CHRONIC TOXICITY TO FISH

TEST SUBSTANCE

Identity: N-ethylperfluorooctane sulfonamidoethanol; may also be referred to as N-EtFOSE Alcohol or FM-3422. (1-Octanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-(2-hydroxyethyl)-, CAS # 1691-99-2)

Remarks: Material is an off-white, waxy solid of uncharacterized purity. The laboratory conducting the study refers to N-EtFOSE Alcohol as "78.01".

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).

Type (test type): Flow-through

GLP: No

Year study performed: 1978

Species/Strain/Supplier: Fathead minnow (*Pimephales promelas*) eggs from the brood stock of the U.S. Environmental Protection Agency's Environmental Research Laboratory in Duluth, Minnesota.

Analytical monitoring: Temperature, dissolved oxygen concentration, and pH were monitored daily, alternating between aquaria such that each aquarium was measured once each week.

Remarks: One thousand ml water samples were taken from each aquarium at the initiation of the test (day 0), when hatching was completed (day 4), and weekly thereafter (days 11, 18, 25, and 32) for determination of N-EtFOSE Alcohol concentration. Samples were taken with a beaker from a point approximately midway between the surface, bottom and sides of each aquarium, and stored in 1000 ml amber glass bottles with foil lined caps. All samples taken during the test were stored at room temperature and shipped on May 31, 1978 to the 3M Company.

Exposure period: 4, 11, 18, 25, and 32 days

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 Condition Remarks:

Exposures were initiated within 48-hours after egg fertilization and continued through 30 days post-hatch.

The diluent water was well water pumped to a concrete reservoir where it was aerated before flowing to the exposure system through aged PVC pipe. The pH of this water ranged from 7.0-7.2; total hardness, 31-38 mg/L as $CaCO_3$; alkalinity, 26-29 mg/L as $CaCO_3$; and specific conductance, 149-170 umhos/cm.

Five nominal concentrations of N-EtFOSE Alcohol, ranging from 1.3-20 µg/L, control water (well water), and control water containing solvent (Dimethylsulfoxide (DMSO) at 24 mg/L) were delivered to duplicate test aquaria. A 50 ml gas-tight syringe was used to deliver 46 µl of a 0.84 mg/ml stock solution of N-EtFOSE Alcohol in DMSO through a stainless steel needle and polyethylene tubing to the mixing chamber of the diluter.

Each glass test aquarium measured 40 X 20 X 25 cm with a 19 cm high standpipe drain which maintained a constant test water volume of 15.2 liters. The diluter delivered 0.50 liters of test water to each aquarium 185 times per day, yielding a 90% test water replacement time of approximately 9 hours. The aquaria rested in a water bath containing circulating water heated by immersion coil heater and regulated by a mercury column thermoregulator designed to maintain the test water temperature at 25°C.

Upon arrival at E G & G , Bionomics, the eggs were allowed to acclimate from 17.5°C to the test temperature of 25°C over a period of two hours. Sixty eggs were then randomly distributed to each of 14 egg cups which were then dipped in a 60 mg/l malachite green solution for 15 seconds to prevent fungal growth. One egg cup was then suspended in each of the 14 test aquaria. Egg incubation cups were glass jars (5 cm O.D., 8 cm high) with 40 mesh Nitex^{R} screen bottoms. An egg cup rocker arm apparatus, as described by Mount (1968), was used to gently oscillate the egg cups in the test water.

Dead eggs were removed and counted daily until hatching was completed. Percentage hatch was calculated based on the number of live fry per aquarium after hatching was completed compared to the number of eggs per aquarium (60) at the initiation of the exposure. To initiate the 30-day fry exposure, forty fry were randomly selected from each egg cup and transferred to the respective aquaria.

Upon transfer of fry to the aquaria, the fry were fed newly hatched San Francisco Bay variety brine shrimp nauplii, ad libitum, three times daily throughout the exposure period. The aquaria were brushed and siphoned to remove excess

food and fecal material three times per week. At 30 days post-hatch, percentage survival, mean total length, and mean wet weight were determined for fry from each aquarium. The fry were measured individually to calculate a mean and standard deviation total length while each fry group (fry from one aquarium) was wet weighed to calculate a mean wet weight.

At the termination of the test, the fry from the control and the high concentration (20 µg/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 3). The remaining preserved fry and frozen fry were analyzed at a later date (by 3M Company) for N-EtFOSE Alcohol concentrations.

RESULTS

The biological data generated in this study indicate that hatchability of eggs, and percentage survival, mean total length and mean wet weight of fry were not adversely affected at any N-EtFOSE Alcohol concentration tested.

Remarks:

Water quality parameters measured during the egg and fry exposure exhibited little variation between test days and test chambers. Dissolved oxygen ranged from 8.2-10.2 mg/L. Temperature ranged from 23.5-26.5°C and pH ranged from 6.9-7.5.

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 N-ETFOSE ALCOHOL FOR 30 DAYS POST-HATCH.

Nominal concentration (µg/l)	30 Days				
	Replicate	Hatch %	Survival %	Mean length in mm (±SD)	Mean weight in mg
Control	A	93	82	21(3)	69
	B	92	92	21(2)	71
Solvent Control	A	92	82	20(3)	64
	B	93	90	20(4)	66
1.3	A	90	92	21(3)	65
	B	92	92	21(3)	70
2.5	A	97	90	21(3)	65
	B	95	95	21(2)	64
5.0	A	88	100	20(4)	58
	B	92	92	21(2)	69
10	A	93	92	21(3)	60
	B	92	88	23(2)	79
20	A	92	98	21(2)	64
	B	87	98	21(2)	66

The 1000 ml water samples taken during the study (described in "Analytical Monitoring") were analyzed at 3M Company. Results indicated excellent recovery. See Table 2.

TABLE 2

ANALYSES FOR N-ETFOSE ALCOHOL IN BIONOMICS SAMPLES TO VERIFY RECOVERY CAPABILITIES

Sample I.D.	Theoretical Conc. (ppb)	Determined (ppb)
A5619	Control A	<0.1
A5620	Control B	<0.1
A5621	Solvent Control A	<0.1
A5622	Solvent Control B	<0.1
A5617	1.25	(a)
A5618	1.25	(a)
A5670	1.25	1.1; 1.0(b)
A5670	1.25	1.0; 1.6(b)
A5615	2.5	2.5; 2.6
A5616	2.5	1.2; 1.3
A5613	5	5.2; 5.2
A5614	5	4.8; 5.1
A5611	10	10.2; 9.9
A5612	10	9.7; 10.1
A5609	20	20.8; 19.8
A5610	20	19.8; 20.6

(a) received broken in shipment
(b) replaces original samples A5617; A5618

TABLE 3

RESULTS OF HISTOPATHOLOGICAL EXAMINATION OF FRY OF FATHEAD MINNOW (PIMEPHALES PROMELAS) EXPOSED TO 20 µg/l OF N-ETFOSE ALCOHOL[a]

Test Material	Number of Observations	Histopathological Findings
Control	10	3/10 Normal 6/10 Liver Fatty Change 3/10 Gill Hyperplasia (Epithelium) I
N-EtFOSE Alcohol	10	9/10 Normal 1/10 Gill Hyperplasia

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

The No Effect Concentration (NOEC) of N-EtFOSE alcohol for hatchability, growth, histopathology and survival of Fathead minnow fry is greater than 19.8-20.8 µg/L measured (20 µg/L nominal), the highest concentration tested.

DATA QUALITY

Reliability: Klimisch ranking = 2. This study meets all the criteria for quality testing, but has several deficiencies. It lacks information on purity of the test substance, the production lot number from which the test sample was taken, and was not done at the reported solubility limit for the test substance.

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 78.01 (N-ETFOSE ALCOHOL) ON HATCHABILITY OF EGGS AND GROWTH AND SURVIVAL OF FRY OF FATHEAD MINNOW (*Pimephales promelas*)." Report # BW-78-6-195, 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 78.01 (N-EtFOSE ALCOHOL) FOR 30 DAYS." Report # BW-78-6-195, E G & G, Bionomics, Aquatic Toxicology Laboratory, 790 Main Street, Wareham, Massachusetts, June 1978.

3M Technical Report Summary, "Analytical Methodology and Support", Fate of Fluorochemicals, Project Number 9970612643, Report Number 008, Arthur Mendel, January 17, 1979

OTHER

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

Last changed: 5/18/00

THE EFFECTS OF CONTINUOUS AQUEOUS EXPOSURE TO 78.01 ON HATCHABILITY OF EGGS AND GROWTH AND SURVIVAL OF FRY OF FATHEAD MINNOW (Pimephales promelas).

FM-3422

RESEARCH REPORT

SUBMITTED TO

3M COMPANY

ST. PAUL, MINNESOTA

REPORT #BW-78-6-195

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

ABSTRACT

Fathead minnow (Pimephales promelas) eggs and fry were continuously exposed to nominal 78.01 concentrations ranging from 20 to 1.3 µg/ℓ through 30 days post-hatch. Data were collected as percentage hatch of eggs, and survival, total length, and average wet weight of fry. Results of these data indicate that none of the above parameters were adversely affected by exposure to any of the 78.01 concentrations tested.

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SECTION I

INTRODUCTION

The objective of this study was to determine the effects of 78.01 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 Stephan (1967). Mount and Stephan's term, however, was estimated after the performance of 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. 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 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). The test material used, labelled 78.01, a white powder, was obtained January 23, 1978 from the 3M Company, St. Paul, Minnesota.

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 pumped to concrete reservoir where it was aerated before flowing to the exposure system through aged PVC pipe. The pH of this water ranged from 7.0-7.2; total hardness, 31-38 mg/ℓ as $CaCO_3$; alkalinity, 26-29 mg/ℓ as $CaCO_3$; and specific conductance, 149-170 μmhos/cm.

The diluter delivered five nominal 78.01 concentrations ranging from 20-1.3 μg/ℓ, control water (well water), and control water containing solvent (Dimethylsulfoxide, DMSO) to duplicate test aquaria. A 50 mℓ gas-tight syringe was used to deliver 46 μℓ of a 0.84 mg/mℓ stock solution of 78.01 in DMSO through a stainless steel needle and polyethylene tubing to the mixing chamber of the diluter. A mariotte bottle-volumetric tube

metering device was used to deliver DMSO into the solvent control aquaria at a concentration equal to the highest concentration of DMSO in the 78.01 exposure aquaria (24 mg/ℓ DMSO).

Each glass test aquarium measured 40 x 20 x 25 cm centimeters (cm) with a 19 cm high standpipe drain which maintained a constant test water volume of 15.2 ℓ. The diluter delivered 0.50 ℓ of test water to each aquarium 185 times per day, yielding a 90% test water replacement time of approximately 9 hours (Sprague, 1969). 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°C.

B. EGG AND FRY EXPOSURE

On March 31, 1978, the exposure of fathead minnow eggs to 78.01 was initiated. Eggs used were from the brood stock of the U.S. Environmental Protection Agency's Environmental Research Laboratory in Duluth, Minnesota. Upon arrival at E G & G, Bionomics, the eggs were allowed to acclimate from 17.5°C to the test temperature of 25°C over a period of two hours. Sixty eggs were then randomly distributed to each of 14 egg cups which were then dipped in a 60 mg/ℓ malachite green solution for 15 seconds to prevent fungus growth. One egg cup was then suspended in each of the 14 test aquaria. Egg incubation cups were glass jars (5 cm O.D.,

8 cm high) with 40 mesh Nitex^{R} screen bottoms. An egg cup rocker arm apparatus, as described by Mount (1968), was used to gently oscillate the egg cups in the test water.

Dead eggs were removed and counted daily until hatching was completed. Percentage hatch was calculated based on the number of live fry per aquarium after hatching was completed compared to the number of eggs per aquarium (60) at the initiation of the exposure. To initiate the 30-day fry exposure, forty fry were randomly selected from each egg cup and transferred to the respective aquaria.

Upon transfer of fry to the aquaria, the fry were fed newly hatched San Francisco Bay variety brine shrimp nauplii, ad libitum, three times daily throughout the exposure period. The aquaria were brushed and siphoned to remove excess food and fecal material three times per week. At 30 days post-hatch, the fry from each aquarium were anesthetized with MS-222 (tricaine methane-sulfonate) and percentage survival, mean total length, and mean wet weight were determined. The fry were measured individually to calculate a mean and standard deviation total length while each fry group (fry from one aquarium) was wet weighed to calculate a mean wet weight.

At the termination of the test, the fry from the control and the high concentration (20 µg/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 were sent to the Environmental Pathology Laboratories, Inc., Carolina, Rhode Island for histopathological examination of 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.

Temperature, dissolved oxygen concentrations, and pH were monitored daily, alternating between aquaria such that each aquarium was measured once each week. Temperature was measured with a mercury thermometer, dissolved oxygen with a YSI Model #54 dissolved oxygen meter and probe, while pH was measured with an Instrumentation Laboratory Model #175 portable pH meter and probe.

One thousand mℓ water samples were taken from each aquarium at the initiation of the test (day 0), when hatching was completed (day 4), and weekly thereafter (days 11, 18, 25 and 32) for determination of 78.01 concentration. Samples were taken with a beaker from a point approximately midway between the surface, bottom and sides of each aquarium, and stored in 1000 mℓ amber glass bottles with foil lined caps. All samples taken during the test were stored at room temperature and shipped on May 31, 1978 to the 3M Company, St. Paul, Minnesota.

C. 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 survival and percentage hatch were transformed to arc sin $\sqrt{\text{percentage}}$ prior to analysis.

SECTION III

RESULTS

Water quality parameters measured during the egg and fry exposure exhibited little variation between test days and test chambers. Mean measured dissolved oxygen was 9.0 $\pm$ 0.5 mg/ℓ and ranged from 8.2-10.2 mg/ℓ. Mean temperature was 25 $\pm$ 1°C and ranged from 23.5-26.5°C and pH ranged from 6.9-7.5.

The biological data generated in this study (Table 1) indicate that hatchability of eggs, and percentage survival, mean total length and mean wet weight of fry were not adversely affected at any 78.01 concentration tested.

Based on this datum, the MTC for fathead minnows and 78.01 is estimated to be >20 µg/ℓ based on nominal concentrations.

SECTION IV

REFERENCES

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.

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

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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: 7 pp.

SECTION V

TABLE

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 78.01 for 30 days post-hatch.

Nominal concentration (µg/ℓ)	Replicate	Hatch (%)	30 Days Survival (%)	30 Days Mean length (mm)	30 Days Mean weight (mg)
20	A	92	98	21(2)	64
	B	87	98	21(2)	66
10	A	93	92	21(3)	60
	B	92	88	23(2)	79
5.0	A	88	100	20(4)	58
	B	92	92	21(2)	69
2.5	A	97	90	21(3)	65
	B	95	95	21(2)	64
1.3	A	90	92	21(3)	65
	B	92	92	21(3)	70
control	A	93	82	21(3)	69
	B	92	92	21(2)	71
solvent control	A	92	82	20(3)	64
	B	93	90	20(4)	66

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June, 1978

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SUMMARY OF HISTOPATHOLOGICAL EXAMINATIONS OF FATHEAD MINNOW (Pimephales promelas) EXPOSED TO 78.01 FOR 30 DAYS.

FM-3422

RESEARCH REPORT

SUBMITTED TO 3M COMPANY

ST. PAUL, MINNESOTA

REPORT #BW-78-9-300

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

INTRODUCTION

An "egg and fry test" was conducted between March 31 and May 4, 1978 (Bionomics Report #BW-78-6-195, June, 1978) to assess the sub-lethal effects of 78.01 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 78.01, 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 78.01 concentration (20 ug/ℓ, nominal) were preserved in 10% buffered formalin and sent to the Environmental Pathology Laboratories, Inc. Carolina, Rhode Island. Control fish from a similar ongoing study were also examined.

The fish were examined for gross lesions, and sagital sections were prepared by a pathologist. Fish were placed with identifying 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 78.03 concentration of 100 mg/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 20 ug/ℓ nominal 78.01 and control water.

Fish #	Sex	Organ Condition	Missing Tissue
20 ug/ℓ 78.01			
1	F[a]	Normal	Ear, thymus
2	Ukn[b]	Normal	Spleen, gonad, nares
3	Ukn	Normal	Spleen, gonad, thymus
4	Ukn	Normal	Spleen, heart, gonad, nares
5	F	Normal	Spleen, nares
6	F	Normal	Spleen, nares, thymus
7	F	Normal	Spleen, heart
8	F	Normal	Spleen, heart, nares
9	F	Normal	Spleen
10	F	Gill Hyperplasia (epithelium) I[c]	Spleen, heart, thymus
Control			
1	Ukn	Liver-fatty change I Gill-hyperplasia (epithilium) I	Gonad, nares, thymus
2	Ukn	Liver-fatty change I	Spleen, gonad, nares
3	F	Gill-hyperplasia (epithilium) I	Thymus
4	F	Normal	Spleen, nares

Table 1 (cont.)

Fish #	Sex	Organ Condition	Missing Tissue
Control			
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

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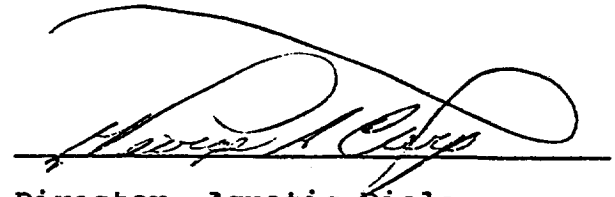

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