CHRONIC TOXICITY TO FISH

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as 78.03, PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 83.
The test sample is FC-143. The testing laboratory refers to it as "78.03". It's purity was not sufficiently characterized, though current information indicates it is a mixture of 96.5 - 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro analogue compounds.

METHOD:

Method Followed: The methodology for the egg and fry exposure closely followed that presented in "Proposed recommended bioassay procedure for egg and fry stages of freshwater fish" (U.S. EPA, 1972).

Test Type: Flow-through
GLP: No
Year study performed: 1978
Species: Fathead minnow (*Pimephales promelas*)
Supplier: U.S. Environmental Protection Agency's Environmental Research Laboratory in Duluth, Minnesota.
Test fish age: Eggs within 48-hours after fertilization

Analytical monitoring: Temperature, dissolved oxygen concentration, and pH were monitored daily. Weekly samples were taken from each aquarium for determination of ammonium perfluorooctanoate concentration. All samples taken during the test were stored in polyethylene bottles and shipped on May 31, 1978 to the 3M Company.

Exposure period: 30 days post-hatch

Statistical Methods: Means of measured biological parameters from duplicate aquaria were subjected to analysis of variance (Steel and Torrie, 1960, completely randomized block design, P=0.05). Data for percentage survival and percentage hatch were transformed to arc sin square root of percentage prior to analysis.
Test conditions:

Exhibit
1176
State of Minnesota v. 3M Co.,
Court File No. 27-CV-10-28862

3M_MN01655650

Dilution water: Well water pumped to a concrete reservoir where it was aerated before flowing to the exposure system through aged PVC pipe.

Dilution water chemistry (0-30 days):

Total hardness:	31 – 38 mg/L (as $CaCO_3$)
Alkalinity:	26 – 32 mg/L (as $CaCO_3$)
pH:	7.0 – 7.4
Specific conductance:	149-170 µmhos/cm

Stock and test solution preparation: A modified, proportional diluter with a 0.50 dilution factor was used. The diluter delivered five nominal concentrations of ammonium perfluorooctanoate ranging from 100 to 6.2 mg/L and control water to duplicate test aquaria. A 4 liter glass mariotte bottle toxicant delivery system was used to deliver 6.6 mL of a nominal ammonium perfluorooctanoate stock concentration of 29.4 mg/mL in distilled water to the mixing chamber of the diluter.

Pretreatment: Eggs were placed in a 60 mg/L malachite green solution for 15 seconds to eliminate possible fungus growth.
Egg cups: acrylic tubes (3 cm O.D., 7 cm long) with 40 mesh Nitex^R screen on one end. An egg cup rocker arm apparatus, as described by Mount (1968), was used to gently oscillate the egg cups in the test water.
Fry exposure vessels: Glass test aquarium measuring 30.5 X 30.5 X 30.5 cm with a 17.5 cm high standpipe drain, water volume of 16 liters.
Diluter: Delivered 0.50 liters of test water to each aquarium 195 times per day, yielding a 90% test water replacement time of approximately 10 hours.
Feeding: fry were fed live brine shrimp nauplii three times daily on weekdays and twice daily on weekends throughout the exposure period. The aquaria were brushed and siphoned to remove excess food and fecal material twice each week.
Temperature control: Water bath containing circulating water heated by immersion coil heater and regulated by a mercury column thermoregulator

Number of replicates: two
Number of eggs for hatchability test: 60
Number of fry for fry exposure test: 40 from each egg cup
Number of concentrations: five plus a blank control

Water chemistry during the study:

Dissolved oxygen: > 95% saturation
pH: 7.0 – 7.3
Temperature: 25 ± 1°C

At the termination of the test, the fry from the control and the high concentration (100 mg/L) were preserved in 10% buffered formalin while the fry from the other test aquaria were frozen. Ten formalin-preserved fry (5

from each replicate) from the control and the high concentration underwent histopathological examination of a transverse section of the nares and cephalic extension of the lateral line (See Table 2). The remaining preserved fry and frozen fry were analyzed at a later date (by 3M Company) for ammonium perfluorooctanoate concentrations.

RESULTS

Nominal concentrations: Bk control, 6.2, 12.5, 25, 50, 100 mg/L
Element value: Not stated

TABLE 1

PERCENTAGE HATCH OF EGGS,
PERCENTAGE SURVIVAL,
MEAN AND STANDARD DEVIATION TOTAL LENGTH,
AND MEAN WET WEIGHT
OF FATHEAD MINNOW (*Pimephales promelas*) FRY EXPOSED
TO AMMONIUM PERFLUOROOCTANOATE FOR 30 DAYS POST-HATCH.

Nominal concentration (mg/l)			30 Days Post-hatch		
	Replicate	Hatch %	Survival %	Mean length in mm (+SD)	Mean weight in mg
Control	A	98	92	20(2)	62
	B	95	95	21(3)	75
6.2	A	95	98	20(2)	59
	B	94	88	22(2)	79
12.5	A	93	95	21(3)	70
	B	88	100	21(2)	72
25	A	98	90	21(2)	74
	B	100	95	21(2)	70
50	A	90	90	20(2)	60
	B	95	98	20(3)	65
100	A	95	88	19(2)	59
	B	97	82	20(2)	60

TABLE 2

RESULTS OF HISTOPATHOLOGICAL EXAMINATION OF PIMEPHALES PROMELAS FRY EXPOSED 30 DAYS TO 100 mg/l OF AMMONIUM PERFLUOROOCTANOATE[a]

Test Material	Number of Observations	Histopathological Findings
Control	10	3/10 Normal 6/10 Liver Fatty Change 3/10 Gill Hyperplasia (Epithelium) I
Ammonium Perfluorooctanoate	10	5/10 Normal 5/10 Liver Fatty Change 2/10 Gill Hyperplasia (Epithelium) I

a. Work performed under contract to EG & G Bionomics Laboratory

NOTE: "Only those tissues which were missing or contained demonstrable change are listed. The only tissue changes observed were hyperplasia of gill lamellar epithelium and fatty change of the liver. These changes were judged to be minimal and consistent with changes seen routinely in healthy fish. Autolipis of gill tissue was observed in several fish. This change was probably due to the poor penetration of the buffered formalin to the posterior dorsal portion of the gill space."

CONCLUSIONS

Biological data generated in this study indicate that the nominal concentration of 100 mg/L had no adverse effect upon the hatchability or eggs or upon the survival and growth of fathead minnow fry through 30 days post-hatch.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking = 2. This study meets all the criteria for quality testing at the time it was conducted, but has several deficiencies. It lacks information on purity of the test substance, and the production lot number from which the test sample was taken. There is no information available on the analysis of the test solution concentrations or on the preserved fry and frozen fry samples.

REFERENCES

This study was conducted by E G & G, Bionomics, Wareham, Massachusetts, 1978 on the request of 3M Company.

Research Report "THE EFFECTS OF CONTINUOUS AQUEOUS EXPOSURE TO 78.03 (AMMONIUM PERFLUOROOCTANOATE) ON HATCHABILITY OF EGGS AND GROWTH AND SURVIVAL OF FRY OF FATHEAD MINNOW (*Pimephales promelas*)." Report # BW-78-6-175, E G & G, Bionomics, Aquatic Toxicology Laboratory, 790 Main Street, Wareham, Massachusetts, June 1978.

Research Report "SUMMARY OF HISTOPATHOLOGICAL EXAMINATION OF FATHEAD MINNOW (*Pimephales promelas*) EXPOSED TO 78.03 (AMMONIUM PERFLUOROOCTANOATE) FOR 30 DAYS." Report # BW-78-9-301, E G & G, Bionomics, Aquatic Toxicology Laboratory, 790 Main Street, Wareham, Massachusetts, September 1978.

OTHER

Last changed: 5/25/00

THE EFFECTS OF CONTINUOUS AQUEOUS EXPOSURE TO 78.03 ON HATCHABILITY OF EGGS AND GROWTH AND SURVIVAL OF FRY OF FATHEAD MINNOW (Pimephales promelas).

FC-143

RESEARCH REPORT

SUBMITTED TO

3M COMPANY

ST. PAUL, MINNESOTA

REPORT #BW-78-6-175

E G & G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
June, 1978

3M_MN01655656

ABSTRACT

Fathead minnow (Pimephales promelas) eggs and fry were continuously exposed to nominal 78.03 concentrations ranging from 100 to 6.2 mg/ℓ through 30 days post-hatch. Observations were made on percentage hatch of eggs and on survival, mean total length and mean wet weight of fry. Results indicated that none of the above parameters were affected by continuous exposure to any of the 78.03 concentrations tested.

3M_MN01655657

TABLE OF CONTENTS

3M_MN01655658

SECTION I

INTRODUCTION

The objective of this study was to determine the effects of 78.03 on fathead minnow (Pimephales promelas) eggs and fry during continuous aqueous exposure. Exposures were initiated within 48-hours after egg fertilization and continued through 30 days post-hatch. The effects on egg hatchability and on survival and growth of fry were measured and would be used to make an estimate of the MTC (minimum threshold concentration). The MTC is virtually synonomous with the term MATC (maximum acceptable toxicant concentration) developed by Mount and Stephen (1967). Mount and Stephan's term, however, was estimated after the performance of a full, life-cycle, chronic test where effects on reproduction and second generation fry were also measured.

Macek and Sleight (1977) and McKim (1977) described egg and fry investigations as being reasonably accurate short-term estimations of potential long-term chemical hazards to fish, and as being similar to those estimations derived from definitive chronic toxicity studies. In the majority of the studies reported by the authors and of those performed at this laboratory, the embryos and fry during early stages of development were generally the most sensitive stages to chemical exposure. Rarely was reproduction or survival and growth of second generation fry reduced at exposure levels lower than those that reduced survival or growth of the first generation fry.

3M_MN01655659

The authors demonstrated that for the great majority of toxicants, the quicker and more economical egg and fry tests yielded estimates of safe concentrations very similar to those derived from chronic toxicity studies.

SECTION II

MATERIALS AND METHODS

The egg and fry study was performed according to methods developed at E G & G, Bionomics (Appendix I), which closely follow those presented in "Proposed recommended bioassay procedures for egg and fry stages of freshwater fish" (U.S. EPA, 1972).

The test material, labelled 78.03, a fine white powder, was obtained from the 3M Company, St. Paul, Minnesota in 2 shipments on March 17 and April 11, 1978.

A. Exposure System

A modified, proportional diluter similar to that described by Mount and Brungs (1967) with a 0.50 dilution factor was used in this study. The diluent water was well water which was pumped to a concrete reservoir where it was aerated before flowing to the exposure system through PVC pipe. This water was characterized as having a total hardness and alkalinity as calcium carbonate ($CaCO_3$) of 31-38 mg/ℓ and 26-32 mg/ℓ, respectively (APHA, et al., 1975), a pH of 7.0-7.4 and a specific conductance of 149-170 micromhos per centimeter (μmhos/cm). The diluter delivered five nominal concentrations of 78.03 ranging from 100 to 6.2 mg/ℓ and control water (well water) to duplicate test aquaria. Each test aquarium measured 30.5 x 30.5 x 30.5

3M_MN01655661

centimeters (cm) and had a standpipe drain 17.5 cm in height to maintain a constant test water volume of 16 ℓ in each aquarium. The diluter delivered 0.50 ℓ of test water to each aquarium 195 times per day yielding a 90% test water replacement time of 10 hours (Sprague, 1969). To minimize the adsorption of 78.03 on surfaces, all exposure system components having contact with 78.03 were constructed of acrylic material rather than glass. Ethylene dichloride was used to cement acrylic components together.

The aquaria rested in a water bath containing circulating water heated by immersion coil heaters and regulated by a mercury column thermoregulator designed to maintain the test water temperature at 25 ± 1°C.

A 4 ℓ glass Mariotte bottle toxicant delivery system was used to deliver 6.6 mℓ of a nominal 78.03 stock concentration of 29.4 mg/mℓ in distilled water to the mixing chamber of the diluter.

B. Egg and Fry Exposure

On March 31, 1978, the exposure of fathead minnow eggs to 78.03 was initiated with eggs obtained within 48-hours after fertilization from the U.S. Environmental Research Laboratory, Duluth, Minnesota. Upon arrival at E G & G, Bionomics, the eggs were allowed to acclimate from 17.5°C to the test temperature

3M_MN01655662

of 25°C over a two hour period. Sixty eggs were then randomly distributed to each of 12 egg cups which were then placed in a 60 mg/ℓ malachite green solution for 15 seconds to eliminate possible fungus growth. One egg cup was then suspended in each of the 12 test aquaria. Egg incubation cups were acrylic tubes (7 cm long, 3 cm O.D.) covered at one end with 40 mesh Nitex® screen. An egg cup rocker arm apparatus, as described by Mount (1968) was used to gently oscillate the egg cups in the test waters. Dead eggs were counted and removed daily until hatching was complete. Percentage hatch calculations were based on the number of live fry per aquarium after hatching was completed compared to the number of eggs (60) per aquarium at the initiation of the exposure.

To initiate the 30 day fry exposure, 40 fry were randomly selected from each egg cup and transferred to the respective aquaria. Upon completion of hatch, fry were fed live brine shrimp nauplii three times daily on weekdays and twice daily on weekends. Aquaria were brushed and siphoned twice each week to remove excess food and fecal matter. Observations on behavior and appearance of fry were made daily and fry counts were made weekly. At 30 days post-hatch the fry from each aquarium were anesthetized with MS-222 (tricaine methanesulfonate) and percentage survival, mean total length, and mean wet weight were determined. The fry were measured individually to calculate mean and standard deviation total length while each fry group (fry from one aquarium) was wet weighed to cal-

3M_MN01655663

culate mean wet weight.

At the termination of the test, the fry from the control and the high concentration (100 mg/ℓ) aquaria were preserved in 10% buffered formalin while the fry from the other test aquaria were frozen. Ten preserved fry (5 from each replicate) from the control and the high concentration were sent to the Environmental Pathology Laboratories, Inc., Carolina, Rhode Island for complete histopathological examination with a transverse section of the nares and cephalic extension of the lateral line. The remaining preserved fry and frozen fry were sent to the 3M Company, St. Paul, Minnesota, May 31, 1978.

C. Test Water Analysis

Dissolved oxygen concentrations were measured in test aquaria using a YSI Model #54 dissolved oxygen meter with a combination electrode polarographic probe while pH was measured with an Instrumentation Laboratory Model #175 pH meter. Temperature was measured with a laboratory thermometer. Measurements were made daily and alternated between aquaria such that each aquarium was measured once each week.

One-hundred mℓ water samples were taken weekly from each test aquarium and stored in polyethylene bottles. Samples were shipped May 31, 1978 to the 3M Company, St. Paul, Minnesota for determination of 78.03 concentration.

3M_MN01655664

D. Statistics

Means of measured biological parameters from duplicate aquaria were subjected to analysis of variance (Steel and Torrie, 1960, completely randomized block design, P=0.05). Data for percentage hatch and percentage survival were transformed to arc sin $\sqrt{\text{percentage}}$ prior to analysis. If treatment effects were indicated, the means of these parameters were compared to those from the controls using Dunnett's procedure (Steel and Torrie, 1960). When a treatment mean was significantly different from the control mean (P=0.05), that treatment was considered to be an effect level.

3M_MN01655665

SECTION III

RESULTS

The daily measurements of water quality parameters demonstrated that the temperature remained at 25 ± 1°C and the dissolved oxygen concentrations above 95% of saturation throughout the entire exposure period. The pH normally ranged from 7.0-7.3 and did not differ significantly between exposure aquaria.

The biological data generated in this study indicate that nominal 78.03 concentrations as high as 100 mg/ℓ had no adverse effects upon the hatchability of eggs or upon the survival and growth of fathead minnow fry (Table 1) through 30 days post-hatch exposure.

3M_MN01655666

SECTION IV

REFERENCES

APHA, AWWA, WPCF. 1975. Standard Methods for the Examination of Water and Wastewater. 14th Edition, New York, Hardness EDTA Titrimetric Method. 309B, pp. 203-206.

Macek, K.J. and B.H. Sleight, III. 1977. Utility of toxicity tests with embryo and fry of fish in evaluating hazards associated with chronic toxicity of chemicals to fishes. Symposium Proceedings, ASTM, Memphis, Tennessee, October, 1976: 137-146.

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Bd. Can. 34: 1148-1154.

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

Mount, D.I. and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 20-29.

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. Amer. Fish. Soc. 96: 185-193.

3M_MN01655667

1176.0018

Sprague, J.B. 1969. Measurements of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793-831.

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

U.S. EPA. 1972. Proposed recommended bioassay procedure for egg and fry stages of freshwater fish: pp. 7.

3M_MN01655668

Table 1 -- Percentage hatch of eggs, percentage survival, mean and standard deviation (S.D.) total length, and mean wet weight of fathead minnow fry (Pimephales promelas) continuously exposed to 78.03.

Nominal concentration (mg/ℓ)	Replicate	Hatch (%)	30 days post-hatch		
			Survival (%)	Total length (mm) and (S.D.)	Weight (mg)
100	A	95	88	19(2)	59
	B	97	82	20(2)	60
50	A	90	90	20(2)	60
	B	95	98	20(3)	65
25	A	98	90	21(2)	74
	B	100	95	21(2)	70
12.5	A	93	95	21(3)	70
	B	88	100	21(2)	72
6.2	A	95	98	20(2)	59
	B	94	88	22(2)	79
control	A	98	92	20(2)	62
	B	95	95	21(3)	75

3M_MN01655669

APPENDIX I

PROCEDURES FOR CRITICAL LIFE STAGE TOXICITY TESTS WITH FRESHWATER FISHES

This describes standard toxicity testing procedures for egg and fry stages of freshwater fishes followed at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts. This procedure closely adheres to the Proposed Recommended Bioassay Procedure for Egg and Fry Stages of Freshwater Fish (EPA, 1972).

A. Physical System

1. Diluter: A proportional diluter (Mount and Brungs, 1967) with a dilution factor of 0.5 is employed for egg and fry exposures. A check is made of diluter function by daily observations. Five toxicant concentrations, a control, and if necessary, a solvent control, are utilized in each test.

2. Toxicant mixing: A container to promote mixing of toxicant bearing solution and diluent water is used between diluter and aquaria for each concentration. Separate delivery tubes are run from this container to each duplicate tank. Calibrations are performed before every test to insure that the correct proportion of toxicant solution and diluent water is delivered to each duplicate tank. Toxicant concentrations are monitored in each duplicate aquarium.

3. Tank: Each duplicate aquarium is constructed of glass and silicone adhesive and measures 39 x 20 x 25 cm. Water depth is maintained by a constant level glass drain tube 19.5 cm from the bottom of each test aquarium. The total test solution volume in each aquarium is thus maintained at 15 l.

4. Flow rate: Five-hundred-ml of test solution are delivered to each duplicate aquarium at a rate of 6-10 tank volumes per 24 hours. This is sufficient to maintain a dissolved oxygen concentration >60% of saturation.

5. Cleaning: All aquaria are brushed and siphoned at least twice weekly.

6. Egg Cup: Egg incubation cups are made from 5 cm O.D. round glass jars with the bottoms cut off and replaced with stainless steel or Nitex^{R} screen (40 mesh per inch). Cups are oscillated in the test water by means of a rocker arm apparatus driven by a 2 RPM electric motor (Mount, 1968).

3M_MN01655670

1176.0021

7. Light: When necessary for egg and fry survival (e.g., salmonids), the aquaria are shielded from all sources of light.

8. Temperature: Temperatures are controlled so as not to deviate from the specified test temperature by more than 1°C throughout the entire test period.

9. Construction materials: Construction materials which contact the test water are chosen which do not either leach of sorb significant amounts of substances from the water. Glass, silicone adhesive, Nitex^{R}, Tygon^{R}, silicone stoppers and unplasticized polyethylene are the construction materials used.

10. Water: A 125 meter deep bedrock well is the source of the diluent water. This water is pumped to a concrete holding tank where it receives extensive aeration and is delivered through aged PVC pipe to the exposure system.

B. Biological System

1. Beginning test: The exposures are initiated as soon as possible after the eggs are fertilized, and the stage of embryo development is recorded. Depending upon availability of eggs, 35 to 50 eggs are randomly distributed to each of two egg cups or 60 eggs are placed in one egg cup per duplicate aquarium. Eggs are exposed for a minimum of 1/2 the expected egg incubation period. Egg mortality in each egg cup is recorded daily. If deemed necessary, eggs will be treated with an appropriate fungicide during incubation.

2. Fry exposure: If handling of eggs permits, a daily record is kept when hatching commences of the number of eggs hatched, the number of dead fry, and the number of deformed fry in each egg cup. After complete hatch, 40 fry are randomly selected from the egg cup or cups and transferred to each aquarium. The fry are exposed to the test solution for a minimum of 30 days post-hatch. This period may be extended if the data warrants a longer investigation. The number of surviving fry is recorded twice weekly. At the end of the fry exposure period, percentage survival, individual mean total length, mean wet weight and deformities are recorded for each fry group.

3. Necessary data: Data that will be reported for

3M_MN01655671

each duplicate in the egg and fry exposure are: a) percentage hatch (number of fry surviving after hatching is complete/number of eggs incubated), b) percentage fry survival at 30 days post-hatch, c) growth (mean total length and weight at 30 days), and d) deformities.

4. Food: Unless otherwise deemed necessary, fish are fed live brine shrimp nauplii twice per day ad libitum supplemented with dry pelleted food when the fish have reached a sufficient size.

5. Disease: Disease outbreaks are handled according to their nature. When treatment is deemed necessary, all aquaria will receive the same treatment.

6. Special examinations: If required, extra fish and eggs are preserved for possible future physiological, biochemical, and histological investigations which may indicate certain toxicant related effects.

C. Chemical System

1. Preparing a stock solution: Stock solutions are prepared by dissolving the toxicant in water or in an organic solvent if insoluble in water. The amount of solvent (reagent-grade or better) is kept at a minimum. If solvent is used, a solvent control is also established. The concentration of solvent in the solvent control is equal to the highest solvent concentration found in any exposure aquarium.

2. Measurement of toxicant concentrations: The concentration of toxicant is measured in each duplicate aquarium at each toxicant concentration at least once per week. Water samples are taken at a point approximately midway between the water surface, bottom and sides of each aquarium. Water samples are either extracted immediately after sampling or appropriately preserved until extractions or analyses can be performed.

3. Measurement of other variables: Temperature and dissolved oxygen are measured in aquaria daily on an alternating basis, such that each aquarium is analyzed once each week. The pH is measured weekly in the high and low test concentration and each control, alternating between replicate tanks from week to week. Total hardness is measured in the high and low concentration and control weekly. If any of these parameters are affected by the toxicant, additional

3M_MN01655672

analyses are performed to more closely monitor that parameter.

4. Residue analysis: When deemed necessary, exposed fish and eggs are analyzed for toxicant residues.

5. Methods: Methods described in Methods for Chemical Analysis of Water and Wastes (EPA, 1971) are used unless other more efficient methods can provide more accurate information. Reference samples are analyzed periodically for each analytical method.

D. Statistics

1. Duplicates: True duplicates are used for each level of the toxicant being tested (i.e., no water connections between duplicate aquaria).

2. Distribution of test concentrations: The toxicant concentrations are assigned to aquaria by stratified random assignment.

3. Analysis of variance/Dunnett's

E. Miscellaneous

1. Additional information: All routine bioassay flow-through methods not covered in this procedure (e.g., physical and chemical determinations, handling of fish) closely followed those described in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1975).

2. References: For additional information concerning flow-through bioassay tests with fish eggs and fry, the following references are listed:

American Public Health Association. 1975. Standard methods for the examination of water and wastewater. 14 Ed. APHA, New York.

Environmental Protection Agency. 1971. Methods for Chemical Analysis of Water and Wastes. Analytical Quality Control Laboratory, Cincinnati, Ohio.

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

3M_MN01655673

1176.0024

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

Mount, Donald I. and William Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Research, 1: 20-29.

Sauter, Scott et al. 1976. Effects of exposure to heavy metals on selected freshwater fish. Ecological Research Series, EPA-660/3-76-105.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York: 481 pp.

U.S. Environmental Protection Agency. 1972. Proposed Recommended Procedure for Egg and Fry Stages of Freshwater Fish.

3M_MN01655674

1176.0025

SUBMITTED BY:

E G & G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
June, 1978

PREPARED BY:

Brenda F. Wilson

Brenda F. Wilson

Aquatic Biologist

Stephen J. Ells

Stephen J. Ells

Aquatic Toxicologist

APPROVED BY:

George A. Cary

Director, Aquatic Biology

3M_MN01655675

SUMMARY OF HISTOPATHOLOGICAL EXAMINATIONS OF FATHEAD MINNOW (Pimephales promelas) EXPOSED TO 78.03 FOR 30 DAYS.

FC-143

RESEARCH REPORT

SUBMITTED TO 3M COMPANY

ST. PAUL, MINNESOTA

REPORT #BW-78-9-301

SUBMITTED BY
E G & G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
September, 1978

3M_MN01655676

INTRODUCTION

An "egg and fry test" was conducted between March 31 and May 4, 1978 (Bionomics Report #BW-78-6-175, June, 1978) to assess the sub-lethal effects of 78.03 on fathead minnows (Pimephales promelas). Effects on egg hatchability and on survival and growth of fry were measured. To further investigate possible effects due to exposure to 78.03, histopathological examinations of exposed fish were also performed. The results of these examinations are reported here.

MATERIALS AND METHODS

Upon termination of the egg and fry study, five fish from each replicate of the high 78.03 concentration (100 mg/ℓ, nominal) and control were preserved in 10% buffered formalin and sent to the Environmental Pathology Laboratories, Inc. Carolina, Rhode Island.

The fish were examined for gross lesions, and sagital sections were prepared by a pathologist. Fish were placed with indentifying numbers into processing cassettes, dehydrated, cleared and infiltrated on an Auto-technicon tissue processor using the method of the Armed Forces Institute of Pathology. The fish were then placed in a vacuum oven and subsequently embedded in paraffin. All blocks were sectioned at 6 microns: one

slide was prepared from each block and stained with hematoxylin and eosin. Special staining procedures such as PAS, Trichrome and Acid Fast were used occasionally when requested by the pathologist. All blocks were sealed with paraffin and stored.

Slides were labeled, boxed and delivered with all work sheets to the pathologist for examination. Two slides were prepared from each fish. Tissues processed and examined included but were not limited to; gill, thymus, liver, spleen, heart, gonad, kidney, foregut, hindgut, olfactory mucosa (nares), brain, ear, skin, muscle, pancreas and pharyngeal mucosa.

RESULTS

A summary of the examination of each fish is reported in Table 1. Only those tissues which were missing or contained demonstrable change are listed.

The only tissue changes observed were hyperplasia of gill lamellar epithilium and fatty change of the liver. These changes were judged to be minimal and consistent with changes seen routinely in healthy fish. Autolipis of gill tissue was observed in several fish. This change was probably due to the poor penetration of the buffered formalin to the posterior dorsal portion of the gill space.

Based on these data, it is concluded that 30 days exposure to a nominal 78.01 concentration of 20 ug/ℓ did not cause any significant, demonstrable, tissue changes in 30 day old fathead minnow fry.

Table 1 -- Histopathological examination of fathead minnow (Pimephales promelas) exposed 30 days to 100 mg/ℓ nominal 78.03 and control water.

100 mg/ℓ 78.03

Fish #	Sex	Organ Condition	Missing Tissue
1	Ukn[a]	Normal	Spleen, gonad, nares, ear
2	Ukn	Normal	Spleen, gonad, thymus
3	Ukn	Normal	Heart, gonad, nares
4	Ukn	Normal	Spleen, gonad, thymus
5	F[b]	Normal	Spleen, nares
6	F	Liver-Fatty change Ic Gill-Hyperplasia (epithelium) I	Nares, thymus
7	F	Liver-Fatty change I Gill-Hyperplasia (epithelium) I	Nares, ear
8	F	Liver-Fatty change I	Spleen, ear
9	Ukn	Liver-Fatty change I	Spleen, gonad, nares, ear
10	F	Liver-Fatty change I	Spleen, nares

3M_MN01655680

Table 1 (cont.)

Fish #	Sex	Organ Condition	Missing Tissue
Control			
1	Ukn	Liver-Fatty change I Gill-hyperplasia (epithelium) I	Gonad, nares, thymus
2	Ukn	Liver-Fatty change I	Spleen, gonad, nares
3	F	Gill-hyperplasia (epithelium) I	Thymus
4	F	Normal	Spleen, nares
5	M[d]	Normal	Spleen, thymus, heart
6	F	Liver-Fatty change I	Spleen, heart
7	Ukn	Liver-Fatty change I	Spleen, heart, gonad, thymus, nares
8	Ukn	Liver-Fatty change I	Spleen, gonad, nares, thymus
9	F	Liver-Fatty change I Gill hyperplasia (epithelium) I	Spleen, nares
10	Ukn	Normal	Gonad, thymus

[a] Female.
[b] Unknown.
[c] Lesions are minimal.
[d] Male

3M_MN01655681

SUBMITTED BY:

E G & G, Bionomics
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
September, 1978

PREPARED BY:

Stephen J. Ells

Aquatic Toxicologist

APPROVED BY:

George A. Cary

Director, Aquatic Biology

3M_MN01655682