using the photographic method of McKim and Benoit (1971). At 60 days, the number of fish in each test chamber was randomly reduced to fifteen. Fathead minnows were fed ad libitum twice daily with a commercially prepared trout starter food which was supplemented with daphnids and brine shrimp nauplii. All tanks were siphoned twice weekly to remove fecal material, excess food, and detritus, and were brushed when algal growth became excessive.

The discovery of bacteria and external parasites on a few

fish prompted the use of flush treatments of tetracycline hydrochloride (4 mg/l active ingredient) and a combination of malachite green and formalin (25 µl/l of formalin containing 3.7 g/l malachite green crystals). These treatments were concentrated between days 70 and 129 of the experiment.

In order to establish the appropriate ratio of male fish to female fish, some of the males were removed after secondary sexual characteristics were well developed. Five spawning sites of halved, 3-inch transite drain tiles were placed in each tank when fish were released from growth chambers at 60 days. The tiles were placed concave surface down at locations that minimized the chance of encounters by separate egg-guarding males. When spawning began, eggs were removed and counted after 1:00 p.m. each day. Fifty eggs from each spawn were oscillated in their corresponding test waters by means of an egg cup and a rocker arm apparatus (Mount, 1968). Dead eggs were removed and counted each day until hatching was completed (3-5 days at 25°C). Percent hatch was based on the number of live fry from 50 eggs.

Forty fry from the earliest two spawns in each tank with at least 80% live hatch were placed in the respective growth chambers. Cumulative mortality and total length of live fish were determined at 30 and 60 days by the photographic method of McKim and Benoit (1971). Fry from all other spawns were discarded unless a growth chamber was later made available by termination of 60-day old fry. Finely ground starter food and brine shrimp nauplii were fed three times daily to fry in the growth chambers.

Parental fish were sacrificed after all spawning had ceased for one week. Total length, weight, sex and gonadal condition were determined for each fish and three samples per concentration of eviscerated fish were retained for residue analysis.

Salvelinus fontinalis

Chronic exposure of brook trout to atrazine began in May 1972, with yearling fish obtained from the National Fish Hatchery, Manchester, New Hampshire. Distribution of fish to the test chambers was delayed by the discovery of external parasites on a few fish. The parasites were controlled by flush treatments of malachite green and formalin (25 µl/l of formalin containing 3.7 g/l of malachite green). These treatments were repeated shortly after the distribution of 15 fish to each duplicate tank to insure control of the parasites. Brook trout were fed twice daily with a measured ration of the largest trout pellet they would take. The feeding rate was based on a

percentage of the initial average biomass per tank and was adjusted after 90 days and 136 days when total weights of fish were again measured. Feeding rates were from a New York State fish hatchery feeding chart (Deuel et al., 1952). Total length and weight of individual fish were measured at the initiation of exposure and after 90 days exposure, utilizing 100 mg/l of tricaine methanesulfonate (MS-222) to quiet the fish during measuring. All tanks were siphoned twice weekly to remove fecal material, excess food, and detritus, and were brushed when algal growth became excessive.

Secondary sexual characteristics were observed on test day 210 and males, females and undeveloped fish were identified in each tank and the number of sexually mature fish was randomly reduced to 2 males and 4 females per tank. All other fish were discarded after being measured and examined for gonadal condition. At this time, two stainless steel spawning substrates, similar to those described by Benoit (1974), were placed in each duplicate tank. The bottom of these box-like substrates (25 x 25 x 15 cm) contained a gridwork of 2.5 cm cubicles made of 20-mesh stainless steel screen to retain the eggs where they were deposited. A 25 x 25 cm square of 4-mesh stainless steel screen was placed on top of the egg retainer and silicone adhesive was used to attach 1.3 - 2.5 cm diameter stream gravel 2 cm apart on the screen.

Substrates were checked after 1:00 p.m. each day and eggs were recovered by removing the gravel screen and egg retainer and pipetting the eggs into a pan containing the test water. Eggs from each spawn were counted and two egg cups containing a group of 50 and 100 eggs, respectively were attached to the rocker apparatus (Mount, 1968) for incubation in their respective test aquaria. Dead eggs were removed and recorded daily and the number of fry hatching each day in the incubation cups with 50 eggs was recorded. After 15 days, the group of 100 eggs was removed and the number of eggs developing a neural keel was observed to determine percent fertilization. Hatchability (percent of 50 eggs) and mean time to hatch (degree days) were determined.

Twenty five fry from the earliest two spwans in each tank with at least 50% live hatch were placed in the respective growth chambers. Fry from all other spawns were discarded after determining percent hatchability. Cumulative mortality and total length of live fry were determined after 30, 60 and 90 days using the photographic method of McKim and Benoit (1971). Brook trout fry were fed five times daily ad libitum with a commercially prepared trout starter food.

After spawning had ceased in all tanks for a period of three weeks, parental fish were removed and the condition of the gonads was observed. Total lengths and weights were determined for each individual fish before obtaining muscle samples for residue analysis and discarding the remaining fish.

SECTION V

RESULTS

ACUTE BIOASSAYS

Analyses of the results of acute static bioassays with the invertebrate species indicated the 48-hour LC50 (95% confidence interval) of atrazine for Chironomus, Daphnia, and Gammarus was 0.72 (0.36-1.44), 6.9 (5.2-8.1), and 5.7 (3.6-8.0) mg/1, respectively. Mortality was observed with Chironomus exposed to 0.5 mg/1, with Daphnia exposed to 3.0 mg/1, and with Gammarus exposed to 2.4 mg/1. Based on these data, the highest nominal concentrations selected for the chronic exposures were 2.0 mg/1 (Chironomus), 1.2 mg/1 Daphnia and 1.0 mg/1 (Gammarus).

A 7-day continuous-flow bioassay conducted at 19 ± 1°C with 6.5-gram bluegill indicated the 96-hour LC50 for atrazine and bluegill was >8.0 mg/1, and the incipient LC50 was 6.7 (5.4-8.4) mg/1. Fathead minnows were not killed in 8 days by 8 mg/1, the highest concentration of atrazine that could be maintained in test chambers in a flow-through test. A 120-hour acute bioassay conducted at 19 ± 1°C with 1.8-gram fathead minnows and utilizing the 24-hour renewal technique indicated that both the 96-hour and incipient LC50 values were 15 (11-20) mg/1. An 8-day continuous-flow bioassay at 13 ± 1°C with 52-gram brook trout indicated the 96-hour LC50 for atrazine and brook trout was 6.3 (4.1-9.7) mg/1, and the incipient LC50 was 4.9 (4.0-6.0) mg/1. In each of these tests, fish appeared darkened and stressed at concentrations as low as 1.4 mg/1. In order to select concentrations for chronic studies which would include safe concentrations, other acute studies were performed on each species. Bluegill (15 g) exposed to a nominal concentration of 0.5 mg/1 atrazine for 28 days in a flow-through test became lethargic, fed poorly, and exhibited partial loss of equilibrium. Toxicity was dose-related since effects occurred earlier and were more severe at higher concentrations and no effects were observed at lower concentrations nor in the controls. We observed 25 percent mortality among fathead minnow fry (approximately 3-5 day old) statically exposed to a measured concentration of 0.52 mg/1 atrazine for 96 hours. Mortality was dose-related with more occurring at higher concentrations and none at lower ones or in the controls. Based on these data, the highest nominal concentrations selected for chronic exposures were 0.1 mg/1 (bluegill), 0.25 mg/1 (fathead minnow) and 1.0 mg/1 (brook trout).

WATER CHEMISTRY

The results of the chemical analyses of water samples taken during the various chronic exposures indicate that hardness, alkalinity, acidity, pH and dissolved oxygen varied minimally within any one chronic exposure. Statistical analysis showed no significant differences for any of the above parameters between treatments within a chronic; therefore, only means and ranges for the various parameters are presented (Table 4).

The results of gas chromatographic analyses of water samples taken periodically during chronic exposure of the fishes and aquatic invertebrates to atrazine indicated that mean measured atrazine concentrations closely approximated nominal concentrations (Table 5).

CHRONIC EXPOSURE

Chironomus tentans

The continuous exposure of first generation chironomid eggs to mean measured concentrations of atrazine as high as 1.33 mg/l had no significant effect on hatchability (Table 6). However, about 60% mortality was observed among first instar larvae developing from eggs exposed to 1.33 mg/l atrazine. Furthermore those first instar larvae surviving exposure to this concentration of atrazine never developed beyond late first instar-early second instar during 37 days exposure. Larvae hatched from eggs exposed to a mean measured atrazine concentration of 0.78 mg/l did not experience significant first instar larval mortality but the majority of these larvae never developed beyond late second instar-early third instar.

Larvae that experienced developmental retardation when compared to controls were considered affected by chemical exposure, and no assessment of the potential for emergence of these retarded forms was made. All data regarding pupation and emergence represent comparable data for all groups during 37 days exposure since pupation and emergence were completed among control organisms within that time. Based on these criteria and an analysis of variance of comparable data, we observed a significant reduction in the number of adults emerging among those groups exposed to mean measured atrazine concentrations of 0.42 and 0.23 mg/l. No significant differences in hatchability, survival, pupation and emergence were observed between midges exposed to a mean measured atrazine concentration of 0.12 mg/l and controls during the first generation exposure (Table 6).

TABLE 4. MEAN AND RANGE OF MEASURED CONCENTRATIONS OF HARDNESS, ALKALINITY, ACIDITY, DISSOLVED OXYGEN AND pH (RANGE ONLY) FROM WATER SAMPLES TAKEN PERIODICALLY DURING CHRONIC EXPOSURE OF AQUATIC INVERTEBRATES AND FISHES TO ATRAZINE

Species	Hardness (mg/l)	Alkalinity (mg/l)	Acidity (mg/l)	D.O. (mg/l)	pH
Chironomus tentans					
Mean ± S.D.	43.0±2.6	39.0±2.5	4.8±1.3	6.8±0.5	-
Range	(40-45)	(36-40)	(4.3-5.1)	(5.8-8.2)	(6.8-7.2
# of Samples	5	5	5	29	16
Daphnia magna					
Mean ± S.D.	32.2±0.81	30.3±1.0	4.1±0.45	6.7±0.23	-
Range	(27-35)	(24-35)	(1.9-6.0)	(6.4-7.3)	(6.3-7.5)
# of Samples	18	18	18	10	45
Gammarus fasciatus					
Mean ± S.D.	34.7±0.53	32.8±0.75	4.4±0.44	8.4-0.11	-
Range	(29-39)	(27-46)	(1.9-9.6)	(6.0-9.9)	(6.4-7.2)
# of Samples	42	42	42	162	120
Lepomis macrochirus					
Mean ± S.D.	33.9±4.5	32.7±3.7	4.7±1.8	6.3±0.8	-
Range	(25-41)	(34-46)	(2.9-9.0)	(3.6-9.1)	(6.3-7.4)
# of Samples	20	20	24	1764	30
Pimephales promelas					
Mean ± S.D.	36.2±2.3	34.6±2.7	4.4±1.3	6.9±0.7	-
Range	(33-40)	(27-48)	(2.9-6.7)	(3.4-10.2)	(6.5-7.3)
# of samples	13	13	8	238	14
Salvelinus fontinalis					
Mean ± S.D.	35.7±3.4	34.6±7.3	4.5±1.8	8.1±0.8	-
Range	(30-43)	(23-46)	(1.9-7.7)	(5.9-11.2)	(6.4-7.4)
# of Samples	16	16	20	1176	24

TABLE 5. NOMINAL AND MEASURED ATRAZINE CONCENTRATIONS (mg/1) IN WATER DURING CHRONIC EXPOSURE OF AQUATIC INVERTEBRATES AND FISHES TO ATRAZINE

Species and Nomin. Conc.	Measured Concentration mg/liter Mean S.D.	Range	of Samples
Chironomus tentans			
2.00	1.33 ± 0.930	0.69 - 3.3	20
1.00	0.78 ± 0.310	0.24 - 2.8	20
0.50	0.42 ± 0.170	0.13 - 0.63	19
0.25	0.23 ± 0.062	0.15 - 0.33	20
0.12	0.11 ± 0.025	0.082- 0.19	20
Daphnia magna			
1.20	1.15 ± 0.370	0.41 - 1.70	21
0.60	0.55 ± 0.122	0.35 - 0.79	21
0.30	0.25 ± 0.057	0.14 - 0.35	18
0.15	0.14 ± 0.036	0.08 - 0.21	15
0.08	0.06 ± 0.012	0.05 - 0.09	16
Gammarus fasciatus			
1.00	0.94 ± 0.167	0.50 - 1.30	40
0.50	0.49 ± 0.208	0.29 - 0.80	40
0.25	0.24 ± 0.069	0.16 - 0.53	38
0.12	0.14 ± 0.029	0.08 - 0.21	40
0.06	0.06 ± 0.011	0.03 - 0.08	35
Lepomis macrochirus			
0.100	0.095 ± 0.026	0.034 - 0.17	106
0.050	0.049 ± 0.011	0.031 - 0.084	63
0.025	0.025 ± 0.008	0.011 - 0.053	58
0.012	0.014 ± 0.004	0.005 - 0.031	58
0.006	0.008 ± 0.004	0.004 - 0.022	57
Pimephales promelas			
0.250	0.213 ± 0.075	0.11 - 0.41	35
0.125	0.112 ± 0.044	0.05 - 0.23	31
0.062	0.054 ± 0.017	0.031 - 0.084	28
0.031	0.033 ± 0.016	0.011 - 0.057	35
0.015	0.015 ± 0.008	0.004 - 0.045	30
Salvelinus fontinalis			
1.00	0.72 ± 0.278	0.18 - 1.10	46
0.05	0.45 ± 0.156	0.12 - 0.66	28
0.25	0.24 ± 0.042	0.17 - 0.32	23
0.12	0.12 ± 0.039	0.03 - 0.20	28
0.06	0.065 ± 0.013	0.021- 0.081	21

TABLE 6. SUMMARY OF THE EFFECT OF VARIOUS CONCENTRATIONS OF ATRAZINE ON HATCHABILITY, PUPATION AND EMERGENCE OF *Chironomus tentans* CONTINUOUSLY EXPOSED FOR TWO GENERATIONS

Mean measured atrazine conc. (mg/l)	Rep	Generation I			Generation II		
		%Hatch	Pupa	Adult	%Hatch	Pupa	Adult
Control	A	91	89	89	90	87	87
	B	93	95	93	83	83	80
0.12	A	94	91	90	95	93	89
	B	90	83	80	90	81	90
0.23	A	86	75	70	89	71	65
	B	90	63	59	93	69	56
0.42	A	91	31	30	70	11	10
	B	86	26	26	63	29	26
0.78	A	90	0[a]	-	-	-	-
	B	86	0[a]	-	-	-	-
1.33	A	84	0[b]	-	-	-	-
	B	92	0[b]	-	-	-	-

[a]Only late second-instar larvae present after 37 days continuous exposure.

[b]Only late first-instar larvae present after 38 days continuous exposure.

Egg masses for the second generation exposure were obtained from those groups where first generation adults emerged during the same period as the controls (day 33-36 of exposure). Continuous exposure of second generation eggs to 0.42 mg/l of atrazine significantly reduced hatchability of eggs. Reduced hatchability was not observed during the first generation exposure to 0.42 mg/l and is indicative of a cumulative effect of atrazine on the midges during the exposure of successive generations. As was observed during the first generation exposure, continuous exposure of midges to 0.42 and 0.23 mg/l of atrazine significantly reduced the number of midges pupating and emerging. The data do not indicate a cumulative toxic effect on these processes due to exposure to these two concentrations of atrazine.

The results of continuous exposure to *C. tentans* to mean measured concentrations of atrazine of 0.23 mg/l and greater through two successive generations resulted in reduced hatching success, increased larval mortality, developmental retardation, or reduction in the number of organisms pupating and emerging. Exposure to 0.11 mg/l atrazine had no observable effect on the growth and development of these organisms

when compared to control groups. Based on these data, the maximum acceptable toxicant concentration of atrazine for C. tentans is estimated to be >0.11 and <0.23 mg/l.

Daphnia mgana

Statistical analysis of the mean percent survival of original Daphnia from each of three successive generations indicated that continuous exposure to mean measured concentrations of atrazine as high as 1.15 mg/l had no significant effect on survival (Table 7). Also, these data indicate that no atrazine-induced cumulative effects on mortality were observed in second or third generation daphnids.

TABLE 7. MEAN[a] PERCENT SURVIVAL OF Daphnia magna CONTINUOUSLY EXPOSED TO ATRAZINE FOR 64 DAYS

Mean measured conc. (mg/l)	Generation[b] I			Generation[b] II			Generation[b] III		
	Day			Day			Day		
	8	15	70	29	38	43	50	57	65
Control	89	61	49	92	88	84	69	65	55
0.06	81	72	51	95	92	91	70	64	55
0.14	84	75	65	82	79	78	39	36	36
0.25	78	75	60	84	82	80	78	72	59
0.55	68	54	34	64	56	52	50	45	44
1.15	88	85	82	91	89	71	82	76	74

[a]Each value represents the mean of four replicates.

[b]Duration of exposure for generations, I. II and III were days 1-22, 22-43, 43-64, respectively.

Analysis of variance indicated that the mean number of young produced per female daphnid during the first generation was significantly reduced by exposure to atrazine (Table 8). Continuous exposure of the first generation Daphnia to mean measured concentrations of 1.15, 0.55, and 0.25 mg/l atrazine significantly reduced the production of young. Although reproduction among second and third generation daphnids exposed to these concentrations was generally less than other groups, variability in the data precluded ascribing statistical significance to these observations.

We are, at this time, unable to explain the declining rate of progeny production observed in every experimental group during the successive generation experiment. It is obviously not toxicant related, but is apparently related to inadequacies in the laboratory life-support systems.

TABLE 8. MEAN[a] PRODUCTION OF YOUNG PER FEMALE *Daphnia magna* CONTINUOUSLY EXPOSED TO ATRAZINE FOR 64 DAYS

Mean measured conc. (mg/l)	Generation[b] I		Generation[b] II		Generation[b] III	
	Day		Day		Day	
	15	22	36	43	57	67
Control	16.3	40.0	10.8	15.8	16.6	11.8
0.06	24.4	33.0	6.3	12.2	6.3	9.7
0.14	19.0	30.5	4.9	14.4	10.4	14.9
0.25	14.6	18.3	3.6	10.1	4.8	3.3
0.55	18.8	24.8	8.8	10.1	6.6	8.8
1.15	13.7	8.0	3.7	13.2	6.3	8.2

[a]Each value represents the mean of four replicates.

[b]Duration of exposure for generations I, II, and III were days 1-22, 22-43, 43-64, respectively.

The results of this research indicate that reproduction of *Daphnia mgana* is a much more sensitive measure of species susceptibility to atrazine than is survival. Some of the adult daphnids in each generation were males, and data on reproduction was adjusted to represent that portion of the population, in each experimental unit, directly involved in production of progeny. The incidence of males induced from parthenogenetic forms appeared to be higher in groups exposed to atrazine than in the control groups. Hutchinson (1967) considered the presence of males in test populations a reaction to an environmental stress, which atrazine may be in this case.

Based on the statistical analysis of the survival and reproductive success of daphnids continuously exposed to atrazine for three successive generations, the maximum acceptable toxicant concentration of this chemical for *Daphnia magna* is estimated to be >0.14 and <0.25 mg/l.

Gammarus fasciatus

Statistical analysis indicated that survival of original gammarids continuously exposed to a mean measured concentration of 0.94 mg/l atrazine for 30 days was significantly less than all other experimental groups (Table 9). No significant difference between survival of control groups and gammarids continously exposed to 0.49 mg/l for 119 days was observed.

TABLE 9. PERCENT SURVIVAL AND REPRODUCTIVE SUCCESS OF Gammarus fasciatus EXPOSED TO ATRAZINE FOR 17 WEEKS

	Mean Measured Atrazine Concentration (mg/l)											
	0.94		0.49		0.24		0.14		0.06		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
Adult Survival (%)												
Day 30	40	40	58	60	64	82	56	66	74	66	64	74
60	20	18	30	42	32	60	26	44	26	58	24	38
91	16	16	24	32	24	56	16	34	16	42	30	30
119	4	10	24	14	14	34	12	32	16	32	12	18
Total # young	-	-	18	1	33	40	39	43	56	14	18	-
Survival after 30 days (%)	-	-	22	0	52	38	82	65	86	43	33	-

Complete reproductive impairment of surviving adult gammarids was observed among groups exposed to 0.94 mg/l atrazine, and the number of progeny produced per female appeared reduced among groups exposed to 0.49 mg/l atrazine. Additionally, the survival during the first 30 days of development of young produced by gammarids exposed to 0.49 and 0.24 mg/l atrazine appeared to be less than that of young produced by gammarids exposed to 0.14 and 0.06 mg/l atrazine (Table 9).

The growth of second generation gammarids during the 30 day period following shedding by the females was significantly reduced by continuous exposure to concentrations of atrazine as low as 0.14 mg/l. None of the young organisms exposed to 0.49 mg/l atrazine developed beyond sixth instar stage and half of them never developed beyond the fifth instar. Of the young gammarids continuously exposed to 0.24 mg/l and 0.14 mg/l atrazine, only 75% developed to seventh instar, while 100% of the control organisms and 93% of those exposed to 0.06 mg/l developed to seventh instar.

The small number of young gammarids produced among control groups was due to the fact that in these aquaria males greatly outnumbered females. Consequently, we occasionally observed moribund gravid females apparently due to continued pursuit by various males and repeated copulation and separation. Also, nearly 60% of the gravid females isolated during the experiment failed to yield young gammarids.

Based on a statistical evaluation of the above data, and consideration of the effects of continuous exposure to atrazine on the survival and reproductive potential of adult gammarids, and on the morphological development of second generation gammarids, we estimate the maximum acceptable toxicant concentration of atrazine for Gammarus fasciatus is >0.06 and <0.14 mg/l.

Lepomis macrochirus

Survival and growth of bluegill exposed to atrazine for 6 and 18 months was similar for all concentrations (Table 10). Spawning activity occurred between days 594 and 716 of exposure. Although spawning activity was too sporadic to be conclusive, the fact that egg numbers in the highest concentration (.095 mg/l) were comparable to the controls suggests that atrazine had no effect on spawning within the range of concentrations tested. An attempt was made to induce further spawning from bluegill by administering interperitoneal injections of carp pituitary extract on test day 716. All

TABLE 10. SURVIVAL AND GROWTH DURING EXPOSURES OF 6 AND 18 MONTHS, AND RESULTS OF SPAWNING ACTIVITY OF BLUEGILL[a] (*Lepomis macrochirus*) CONTINUOUSLY EXPOSED TO ATRAZINE

Item	Mean Measured Atrazine Concentration (mg/liter)											
	0.095		0.049		0.025		0.014		0.008		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
6 MONTHS												
Survival (%)	100	85	95	80	100	95	95	100	95	85	75	95
Total Length (mm)	124	129	127	128	126	128	129	130	130	126	133	129
Total Weight(g)	35	40	40	40	37	38	41	40	41	37	43	41
18 MONTHS												
Survival (%)[b]	90	80	90	100	100	90	100	100	100	70	90	100
♂/♀	3/6	3/5	3/6	5/5	3/7	2/7	3/7	4/6	3/7	2/5	3/6	3/7
Total Length (mm)												
♂	185	199	200	193	172	179	192	192	198	199	182	207
♀	157	153	155	153	158	166	158	161	156	165	160	158
Total Weight(g)												
♂	120	146	165	147	102	112	143	138	146	163	122	172
♀	77	69	62	63	73	86	74	76	72	78	76	72
# of Spawnings	4	3	0	4	8	0	7	8	0	7	8	2
Eggs/♀	12920	2432	-	7331	5153	-	6703	8218	-	15254	16890	580
Eggs/Spawn	19380	4053	-	9164	4509	-	6703	6163	-	10895	12668	2031

[a]Mean initial length and weight 100 mm, 15 g.

[b]Based on 10 fish per aquarium after thinning on day 185 of exposure.

fish were injected with 0.5-1.0 cc of a 200 µg/100 ml solution of carp pituitary in physiological saline. The injections failed to induce the desired increase in spawning and may have been administered too late in the spawning period to be effective. Parental fish were sacrificed on test day 736 and samples of muscle were retained for residue analysis.

The percent of eggs successfully hatching among groups of eggs exposed to each of the four highest concentrations of atrazine tested was similar to that observed among control groups and indicates that continuous exposure to mean measured concentrations of atrazine as high as 0.095 mg/l did not significantly affect hatchability of eggs (Table 11). Survival of bluegill fry was significantly lower during 30 days exposure to 0.095 and 0.049 mg/l than to the other three concentrations of atrazine, suggesting a toxicant related effect. Unfortunately, survival of fry in control groups was very low and it is impossible to confirm whether fry survival was toxicant related. Survival of fry in all groups during this period appeared to be related to an early acceptance of the appropriate available food rather than toxicant related. This interpretation is based on the observation that survival in all groups was excellent during the final 60 days of exposure when feeding habits became well established and food was readily accepted.

Total lengths of bluegill fry at 30 days did not vary significantly between controls and any concentration. Total lengths at 60 and 90 days were significantly lower among fish exposed to 0.049 mg/l of atrazine, however these were the only fry groups remaining in the experiment when a heater malfunction caused a decrease in temperature in the experimental system. The malfunction could not be corrected soon enough to bring growth rate of these fish back to a rate comparable with other groups. Total lengths of bluegill fry in all other tanks were similar after 60 and 90 days exposure, and we conclude that continuous exposure of bluegill fry to atrazine concentrations as high as 0.095 mg/l for 90 days had no significant effect on growth (Table 11).

Based on the data obtained from continuous exposure, it appears that the maximum acceptable toxicant concentration is greater than the highest measured atrazine concentration (0.095 mg/l) to which bluegill were exposed during the chronic test. Since we observed loss of equilibrium among bluegill continuously exposed to 0.50 mg/l atrazine for 28 days, the maximum acceptable toxicant concentration of atrazine for this species is estimated to be between 0.095 and 0.50 mg/l.

TABLE 11. HATCHABILITY, SURVIVAL, AND GROWTH OF BLUEGILL (Lepomis macrochirus) FRY EXPOSED TO ATRAZINE

ITEM	Mean Measured Atrazine Concentration (mg/l) 0.095	0.049	0.025	0.014	0.008	Control
Hatchability(%)	91	90	87	81	66	84
# of egg groups[a]	4	3	6	12	3	7
Survival (%)						
30 days	18	22	45	36	48	22
60 days	18	20	45	35	46	22
90 days	18	20	42	35	43	22
Mean Total Length ± S.D.[b]						
30 days	20±2.9	17±1.8[c]	22±3.2	20±3.2	21±2.4	18±2.0
60 days	30±3.1	18±2.0	30±6.0	30±3.1	25±3.7	29±3.1
90 days	40±5.9	18±2.0	38±8.0	38±4.9	35±3.5	40±7.2
# of fry groups[d]	1	1	2	4	2	2

[a]Each group contained 200 one day old eggs.

[b]Standard deviation.

[c]Fry groups exposed to lower water temperature due to heater malfunction.

[d]Each group contained 50 one day old fry.

Pimephales promelas

Statistical analysis of data on survival and growth of fathead minnows after 30 and 60 days exposure to atrazine indicated that survival was significantly less and growth significantly greater among controls than all treated groups (Table 12). We believe these to be related phenomena. Although no explanation for the lower survival among controls exists, we suggest the greater growth among fish remaining in these tanks is related to reduced competition for food. This hypothesis is supported by the observation that during the period of exposure from 9-43 weeks no significant differences in growth and survival among any of the experimental groups were evident.

Spawning activity of fathead minnows in all experimental groups occurred between days 164 and 280. Virtually no spawning activity occurred among fathead minnows exposed to 0.033 mg/l atrazine, and spawning among fish exposed to 0.213 mg/l in replicate A was reduced (Table 13). These were the only groups in which the number of males was equal

TABLE 12. SURVIVAL AND GROWTH OF FATHEAD MINNOWS (Pimephales promelas) EXPOSED 30 DAYS, 60 DAYS, AND 43 WEEKS TO VARIOUS ATRAZINE CONCENTRATIONS

Item	Mean Measured Atrazine Concentration mg/liter											
	0.213		0.112		0.054		0.033		0.015		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
30 DAYS												
Survival (%)	86	89	86	86	80	69	100	97	63	83	60	47
Total Length (mm)	16	13	16	14	14	13	14	13	14	13	20	19
(S.D.)	(3.9)[a]	(2.1)	(3.8)	(3.5)	(3.2)	(3.8)	(2.8)	(3.5)	(4.5)	(3.8)	(4.2)	(3.9)
60 DAYS												
Survival (%)[b]	80	86	71	69	69	66	90	70	57	67	60	47
Total Length (mm)	21	22	18	21	19	20	20	21	19	21	24	26
(S.D.)	(3.2)	(3.3)	(3.9)	(4.8)	(3.6)	(4.0)	(3.5)	(5.5)	(6.5)	(5.1)	(4.2)	(4.1)
43 WEEKS												
Survival (%)[c]	93	67	87	67	80	80	87	80	80	87	93	93
# ♂ removed	0	1	0	1	1	1	2	3	0	3	0	2
♂/♀ at term.	8/6	3/6	3/10	3/6	4/7	2/9	5/6	4/5	4/8	4/6	5/9	2/9
Total Length (mm)	66	63	66	70	63	65	64	69	62	69	64	60
	54	52	51	54	48	54	57	53	50	55	52	55
Total Weight (g)	2.4	2.0	2.7	3.3	2.5	2.5	2.4	2.8	1.8	3.0	2.4	2.0
	1.8	1.8	1.0	1.5	0.9	1.3	1.5	1.0	1.3	1.3	1.1	1.3

[a]Standard Deviation.

[b]Survival based on 40 fish/duplicate tank.

[c]Survival based on 15 fish/duplicate tank after thinning on day 60 of exposure.

TABLE 13. SPAWNING RESULTS, EGG HATCHABILITY, SURVIVAL AND GROWTH OF OFFSPRING AFTER 30 AND 60 DAYS, FOR FATHEAD MINNOW (Pimephales promelas) CONTINUOUSLY EXPOSED TO ATRAZINE

Item	Mean Measured Atrazine Concentration mg/liter											
	0.213		0.112		0.054		0.033		0.015		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
Spawnings/♀	1.6	7.8	5.5	10.8	3.5	5.3	0.2	0	4.3	7.7	3.5	9.9
Eggs spawned/♀	133	1208	757	1255	399	1160	7.5	0	450	862	446	1284
Eggs/Spawn	100	154	138	116	133	217	45	0	120	112	143	130
% Hatch[a]	82	76	77	81	74	87	-	-	76	84	83	74
(N)	5	27	22	27	8	23	0	0	11	22	19	37
30 DAYS												
Survival (%)	56	64	68	73	65	60	-	-	50	69	62	89
Length (mm)	12	10	11	10	13	12	-	-	11	11	9	10
(S.D.)[b]	(2.3)	(2.7)	(1.5)	(2.5)	(2.6)	(2.3)	-	-	(1.8)	(2.7)	(1.7)	(2.1)
60 DAYS												
Survival (%)	45	28	57	69	48	55	-	-	25	67	62	84
Length (mm)	17	21	17	16	20	19	-	-	15	18	16	19
(S.D.)	(2.9)	(3.7)	(3.3)	(4.5)	(3.8)	(3.6)	-	-	(4.0)	(4.4)	(4.0)	(4.5)
# fry groups[c]	1	3	2	4	2	2	0	0	2	4	3	2

[a]Each sample contained 50 one day old eggs.

[b]Standard Deviation.

[c]Each group contained 40 one day old fry.

to or greater than the number of females and suggests that such sex ratios may encourage competition among males and discourage spawning by females. With the exception of these groups, there were no significant differences in the mean number of eggs produced per female, and the mean number of eggs per spawn indicating that continuous exposure to atrazine had no effects on these parameters. Hatchability, survival and growth of second generation fathead minnows through 30 and 60 days was similar among all treatments.

The available evidence, based on the chronic exposure of fathead minnows to atrazine, indicates that the maximum acceptable toxicant concentration is greater than 0.213 mg/l. Since we observed 25% mortality among 3 to 5 day old fathead minnow fry during 96 hours exposure to 0.87 mg/l atrazine in a static system, the estimated maximum acceptable toxicant concentration of atrazine for the fathead minnow is between 0.21 and 0.87 mg/l.

Salvelinus fontinalis

Continuous exposure for 44 weeks to mean measured atrazine concentrations as high as 0.72 mg/l had no significant effect on survival of brook trout. Continuous exposure to 0.72, 0.45, and 0.24 mg/l atrazine for 90 days significantly reduced the weight and total length of brook trout when compared to controls. Total lengths and weights of brook trout fry were significantly reduced by 306 days exposure to concentrations of 0.72, 0.45, 0.24 and 0.12 mg/l atrazine (Table 14). Brook trout in these tanks appeared lethargic when compared to fish in the controls and those exposed to 0.065 mg/l atrazine, and did not feed as well.

Spawning activity of yearling brook trout in all experimental units was delayed approximately one month due to a malfunction in the lighting system which exposed fish in all tanks to dim lighting during scheduled night hours. After correcting the problem, spawning occurred in all tanks between days 222 and 302 of exposure. Total number of eggs spawned, number of eggs per female, and percent fertilization and hatchability appeared to be unaffected by exposure to all concentrations of atrazine. Variability between replicate tanks of the same concentration precluded ascribing statistical significance to values which appeared reduced at higher atrazine concentrations (Table 15). The percentage of eggs developing a neural keel after 15 days was extremely variable between replicate tanks of the

TABLE 14. MEAN INCREASE IN LENGTH AND WEIGHT OF YEARLING BROOK TROUT (Salvelinus fontinalis) DURING 90 AND 306 DAYS CONTINUOUS EXPOSURE TO ATRAZINE

Item	Mean Measured Atrazine Concentration mg/liter											
	0.72		0.45		0.24		0.12		0.065		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
90 DAYS												
Mean increase in length (mm)	10	5	10	16	12	16	18	22	17	24	27	26
Mean increase in weight(g)	22	8	20	36	24	39	40	46	44	52	60	61
306 DAYS												
Mean increase in length (mm)	70	57	51	70	54	64	66	78	87	86	101	92
Mean increase in weight(g)	149	122	100	157	106	155	153	180	214	204	253	216

TABLE 15. RESULTS OF SPAWNING ACTIVITY OF YEARLING BROOK TROUT (Salvelinus fontinalis) DURING CONTINUOUS EXPOSURE TO ATRAZINE

Item	Mean Measured Atrazine Concentration mg/l											
	0.72		0.45		0.24		0.12		0.065		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
# ♂/♀	2/4	2/4	2/4	3/3	2/4	1/3	2/4	3/3	2/4	2/4	3/3	2/4
# spawning	4	2	4	2	4	3	4	3	4	4	2	4
# spawns/♀	2	2	1	2	2	3	2	2	2	2	2	3
Total # eggs spawned	1485	589	531	814	1790	1710	1606	1506	1412	1782	1122	1492
# eggs spawned/♀	371	295	133	407	448	570	402	502	353	446	561	373
Neural keel developed(%)	26	40	0	83	74	63	27	66	65	50	70	41
Incubation time (degree days)	387		405		432		432		450		441	
Mean Hatchability (%)	58	8	-	48	67	26	62	78	25	34	65	37
(N)[a]	1	1	0	3	5	2	1	3	2	2	2	1

[a]Indicates the number of groups of 50 eggs in which at least half successfully developed a neural keel.

same treatment but indicated that about half of the eggs spawned successfully developed to this stage. Incubation in degree-days was significantly reduced for brook trout eggs spawned and incubated in 0.72 and 0.45 mg/l of atrazine.

Survival of brook trout fry after 30 days exposure was similar for all treatments. After 60 and 90 days exposure, survival of fry was reduced for brook trout exposed to 0.72, 0.45 and 0.24 mg/l of atrazine (Table 16).

As was observed during the first generation exposure, mean length and weight of fry, including those from unexposed parents, was significantly reduced after 90 days exposure to 0.72, 0.45 and 0.24 mg/l of atrazine.

As in parental fish, the lethargy and reduction in feeding activity appeared to be the underlying causes for the measured response.

Based on the available evidence relating to pesticide-induced reduction in growth of yearling brook trout, we estimate that the maximum acceptable toxicant concentration of atrazine for brook trout is between 0.065 and 0.12 mg/l.

RESIDUE ANALYSIS

Three samples of muscle tissue from adult bluegill and brook trout taken at the end of the exposure period were analyzed to determine the concentration of atrazine residues in fishes exposed to the highest concentration of atrazine in each chronic. The small size of the individual fathead minnows prompted the use of three samples of pooled eviscerated carcasses of adults exposed to the highest concentration of atrazine. In all cases the results indicated that the concentrations of atrazine in tissues were below the minimum detectable limits. Brook trout exposed to a mean measured concentration of 0.74 mg/l atrazine for 44 weeks contained <0.20 mg/kg atrazine in the muscle tissue. Bluegill exposed to 0.094 mg/l for 78 weeks contained <0.20 mg/kg atrazine in the muscle. Fathead minnows exposed to 0.21 mg/l atrazine for 43 weeks contained <1.7 mg/kg atrazine in the eviscerated carcass. These data clearly indicate that the fishes tested do not bioconcentrate atrazine significantly.

TABLE 16. SURVIVAL AND GROWTH OF SECOND GENERATION BROOK TROUT (Salvelinus fontinalis) DURING THE FIRST 90 DAYS DEVELOPMENT OF FRY CONTINUOUSLY EXPOSED TO ATRAZINE

Item	Mean Measured Atrazine Concentration mg/l											
	0.72		0.45		0.24		0.12		0.065		Control	
	A	B	A	B	A	B	A	B	A	B	A	B
No. groups[a]	1	3[b]	1[b]	3	2	2	2	2	2	2	2	2
Survival (%)												
30 days	91	95	100	85	86	92	78	96	84	84	96	88
60 days	9	39	68	27	72	16	32	76	66	52	80	74
90 days	4	15	48	9	32	8	22	58	46	38	52	50
Mean Length (mm)												
30 days	21	23	24	20	21	20	21	23	22	21	24	25
60 days	22	25	26	23	24	22	25	27	26	24	27	27
90 days	25	28	29	28	27	25	31	33	34	32	34	35
Mean Weight (g)												
90 days	0.10	0.11	0.14	0.11	0.11	0.10	0.20	0.28	0.30	0.22	0.27	0.30

[a] Fry groups contained 25 one day old fry.

[b] Fry from unexposed parents.

CALCULATION OF APPLICATION FACTORS

A summary of the estimated LC50 value, the maximum acceptable toxicant concentration, and the application factor derived therefrom for atrazine and all species studied is presented (Table 17). The MATC for each species except bluegill and fathead minnows is estimated between the highest mean measured concentration having no significant effect and the lowest mean measured concentration significantly affecting the organism during chronic exposure. The MATC for bluegill and fathead minnows is estimated between the highest mean measured concentration applied during chronic exposure and the minimum concentration which produced harmful effects during acute exposure. Application factors describing the relationship between the acute and chronic toxicity of atrazine for the species studied are calculated using concentrations bracketing the MATC and the 48-hour LC50 for invertebrates. Similar application factors are calculated for fishes utilizing the values bracketing the MATC and the incipient LC50 which Eaton (1970) suggests to be a better measure of acute toxicity for this type of calculation.

Application factors are similar for five of the six species tested. Application factors for the fishes are remarkably similar. However, the factors calculated for one of the invertebrates (midges) are an order of magnitude greater than those calculated for the fishes, and the other two invertebrates.

TABLE 17. SUMMARY OF CONCENTRATIONS OF ATRAZINE (mg/l) PRODUCING ACUTE AND CHRONIC TOXICITY TO AQUATIC SPECIES, AND CALCULATED APPLICATION FACTORS DESCRIBING THE RELATIONSHIP BETWEEN ACUTE AND CHRONIC TOXICITY (MATC/LC50)

Species	Common Name	LC50[a]	MATC	Limits on application factor
Chironomus tentans	midge	0.72 (0.36-1.4)[b]	>.11<.23	0.15&0.32
Daphnia magna	water flea	6.9 (5.2-8.1)	>.14<.25	0.02&0.04
Gammarus fasciatus	scud	5.7 (3.6-8.0)	>.06<.14	0.01&0.02
Lepomis macrochirus	bluegill	6.7 (5.4-8.4)	>.10<.50	0.01&0.07
Pimephales promelas	fathead minnow	15 (11-20)	>.21<.52	0.01&0.03
Salvelinus fontinalis	brook trout	4.9 (4.0-6.0)	>.06<.12	0.01&0.02

[a]48-hour LC50 for invertebrates, incipient LC50 for fishes.
[b]95% confidence interval.

SECTION VI

DISCUSSION

The estimated 48-hour LC50 value of 6.9 mg/l determined for Daphnia is higher than the previously estimated value of 3.6 mg/l (FWPCA, 1968). The difference may be attributable to any number of variables (e.g. diluent water quality, age of test organisms, temperature, etc.). No previous estimates of the acute toxicity of atrazine to chironomids and gammarids are available.

The 96-hour LC50 of atrazine to rainbow trout (Salmo gairdneri), based on the results of a static bioassay, was previously estimated to be 12.6 mg/l (FWPCA, 1968). The difference between this value and our 4.9 mg/l estimated incipient LC50 for brook trout is probably due to species differences and/or the fact that the rainbow trout were tested under static conditions while the brook trout were tested in a dynamic system. Our estimate of the acute LC50 of 6.7 mg/l for bluegill compares favorably with a previously estimated 96-hour LC50 of about 6 mg/l, (i.e. 12 mg/l 50% Wettable Powder Formulation) reported by Walker (1964).

The results of these investigations indicated that the three invertebrate species were very similar in their susceptibility to chronic exposure to atrazine despite the fact that there were order of magnitude differences in their susceptibility to acute exposure. It is interesting to note that this similarity existed despite the fact that the MATC for each species was estimated on the basis of different criteria. The most sensitive criterion for determining the effects of atrazine on these invertebrates was developmental retardation for Chironomus, production of young for Daphnia, and survival of young for Gammarus.

The fishes tested were not only similar to each other in their susceptibility to chronic exposure to atrazine but also were remarkably similar to the invertebrates studied. Only one other study has been reported describing the susceptibility of the above species to chronic exposure to a pesticide (Macek et al., 1975). In that study, we found that although the estimated MATC of lindane to the fishes tested were similar, the MATC estimates for two of the three invertebrates tested were significantly lower.

Except for Gammarus, little other previous information is available to provide a basis for making generalizations about the relative susceptibility of fishes and invertebrates to chronic exposure to chemicals in water. However, much of

the previously reported information leads one to the conclusion that invertebrates generally are more susceptible to chronic exposure than fishes. Pickering and Thatcher (1970) reported the MATC of LAS for fathead minnows was >0.63 <1.2 mg/l, while Arthur (1970) reported the MATC of LAS for Gammarus pseudolimnaeus to be >0.2 <0.4 indicating that this invertebrate species may be about 3 times more susceptible to LAS than the fathead minnow. Mount and Stephan (1969) reported the MATC of copper in soft water for fathead minnows was >10.6 <18.6 µg/l, while Arthur and Leonard (1970) reported the MATC of copper in soft water for G. pseudolimnaeus was >4.6 <8.0 indicating again that the invertebrate species may be at least 2 times more susceptible than the minnow. More recently, Arthur et al. (1973) have reported that gammarids may be 3 times more susceptible to chronic exposure to NTA than were fathead minnows.

Only one similar chronic toxicity study with bluegill has been previously described (Eaton, 1970). In comparing the data from our study with those previously reported, it appears that the bluegill in our study were significantly larger and older than those in Eaton's work. As a result, when spawning did occur in our study, we observed more spawns per female and greater egg production per female than reported in the previous work. It is extremely unfortunate that the factors influential in stimulating or inhibiting spawning activity in our study could not be adequately identified. The percent hatch of bluegill eggs which we observed (generally >80%) was comparable to that observed by Eaton (generally>72%). As in our previous study with lindane (Macek et al. 1975) we experienced difficulty in maintaining and feeding bluegill fry during the initial 30-day developmental period. We attribute this primarily to an inability to provide adequate amounts of suitable food forms during the various stages of fry development. Eaton (1970) experienced the same difficulties as described above and suggested that lack of suitable food was the primary factor responsible for poor fry survival. Before additional effort is expended to investigate chronic toxicity of chemicals to bluegill, definitive information to determine what are suitable food sources for developing bluegill fry, and how adequate numbers of these can be effectively made available to the fry, must be developed.

The results of our investigation of the chronic toxicity of atrazine to fathead minnows, though not without its problems, reinforces our opinion that the species provides the best opportunity for evaluating chronic toxicity of chemicals to freshwater fishes. Certainly more work has been reported with

this species than any other (Mount, 1968; Brungs, 1969; Mount & Stephan, 1969; Hermanutz et al. 1973) and procedures for utilizing this species in long-term laboratory assays are well defined. The results of our fathead minnow chronic study in terms of performance of the test organisms are excellent and indicate again that subtle long-term effects of chemicals can be evaluated using this technique.

The number of spawnings per fathead minnow female, the number of eggs per spawn, the percent hatchability, and the survival of fry during the first 60 days development are as good or better than that generally observed in previous studies. In view of these facts, and the fact that the fathead minnow procedure represents the only "true chronic bioassay" of the three species tested (i.e. at least one complete life cycle) we feel strongly that fathead minnows should be one of the freshwater fish species of choice until methods suitable for using other species are sufficiently defined.

We feel the brook trout offers significant potential for use in "partial chronic bioassays". Generally the numbers of fish participating in spawning and the number of eggs spawned were adequate. Although admittedly variable, the percent fertilization of eggs, the percent hatch and survival through the first 30 days (to swim-up) were generally good. We feel that valid information can be generated utilizing this species and these procedures. However, obvious critical areas in the performance of such assays exist. The accidental exposure of parent brook trout to increased day length at a time which coincided with the period of late gametogenesis and the onset of spawning activity verified Henderson's (1963) observation that functional maturity in brook trout is delayed or inhibited by increasing day length. Additionally we suggest that separate systems, free of light, be provided for egg incubation as Letritz (1959) has suggested that both trout and salmon eggs are sensitive to direct light.

We also suspect that the decreased survival of brook trout fry observed in all groups during the period 30-90 days after hatch may be related to the stress associated with handling fry at 30 and 60 days to count and measure them. We suggest that a single measurement after 60 days exposure would provide the same useful information regarding potential effects of chemicals on second generation trout yet would minimize possible adverse effects of handling. We have recently conducted several similar studies with brook trout eggs and fry and achieved excellent success in hatching and fry survival by shielding the developing

eggs from light and minimizing handling of fry. We have observed that continuous exposure of developing brook trout eggs to 0.72 and 0.45 mg/l atrazine significantly reduced incubation time (degree-days). A similar phenomenon has recently been reported by Halter and Johnson (1974). These investigators observed that exposure of coho salmon to Aroclor 1254 resulted in premature hatching of eggs.

The results of the analysis of fish tissue samples to determine the concentration of atrazine residues showed that residues in fish tissues were below minimum detectable limits in all cases. Certainly such findings preclude concern over bioconcentration of atrazine by fishes, a subject of considerable concern for other types of pesticides. These data are, in fact, consistent with other studies that we have conducted which suggest that bioconcentration of triazine herbicides by fish is relatively low (Bionomics, unpublished data).

During the last two years, we have had the opportunity to investigate the bioconcentration of some 50 pesticides by bluegill during a minimum of 30 days continuous exposure (Bionomics, unpublished data). With all but three of the pesticides studied, we observed bioconcentration factors of at least 10X, and with more than half of those studied we observed bioconcentration factors >50X. The absence of detectable concentrations of atrazine residues from our experiments suggest that fishes do not bioconcentrate atrazine to the extent they do many pesticides. Certainly, bioconcentration of atrazine does not appear as important a phenomenon as it appears to be for certain chemicals which have recently been the cause of great concern among regulatory agencies and environmentalists. For example, Macek and Korn (1970) reported brook trout continuously exposed to 3 ng/l DDT for 120 days concentrated the chemical approximately 8500X. Dr. R. Reinert, U.S.D.I., Great Lakes Research Lab., Ann Arbor, Michigan has found that rainbow trout bioconcentrate methylmercury by as much as 8000X (personal communication). Hansen et al. (1971) reported marine fish exposed to polychlorinated biphenyls concentrated the material 30,000X. Mayer et al. (1975) reported brook trout exposed continuously to toxaphene concentrate that chemical 5,000-16,000X.

The value of estimating the MATC for a chemical and an aquatic organism is clearly demonstrated in the case of atrazine. All of the previously reported acute toxicity

information suggested response to atrazine exposure in the range of 5-10 mg/l (Walker, 1964; FWPCA, 1968; Sanders, 1969). Based on field observation Walker (1964) suggested that atrazine concentrations of 0.5-1.0 mg/l had no effect on fishes and only temporarily affected benthic forms. Hiltibrand (1967) suggested that 10 mg/l atrazine had no discernible effect on bluegill and green sunfish fry during 8 days post-hatch exposure. All of these data suggest safe concentrations of atrazine generally an order of magnitude greater than the estimated MATC values for the six species tested in chronic studies. Although the time and effort is considerably greater in developing and estimating MATC values for chemicals based on chronic exposure, it appears to be the only currently available method for generating the information required to establish meaningful and realistic water quality criteria.

Mount and Stephan (1967) have reported the utilization of application factors to estimate chronic toxicity of chemicals to fish based on acute toxicity data, and considerable information based on the chronic exposure of a variety of fishes to pesticides and heavy metals supports this hypothesis. The similarity of the limits of the application factors for each of the fishes and two of the three invertebrates tested with atrazine lends confidence to the general validity of the application factor concept.

SECTION VII

REFERENCES

American Public Health Association, 1971. Standard Methods for the Examination of Water and Wastewater, 13th ed. Am. Public Health Assoc., New York, 874 p.

Arthur, J. W., 1970. Chronic effects of linear alkylate sulfonate detergent on Gammarus pseudolimnaeus, Campeloma decisum and Physa integra. Water Research 4:251-257.

Arthur, J. W., A. E. Lemke, V. R. Mattson and B. J. Halligan, 1973. Toxicity of sodium nitrilotriacetate (NTA) to the fathead minnow and amphipod in soft water. Water Research 8:187-193.

Arthur. J. W. and E. N. Leonard, 1970. Effects of copper on Gammarus pseudolimnaeus, Physa integra and Campeloma decisum in soft water. J. Fish. Res. Bd. Canada 27: 1277-1283.

Benoit, D. A., 1974. Artificial laboratory spawning substrate for brook trout (Salvelinus fontinalis, Mitchill). Trans. Am. Fish. Soc. 103:144-145.

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

Bioassay Committee, 1971a. Recommended bioassay procedures for fathead minnow (Pimephales promelas, Rafinesque) chronic tests. National Water Quality Laboratory, Duluth, Minnesota, 13 p.

Bioassay Committee, 1971b. Recommended bioassay procedures for bluegill (Lepomis macrochirus, Rafinesque) partial chronic tests. National Water Quality Laboratory, Duluth, Minnesota. 12 p.

Bioassay Committee, 1971c. Recommended bioassay procedures for brook trout (Salvelinus fontinalis, Mitchill) partial chronic tests. National Water Quality Laboratory, Duluth, Minnesota, 12 p.

Brungs, W. A., 1969. Chronic toxicity of zinc to the fathead minnow Pimephales promelas, Rafinesque. Trans. Am. Fish. Soc. 98:272-279.

Clemens, H. P., 1950. Life cycle and ecology of Gammarus fasciatus, Say. Ohio State Univ., Franz Theo. Stone Inst. Hydrobio-Contrib. 12. 63 p.

Cope, O. B., 1966. Contamination of the freshwater ecosystem by pesticides. J. Appl. Ecol. 3 (Suppl.): 33-44.

Deuel, C. R., D. C. Haskell, D. R. Brockway and O. R. Kingsbury, 1952. New York State fish hatchery feeding chart, N.Y. State Conserv. Dept. Fish. Res. Bull. No. 3. 26 p.

Drummond, R. A. and W. F. Dawson, 1970. An inexpensive method for simulating diel patterns of lighting in the laboratory. Trans. Am. Fish. Soc., 99:434-435.

Duncan, D. B., 1955. Multiple range and multple F-tests. Biometrics 11:1-42.

Eaton, J. G., 1970. Chronic malathion toxicity to the bluegill (Lepomis macrochirus, Rafinesque). Water Research 4:673-684.

Eisler, R., 1973. Annotated bibliography on biological effects of metals in aquatic environments. EPA, Ecol. Res. Ser. EPA-R3-73-007, Washington, D.C., 287 p.

FWPCA, 1968. Water Quality Criteria. Report of the National Tech. Adm. Comm. to Secr. of the Interior Fed. Water Poll. Contr. Adm. U.S.D.I., 234 p.

Hall, J. K., M. Pawlus and E. R. Higgins, 1972. Losses of atrazine in run-off water and soil sediment. J. Environ. Qual. 1:172-176.

Halter, M. T. and H. E. Johnson, 1974. Acute toxicities of polychlorinated biphenyl (PCB) and DDT alone and in combination to early life stages of coho salmon (Oncorhyncus kisutch). J. Fish. Res. Bd. Canada 31:1543-1547.

Hansen, D. J., P. R. Parrish, J. I. Lowe. A. J. Wilson, Jr. and P. D. Wilson, 1971. Chronic toxicity, uptake and retention of Aroclor 1254 in two estuarine fishes. Bull. Environ. Contam. Toxicol. 6:113-119.

Henderson, N. E., 1963. Influence of light and temperature on the reproductive cycle of the eastern brook trout, Salvelinus fontinalis. J. Fish. Res. Bd. Canada 20:859-897.

Hermanutz, R. O., L. H. Mueller amd K. D. Kempfert, 1973. Captan toxicity to fathead minnows (Pimephales promelas), bluegills (Lepomis macrochirus) and brook trout (Salvelinus fontinalis). J. Fish. Res. Bd. Canada 30:1811-1817.

Hesselberg, R. J. and J. L. Johnson, 1972. Column extraction of pesticide from fish food and mud. Bull. Environ. Contam. Toxicol. 7:115-120.

Hiltibrand, R. C., 1967. Effects of some herbicides on fertilized fish eggs and fry. Trans. Am. Fish. Soc. 96:414-416.

Hutchinson, G. E., 1967. A Treatise on Limnology, Vol. 2 John Wiley & Sons, New York, 1115 p.

Johnson, D. W., 1968. Pesticides and fishes - A review of selected literature. Trans. Am. Fish. Soc. 97:398-424.

Leitritz, E., 1959. Trout and salmon culture. State of California, Dept. Fish & Game, Fish Bull. No. 107, 169 p.

Macek, K. J. and S. Korn, 1970. Significance of the food chain in DDT accumulation by fish. J. Fish. Res. Bd. Canada 27:1496-1498.

Macek, K. J., K. S. Buxton, S. K. Derr, J. W. Dean and S. Sauter, 1975. Chronic toxicity of lindane to selected aquatic invertebrates and fishes. EPA, Ecol. Res. Series (In Press).

Mayer, R. L., P. M. Merhle and W. P. Dwyer, 1975. Toxaphene effects on reproduction, growth and mortality of brook trout. EPA, Ecol. Res. Series (In Press).

McComish, T. S., 1968. Sexual differentiation of bluegills by the urogenital opening. Prog. Fish. Cult. 29:28

McKim, J. M. and D. A. Benoit, 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (Salvelinus fontinalis). J. Fish. Res. Bd. Canada 28:655-662.

Mount, D. I., 1968. Chronic toxicity of copper to fathead minnows (Pimephales promelas, Rafinesque). Water Research 2:215-223.

Mount, D. I. and W. A. Brungs, 1967. A simplified dosing apparatus for fish toxicological studies. Water Research 1:21-29.

Mount, D. I. and C. E. Stephen, 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96:185-193.

Mount, D. I. and C. E. Stephan, 1969. Chronic toxicity of copper to the fathead minnow (Pimephales promelas) in soft water. J. Fish. Res. Bd. Canada 26:2449-2457.

Pickering, Q. H. and T. O. Thatcher, 1970. The chronic toxicity of linear alkylate sulfonate (LAS) to Pimephales promelas, Rafinesque. J. Water Pollut. Control Fed. 42: 243-254.

Sanders, H. O., 1969. Toxicity of pesticides to the crustacean Gammarus lacustris. U.S. Bur. Sport. Fish Wild, Tech. Rep. 25. 18 p.

Steel, R. G. D. and J. H. Torrie, 1960. Principles and procedures of statistics. McGraw Hill, New York. 481 p.

Walker, C. A., 1964. Simazine and other s-triazine compounds on aquatic herbicides in fish habitats. Weeds 12:134-139.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-76-047		

4. TITLE AND SUBTITLE	5. REPORT DATE
Chronic Toxicity of Atrazine to Selected Aquatic Invertebrates and Fishes	May 1976 (Issuing Date)
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Kenneth J. Macek, Kenneth S. Buxton, Scott Sauter, Sarah Gnilka, Jerry W. Dean	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Bionomics EG & G Inc. 790 Main Street Wareham, Massachusetts 02571	1BA608
	11. CONTRACT/GRANT NO. 68-01-0092 and 68-01-1844

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory-Duluth Office of Research and Development U.S. Environmental Protection Agency Duluth, Minnesota 55804	Final
	14. SPONSORING AGENCY CODE EPA/ORD

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Representatives of the aquatic invertebrate species of water flea (Daphnia magna), midge (Chironomus tentans), and scud (Gammarus fasciatus); and the fish species bluegill (Lepomis macrochirus), fathead minnow (Pimephales promelas), and brook trout (Salvelinus fontinalis) were chronically exposed to various concentrations of atrazine in separate flowing-water systems.

Maximum acceptable toxicant concentrations (MATC) of atrazine for the selected species in soft water were estimated using survival, growth, and reproduction as indicators of toxic effects. The MATC was estimated to be between 0.11 and 0.23 mg/1 for midges, between 0.14 and 0.25 mg/1 for water fleas, and between 0.06 and 0.14 for the scud. For fishes the MATC was estimated to be between 0.09 and 0.50 mg/1 for bluegills, between 0.21 and 0.52 mg/1 for fathead minnows, and between 0.06 and 0.12 mg/1 for brook trout. The incipient-LC50 for fishes and the 48-hour LC50 for invertebrates was estimated from acute exposures and was used to calculate application factors (MATC/LC50). For aquatic invertebrates and atrazine the estimated application factors were between 0.15 and 0.32 for midges, between 0.02 and 0.04 for water flea, and between 0.01 and 0.02 for scud. Application factors were estimated between 0.01 and 0.07 for bluegills, between 0.01 and 0.03 for fathead minnows, and between 0.01 and 0.02 for brook trout.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Toxicity Fishes Invertebrates Pesticides	Chronic toxicity Tissue residues Flowing water system Application factors Atrazine	06T

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release unlimited	Unclassified	58
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

☆ U.S. GOVERNMENT PRINTING OFFICE: 1976-657-695/5441 Region No. 5-11