U. S. ENVIRONMENTAL PROTECTION AGENCY
NATIONAL WATER QUALITY LABORATORY
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DULUTH, MN 55804

ANNUAL REPORT
1973

ASSOCIATE LABORATORY OF
NERC-Corvallis, OR

Office of Research & Monitoring

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FOREWORD

This is the first annual report prepared by the National Water Quality Laboratory. We have tried to provide an overview of the activities and highlights of the findings of the various studies so that those in the scientific community can learn of our present work and contact staff working on projects of especial interest. All data should be treated as tentative and subject to minor change. We decided that with this provision we could include much more very recently completed work.

Three events in 1973 made this a significant year to us. In June we announced that part of the mineral we had been using to trace tailings in Lake Superior (and in Duluth drinking water) was asbestos and we alerted EPA that there might be serious health concerns. At the time of this writing, the trial is nearly complete and the U. S. District Court has said that in its judgment there is primafacie evidence of a "significant health hazard". Phil Cook, Gary Glass, and Jim Tucker deserve special mention for thise findings.

The second event was the official opening of our Monticello Field Station located at Monticello, Minnesota. At this facility we have eight 1,700 foot long channels in which self-sustaining fish populations will be maintained. Heat can be added to provide an initial Δt of up to 20° C. In this facility we will be able to simulate, on a rather large experimental scale, the effects of a thermal plume in a river and determine its ecological consequences. We also plan use of toxic materials in future research after the initial work with heat. We feel that only after validating in such ways can models of biologic response be considered established.

Finally, the new permanent laboratory building for our Western Fish Toxicology Field Station in Corvallis, Oregon, was ready for occupancy. For the first time in about six years, the staff has a permanent home! Work there is concentrated on problems important in the Pacific Northwest, especially those related to the salmon fishery. Both for the dogged persistence in seeing the building to completion and for the development of the only adult salmon bioassay facility, the staff of Western Fish Toxicology Station deserves special mention.

We would welcome your suggestions on this report, and we encourage you to inquire if you desire further information on any project. We also hope that you will let us know if you do not receive our quarterly newsletter, or if you wish to receive reprints of published work.

Donald I. Mount, Ph.D.
Director

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In 1971 the Office of Research and Development of the Environmental Protection Agency initiated a procedure for research planning. This procedure uses an Environmental Research Objective Statement (EROS) prepared by staff members at Headquarters to relate what research should be performed and the available resources to perform that research. Upon receipt of the EROS by the laboratory, Research Objective Achievement Plans (ROAP) are prepared utilizing the resources as defined in the EROS to plan research programs which will satisfy the objectives as outlined in the EROS.

This report will be divided into the research, both intramural and extramural, performed by the staff to meet the stated objectives of these ROAP's.

ROAP 16AAD - Effects of Heavy Metals Upon Freshwater Aquatic Life

I. An approach to the trace metal analysis of environmental samples utilizing flameless atomic absorption spectroscopy with the graphite tube furnace was outlined in a preliminary NWQL report (December 1973). The method has several advantages, but because it is a relatively new and unproven technique, there can be certain associated dangers and difficulties with respect to the number it produces. The report focuses on some possible problems, but more important it attempts to outline a practical systematic approach which is of aid to NWQL analysts involved in developing methods. The proposed approach consists of three general steps namely, (1) necessary measures to obtain reproducible atomization; (2) varying operating conditions for the purpose of optimization and diagnosing possible problems; and (3) establishing validity, showing agreement of analyses by the flameless technique to those obtained by another established method and analyzing appropriate standard samples. In the report, each of these considerations is expanded to the extent of suggesting specific steps which are oriented toward Perkin Elmer instrumentation. Such considerations as proper charring temperature, atomization temperature, program times, preparation of standard sample alteration techniques and use of background correction are covered. At NWQL the method has been employed to effectively measure

cadmium, lead, copper, managanese, and iron in various samples, which include Lake Superior interstitial waters and overlying waters, bioassay systems and sewage effluent. Comparisons of results to those obtained by flame atomic absorption, anodic stripping voltammetry and/or standard colorimetric methods have been quite favorable.

A minor amount of work has also been done with other metals such as chromium, cobalt, nickel, arsenic, selenium, molybdenum, and silver. (John E. Poldoski and James T. Fiandt).

A method has been developed for preparing mercury film electrodes for use in trace metal analysis by anodic stripping voltammetry. This helped to fulfill NWQL needs for ultrasensitive analytical methods in monitoring cadmium, lead, and copper. Briefly, the preparation scheme consists of plating mercury onto an appropriately prepared surface of wax impregnated graphite rod. The basic electrode construction is accomplished by machining the graphite rod to fit into a tubular teflon electrode body. The overall electrode dimensions are roughly 6mm in diameter by 90 mm long.

In normal use the electrode requires periodic reconditioning, which is easily and reproducibly accomplished in about 15-20 minutes. Typical reproducibility of peak currents resulting from several scans of a single solution containing 10 µg/l each of cadmium, lead, and copper is about 1-2 percent. The sensitivity is characterized by the fact that a five minute plating time is sufficient to determine cadmium, lead, and copper in acidified Lake Superior water, which typically measures 0.02 µg/l, 0.1 µg/l, and 0.5 µg/l, respectively. Measurements made on other sample types such as bioassay waters, well waters, fish tissue, and sewage effluent agree well with the corresponding measurements made by atomic absorption. The bioassay water was monitored routinely over a period of several months. The main advantages compared to other available film electrodes appears to be the ease and reliability of electrode reconditioning, the ability to analyze in highly acidic media (pH = 1), and the high sensitivity required to analyze metal ions in the sub-parts µg/l range. (John E. Poldoski and Edward N. Leonard).

II. The determination of the relative susceptibility of anadromous salmonids to metals is being studied. Swim-up stage (ca. 1 month-old) chinook salmon (Oncorhynchus tshawytscha) were determined to be considerable more susceptible to the lethal effects of cadmium, copper, and zinc than were the newly hatched fry. Data obtained to date are summarized in the following table:

96-hr. LC50 values

		At Hatch	Swim-up	4 mo.-old
Chinook Salmon (O. tschawytscha)	Cd	> 25 µg/l	1.9 µg/l	
	Cu	31 µg/l	18 µg/l	
	Zn	> 700 µg/l	103 µg/l	
Steelhead Trout (Salmo gairdneri)	Cd			0.95 µg/l
	Cu			20 µg/l
	Zn			100 µg/l

A 21-month exposure of sockeye salmon to zinc was terminated. Adult sockeye salmon (O. nerka) were held in zinc for 3 months prior to artificial spawning; the subsequent egg-to-smolt zinc exposure continued for an additional 18 months. No adverse effects were observed at the highest concentrations tested (120 ppb adults through smolts and 240 ppb eggs through smolts). Smolts from all zinc concentrations appeared normal as evidenced by survival at 30 ppt seawater for 96 hours. (A zinc concentration of 240 µg/l is about 1/4 of the 96-hour LC50 for sockeye salmon, but is much higher than the 100 µg/l 96-hour LC50 for the more susceptible chinook salmon and the steelhead trout.)

Because results reported by the Western Fish Toxicology Station indicate very low levels of cadmium, copper, and zinc to be lethal to salmonids, quarterly water samples were collected and analyzed from most major salmonid streams in Western Oregon. The purpose of this program was three-fold: 1) to determine if levels of these metals occurred which were near or above those concentrations reported as lethal (i.e. Cd, 1 µg/l; Cu, 20 µg/l; Zn, 100 µg/l). Existence of such levels would either indicate an area of problem pollution or possibly an unexplained research finding; 2) to check the general acceptability of existing STORET water quality data for these streams; and 3) to collect heavy metal data for these streams using more sensitive analytical methods than those available in the past.

The results of the survey are summarized briefly in the following table:

Summary of 6 water quality parameters from Western Oregon streams

Alkalinity (mg/1 $CaCO_3$)		10-30	31-60	61-100
No. Occurrences		91	20	3
Hardness (mb/1 $CaCO_3$)		10-30	31-60	61-100
No. Occurrences		85	23	3
pH	6.5-7.0	7.01-7.5	7.51-8.0	8.01-8.5
No. Occurrences	47	58	12	3
Cadmium (ppb)		<0.01	0.01-0.1	0.11-0.22
No. Occurrences		81	18	7
Copper (ppb)		<0.1	0.1-1.0	1.1-5.3
No. Occurrences		65	15	28
Zinc (ppb)		<0.1	0.1-1.0	1.1-11
No. Occurrences		35	35	40

On the basis of these data it was concluded that: 1) barring exceptional circumstances, lethal or near-lethal levels of cadmium, copper, or zinc probably do not occur in Western Oregon streams; 2) existing STORET water quality data are generally adequate, although data are included which in most cases should be regarded as aberrant due to sample contamination or analytical or mathematical errors; and 3) a typical Western Oregon stream has alkalinity and hardness below 30 mg/1 as $CaCO_3$, a near neutral pH, no detectable cadmium or copper, and less than 10 µg/1 of zinc.

A grant to the Oregon State Game Commission was awarded in October 1973 for the study of the Effects of Copper and Zinc on the Seawater Adaptation of Juvenile Coho Salmon. The results of this research will be a major first step in determining if this critical life history phase is susceptible to adverse sub-lethal effects of toxic chemicals. In addition, the study will provide specific data on the effects of copper and zinc, as well as develop and test an array of criteria for testing toxic effects from the level of the whole organism to sub-cellular levels. (Gary A. Chapman).

III. The effects of cadmium and lead on brook trout (Salvelinus fontinalis) was studied. The purpose was to determine the maximum acceptable toxicant concentration (MATC) for brook trout exposed to cadmium and lead in Lake Superior water. Survival, growth, and reproduction were used to measure adverse toxic effects upon the fish. The tests were also designed to evaluate the use of cadmium and lead application factors for brook trout. Both tests involve the exposure of first generation trout (juvenile through adults) and the offspring or second generation trout (egg through maturity). Nominal water concentrations ranged from 6.0 to 0.4 µg Cd/liter and 500 to 31 µg Pb/liter. Analysis of each test showed that approximately 80% of the total cadmium and 70% of the total lead was available as dissolved cadmium and lead (Cd, Pb passing through a 0.10 µ filter). Anodic stripping voltammetry (ASV) was used as a comparison with atomic absorption on some samples (10); the methods were found in good agreement.

Several attempts to calculate a 96-hour TL50 for brook trout and cadmium failed because the fish always died at approximately the same rate in all concentrations tested (400 to 12 µg Cd/liter) and this produced an essentially flat mortality-concentration curve.

Two months after the first generation exposure of brook trout to cadmium was initiated all trout at 6 µg Cd/liter began to experience periods of severe hypertensive activity in which the fish would frantically swim around the tank with totally uncontrollable movements. All fish at this concentration died prior to spawning. During the spawning period all males and several females at 3 µg Cd/liter also became very hyperactive and died. Males seemed most sensitive to cadmium during courting activity, especially when the females began digging in the gravel substrates. Adult survival, spawning, egg hatchability, and 90-day second generation alevin survival and growth were unaffected at 1.5 µg Cd/liter and below. The second generation exposure was continued beyond the 90-day period in order to study second generation effects on adult survival and reproduction. Results of this exposure are not yet available.

The 96-hour TL50 for brook trout to lead was 4.5 mg Pb/liter.

During the first generation exposure of brook trout to lead, most trout at 500 µg Pb/liter became somewhat hypertensive and some were subject to periodic involuntary muscle spasms. Viable eggs spawned, egg hatchability and 90-day survival of second generation offspring were also affected at 500 µg Pb/liter. Significant numbers of second generation alevins with deformed spines (scoliosis) were noted at hatch in 250 and 500 µg Pb/liter. Three to four

months after hatch the caudal peduncle of normal looking alevins at 250 and 500 µg Pb/liter began to turn black and each fish eventually suffered from severe scoliosis. Six months after hatch, black tails and spinal deformities also began occurring to a lesser extent in second generation trout exposed to 125 µg Pb/liter. The second generation exposure is currently being continued in order to study the effects on reproduction. Complete results of this exposure are not yet available. (Duane A. Benoit and Edward N. Leonard)

IV. The acute and chronic toxicity of methylmercuric chloride to brook trout (Salvelinus fontinalis) was determined. Nominal water concentrations were 3.0, 1.0, 0.3, 0.1, 0.03µg/liter mercury and controls. All measured concentrations were slightly less than nominal concentrations. Water analyses indicated that 90-95% of the mercury in the test water was organic mercury.

Two 96-hour acute toxicity tests were run--one on yearling brook trout (200 grams each) and one on five-month old juvenile brook trout (12.0 grams each). The calculated TL50's were 65.0 µg/l for the 200 gram trout, and 84 µg/l for the 12 gram trout.

A brook trout partial life-cycle chronic with methylmercuric chloride was completed in March 1972. The offspring of these trout were carried on, under exposure, to investigate second generation effects. The second generation study will terminate in March 1974. In the first generation study (partial chronic), all trout exposed to 3.0 µg/l of mercury died prior to spawning. Spawning was good at all other mercury concentrations except 1.0 µg/l where egg mortality, fry survival and growth were affected. Second generation data on spawning is not yet available. These preliminary first generation exposure data suggest that 1.0 µg/l mercury is definitely unsafe for brook trout. (James M. McKim)

V. Acute and chronic tests to determine the effects of cadmium on growth, reproduction, and survival of Jordanella floridae, (flagfish) were completed

Fourteen-and 30-day acute tests were run. Median tolerance limits (TL50) were similar for adult fish and one-day old fry. Values ranged from 18.4 to 25.5 µg Cd/l.

Two chronic tests approximately 16-weeks in length were completed to determine effects on growth and reproduction. The second test was done at higher concentrations to obtain effects not apparent in the first chronic test. All significant effects noted

were calculated by using an analysis of variance and Dunnetts' Test, (P = .05). Concentrations in Test 1 ranged from 6.6 µg/l to 0.06 µg/l with a .5 reduction between concentrations. There was no effect at any level on growth or reproduction. Only the number of spawnings was significantly different from control at 6 µg/l.

Cadmium concentrations in chronic Test 2 ranged from 31 µg/l (high) to 0.11 µg/l (control). At these concentrations there was no significant effect on growth in initial 30-day tests and 30-day second generation tests at concentrations less than 31 µg/l. At 31 µg/l there were no eggs produced and thus no second generation. Growth of adult fish was significantly (P = .05) decreased at 16 and 31 µg/l. All males at these levels died and females showed a marked decrease in length and weight. Both males and females also became highly convulsive for short periods of time at these concentrations.

Reproduction studies were based on spawning results, eggs produced, and hatchability. There were significant effects on all aspects of spawning and egg production at concentrations of 8.1 µg/l and above. There was a thirty percent reduction in the total number of eggs produced at 8.1 µg/l. The two higher levels, however, severely affected growth and caused mortalities leading to significant decreases in spawning or no spawning at all. Fish in the two high concentrations showed more convulsive activity when they began spawning indicating an additional stress especially in the males.

There were no detrimental effects on hatchability at any cadmium concentration. There was, however, an increase in hatchability at higher concentrations. The highest hatchability was found when control eggs were transferred to the high concentration for incubation. Percentages ranged from 65 percent in the control to 83.1 percent for eggs transferred to the high concentration (31.1 µg/l). Since a concentration of 8 µg/l affected reproductive activity in chronic test 2, an effect at 6 µg/l in chronic Test 1 would indicate the lowest possible effect level of cadmium to this species. However, only the number of spawnings were affected, not egg production in general. (Robert L. Spehar)

VI. A method was developed to measure total mercury in water at concentrations below 0.2 µg/l. During the past 3 years total mercury has been routinely measured in experimental waters containing less than 0.2 µg of Hg/liter, which is the lowest limit for many analytical laboratories. To achieve detection limits of 0.012-0.020 µg/liter.r, chemicals are used from those manufacturers which yield the lowest amount of mercury contamination. A sample of 150 ml is

analyzed in a 250 ml flat bottom boiling flask, which has been cleaned with hot nitric acid. To the sample is added 2 ml concentrated HNO_3, 2 ml concentrated H_2SO_4, 1 ml 6% $KMnO_4$, and 2 ml 5% $K_2S_2O_8$, This mixture is heated just to boiling, cooled to room temperature and then reduced to the elemental mercury with 1 ml sodium chloride-hydroxylamine sulfate solution and 5 ml stannous sulfate. By aerating the solution using a Neptune Dyna-Pump, the mercury vapors are released into a closed circulating system for measurement of the concentration in the sample. The vapor passes through a magnesium perchlorate drying tube, into an absorption cell of 2.0 cm O.D. X 18 cm. which is aligned in the light beam of a Perkin-Elmer 403 Atomic Absorption Spectrophotometer and continues to circulate until a maximum absorbance value is reached. The absorbance values are expanded 10 times by the concentration mode on the instrument. A minimal of 1/16 inch I.D. tygon tubing is used for all the connections in the apparatus.

Using this procedure to analyze water samples from a fathead minnow bioassay with methyl mercuric chloride, the following table summarizes the analyses for 37 weeks.

Nominal Concentration as µg Hg/l in Test Water	Measured Concentration µg Hg/l	
	Mean	95% Confidence Interval
0.015	0.018	.015 - .021
0.030	0.036	.031 - .041
0.060	0.063	.055 - .071
0.120	0.114	.102 - .126
0.240	0.247	.225 - .269

Mercury analyses at these concentrations in natural waters are important because there is significant uptake by aquatic organism, especially fish. The total body residues of the fish from this exposure ranged from 1.5 µg of Hg/gram in the 0.018 µg/liter concentration to 10.9 µg/gram in the fish from the 0.247 µg/liter. The control fish from water less than 0.01 µg/liter contained only 0.2 µg/gram total body residues. (Gayle F. Olson)

VII. A chronic bioassay with fathead minnows exposed to mercuric chloride has been running for about 8 months. The nominal concentrations are 12.0, 6.0, 3.0, 1.5, 0.75, and 0 µg Hg/liter. All fish were dead in the 12.0 µg/l concentration after 8 days. Only 4 stunted and deformed fish survived at 6.0 µg/liter.

The incidence of deformities appears to increase with concentration among the adult fish. Thirty and sixty day growth and survival effects have not been completed on the hatched fry. After termination of exposure, residue analyses will be run on the adult fish for total and methyl mercury (Gayle F. Olson)

Two chronic tests with Jordanella and methyl mercury were completed. In the first test, concentrations tested were .6, 1.25, 2.5, 6.0, 15.0 µg/1 Hg and controls. At the completion of the test all fish were dead at concentrations of 2.5 µg/1 and above. There was 70% mortality at 1.25 µg/1 and 20% mortality at .6 µg/1. There was no spawning at 2.5 µg/1 and a nearly 50% reduction of spawnings at the low concentration of .6 µg/1.

In the second test concentrations tested were 1.5, .75, .375, .187 µg/1 Hg, and controls. At 16 weeks there was a 70% mortality at 1.5 µg/1 and 25% mortality at .75 µg/1. Spawning was inhibited at a concentration of .375 µg/1 and above and no spawning occurred at 1.5 µg/1. In a 30-day growth study with fry at these concentrations, all levels of Hg, including the low concentration of .187 ppb, inhibited growth. Adult fish at 16 weeks from the same concentrations were found to be of normal length and weight at the three low concentrations tested.

Jordanella exposed to Hg and containing Hg in their tissues were placed in clean water in a continuous flow system. The fish were sampled monthly for tissue analysis. At six months there was no appreciable loss of Hg from tissues.

A mercury uptake study designed to show the relative significance of Hg uptake by fish from food as compared to that from water using bluegills was completed. The Hg test water contained .25 µg/1 Hg. The food source was clam tissue containing 2.62 mg/kg Hg. Ten fish were maintained for 30 days under each of four test conditions, the fish tissues were then analyzed for Hg. In clean water and receiving clean food the fish tissue contained 0.32 mg/kg Hg. In clean water and receiving Hg food the fish contained 2.9 mg/kg Hg. In Hg water with clean food the fish contained 1.6 mg/kg Hg. In Hg water with Hg food the fish contained 4.4 mg/kg Hg. A concentration of .187 ppb would permit maintenance of normal populations of Jordanella and .375 ppb would have a definite inhibitory effect. (Wesley E. Smith)

VIII. Bionomics, Inc., Wareham, Massachusetts, was awarded a contract to determine the maximum acceptable toxicant concentrations of lead ($Pb(NO_3)_2$), cadmium ($CdCL_2$), chromium (Na_2CrO_7), and copper ($CuSO_4$) on the egg or fry of nine species of fish exposed for 60 days post-hatch.

Exposures of lake and rainbow trout to lead in soft water have been completed. Based on 60-day fry survival, the estimated MATC for rainbow trout eggs and fry in soft water lies between 61 and 131 µg Pb/liter. Whole body lead residues were dose related and ranged up to 57 mg/kg in the rainbow trout after 60 days exposure to 27 µg Pb/liter and 56 mg/kg in the lake trout after 60 days exposure to 22 µg Pb/liter.

Channel catfish exposures in both hard and soft water have also been recently completed and all six tests indicated a safe and unsafe level; however, the data have not been finalized and are not yet available. (Duane A. Benoit)

IX. In toxicity tests with the "heavy" or transition metals in various natural waters, gross differences in toxicity are observed due to the formation of both soluble and insoluble hydroxide, carbonate, phosphate, and other complexes. In addition, toxicity is moderated by the physiological effects of calcium, magnesium, sodium and changes in pH in such waters. Although variations in metal toxicity have long been recognized as generally related to water quality, few concerted efforts have been made to determine the chemical relationships causing such differences. Attempts were made through acute toxicity tests of copper to Daphnia magna to elucidate the chemical-toxicity relationships that may be applicable to many of the heavy metals in natural waters. The overall objectives were to determine the chemical factors regulating toxicity and the presence of toxic aqueous forms of the metal.

The effects investigated were primarily the effects of the naturally occurring anions: hydroxide, carbonate, bicarbonate, chloride, sulfate, and orthophosphate on ionic copper distributions and resultant toxicity. Tests included varying concentrations of pyrophosphate as a means of determining the mathematical relationship between complex formation and toxicity. Comparative tests with varying sodium, potassium, and pH were also conducted, since experimentally the anions above are added as the appropriate sodium, potassium or acidic form.

Results of analytical measurements and chemical equilibrium calculations indicate that only a small percentage of the copper added even to very soft water (Lake Superior total alkalinity $\tilde{\sim}$ 45 mg/l) remains as the cupric (Cu^{++}) form at moderate pH's. The proportion of the cupric form present decreases markedly with increasing pH and/or carbonate alkalinity, and decreases in proportion to the concentration of added complexing agents and the stability constant of the copper complexes formed.

The principal result of the toxicity tests was the finding that toxicity at a given pH is linearly related to the concentrations of the soluble cationic copper forms (Cu^{++})and $CuOH^{+}$). Additional toxicity tests in waters with widely varying carbonate alkalinities have shown that copper toxicity is inversely related to the total carbonate ($CO_3^{=} + HCO_3^{-}$) concentration, and the degree of copper carbonate formation. All anions tested to date, including chloride, sulfate, orthophosphate, pyrophosphate, carbonate, and bicarbonate reduce cationic copper concentrations (and toxicity) in proportion to the stability of the cupric complex formed. Cupric ion concentrations may be calculated directly from published stability constants and measurements of pH, carbonate alkalinity, etc., and copper toxicity is predictable under conditions of fixed pH, sodium, and calcium concentrations.

The second major finding of the toxicity tests was the indication that toxicity of the cupric ion varied as a logarithmic function of both pH and log sodium (pNa) concentrations (i.e., the slope of toxicity curves varies as a combined function of pH and pNa). Cupric ion toxicity increases approximately tenfold between pH 6.0 and pH 8.0 at low sodium levels, and decreases approximately tenfold with an increase in sodium concentration from 10^{-4} M to 10^{-2} M at pH 8. The effects of pH and sodium are interdependent however, with sodium having less effect at pH 6, and pH having less effect at high sodium concentrations.

The relative toxicity of the cupric ion at various pH's and in the presence of varying sodium appears to be entirely dependent on physiological mechanisms and it is expected that a similar interdependent effect of calcium will be observed. The question of the relative toxicity of the cupric and copper hydoxy ions cannot be answered at the present time, since concentrations of these ions cannot be varied independently of pH. Since interchange of these two ions at the site of toxic action involves only a single proton,

$$CuOH^{+} + H^{+} \rightleftarrows Cu^{++} + H_2O$$

they are probably about equally toxic. (Robert W. Andrew)

ROAP 16AAK - Effects of Selected Hazardous Synthetic Organics in the Freshwater Environment

I. A study was conducted to determine the relationship between the toxicity of DDT to fathead minnows when present in water, in food, and in water plus food. Fish were exposed to each of these conditions for 266 days starting with 45-day old fry through a reproductive period of their life. The contribution of DDT from each source was monitored by tissue residue analysis through both gas chromatographic and liquid scintillation techniques. Two DDT water

concentrations (2.0 µg/l and .5 µg/l) and one DDT concentration for the food (50 µg/g) were used. The food consisted of clams, half of them exposed to C^{14} labeled DDT at the same water concentration as the high DDT fathead water exposure, and the other half were used as a "clean" food containing no DDT.

Residue data agree closely with mortality data, the results indicating higher tissue residues with slightly greater mortality for all fish exposed to DDT in water plus contaiminated food when compared to fish in corresponding DDT water exposures alone. Mortality and residues among fish only fed DDT w re intermediate between those of the groups fed and not fed DDT in the low water concentration. Statistical analysis of the mortality data shows that the presence of DDT in food caused a significant reduction in the probability of survival. Two separate mortality periods occurred, indicating increased susceptibility to DDT at both the fry stage up to 80 days of age, and at spawning time when highly colored males were most susceptible. The percentage of total tissue residues attributable to the DDT food source remained relatively constant after 28 days exposure at about 35% for fish exposed to 2.0 µg/l DDT water plus DDT food, and 60% for fish exposed to 0.5 µg/l DDT in water plus food.

There was no survival of fry at 2.0 µg/l DDT in the water both with and without food containing DDT. All fry died within 5 days of hatch. Groups of fry (40 each) spawned from adult fathead minnows exposed to 2.0 µg/l DDT in the water and 2.0 µg/l DDT in the water plus DDT in the food were transferred to control water for 30 days. Fry from the adults exposed to DDT in both food and water experienced about two times greater mortality than those from adults exposed to DDT in the water only. These data agree with egg residue results that indicate almost two times greater residue levels (40.9 vs 24.0 µg/g) for eggs from adults exposed to 2.0 µg/l DDT in the water plus DDT in the food as compared to those of adults exposed to DDT in the water alone.

Elimination rates were determined for fish exposed to 0.5 µg/l DDT in water and 0.5 µg/l DDT in water plus DDT in food. Initial DDT residue levels were 25.0 and 70.0 µg/g respectively. Essentially, there was zero DDT elimination for the 0.5 µg/l DDT water exposed fish up to 56 days, whereas the fish exposed to 0.5 µg/l DDT in water plus DDT in food had a rapid elimination within the first 28 days followed by a slower elimination. At 56 days, greater than 50% of the total tissue residues were lost and the body burden was equal to that of the non-DDT in food exposed fish. (Alfred W. Jarvinen)

II. Diazinon is an organophosphorus insecticide first introduced in 1952. Initial use was limited but has increased greatly in recent years. It has been recommended as an alternative to several organochlorine insecticides for application to agricultural crops. Although it is considered a "non persistent" insecticide, recent studies have indicated that it may have a half life of several months in water under some conditions. Throughout 1972 and 1973 a number of flow-through bioassays were conducted to determine the effects of diazinon on fishes. Tests in the coming year will begin to establish the relationship between these constant concentration diazinon exposures and long-term exposure to fluctuating concentrations.

Fathead minnows, Pimephales promelas Rafinesque, were exposed to diazinon from hatch through spawning and the progeny for two months. The most pronounced chronic effect on this species was the incidence of spinal deformity (scoliosis and/or lordosis). Percentages of surviving minnows crippled after four months of exposure at various measured concentrations are as follows: 1099 µg/l (87%), 511 µg/l (83%)k,229µg/l (89%), 119 µg/l (89%), 59 µg/l (67%), 27 µg/l (44%), 14 µg/l (40%), 6.8 µg/l (26%), 3.2 µg/l (29%), Control (15%). When disturbed many fish had tetanic convulsions in concentrations as low as 14 µg/l. There was no reproduction among fathead minnows exposed to concentrations of 14 µg/l or higher. Survival of eggs to hatch was reduced in the lowest concentration tested (3.2 µg/l). From acute tests, a 96-hr LC50 of 7.0 mg/l was estimated.

Brook trout, Salvelinus fontinalis (Mitchill) were exposed for six months prior to and during spawning, and progeny for two months as eggs and three months after hatch. After six months adult fish exposed to 10 µg/l and 5 µg/l had an incidence of spinal deformity (scoliosis and/or lordosis) of 33% and 12%, respectively. Mortality was 25% at 10 µg/l and 4% at both 5 µg/l and 2.5 µg/l. During the first three months fish in 10 µg/l lost 4% in weight, those in 5 µg/l gained 11% and the others in lower concentrations and the diazonon-free control gained 40% to 50%. During the subsequent three months, fish exposed to 10 µg/l gained only 32% in weight compared to 47% to 58% for fish in lower concentrations. Data are not yet available for the possible effects on reproduction. Acute tests provided an estimated 96-hr LC50 of 0.6 mg/l.

The estimated 96-hr LC50 for Florida flagfish, Jordanella floridae (Goode and Bean) is 1.5 mg/l. For bluegills, Lepomis macrochirus Rafinesque the estimated 96-hr LC50 is 0.5 mg/l. (Donald T. Allison)

III. The toxic effects of captan on survival, growth, and reproduction of fathead minnows (Pimephales promelas) and on the survival of bluegills (Lepomis macrochirus) and brook trout (Salvelinus fontinalis) were determined in a flow-through system. In a 45-week exposure of fathead minnows, survival and growth were adversely affected at 39.5 µg/1. Adverse effects on spawning were suspected but not statistically demonstrated at 39.5 and 16.5 µg/1. The maximum acceptable toxicant concentration (MATC), based on survival and growth, lies between 39.5 and 16.5 µg/1. The lethal threshold concentration (LTC) derived from acute exposures was 64 µg/liter, resulting in an application factor (MATC/LTC) between 0.26 and 0.62. LTC values for the bluegill and brook trout were 72 and 29 µg/liter, respectively. The estimated MATC is between 44.6 and 18.7 µg/liter for the bluegill and between 18.0 and 7.5 µg/liter for the brook trout. The half-life of captan in Lake Superior water with a pH of 7.6 is about 7 hours at 12° C. and about 1 hour at 25° C. Breakdown products from an initial 550 µg/liter of captan were not lethal to 3-month-old fathead minnows.

Acute toxicity tests have been completed in a study of the acute and chronic toxicity of endrin and malathion mixtures to flagfish (Jordanella floridae). The data hasn't been completely analyzed, but it appears that the acutely toxic effects of the mixtures are additive. Tests are in progress to determine if the chronic effects are additive. (Roger O. Hermanutz)

IV. A research contract with Union Carbide Corp., under Miss Anne Spacie as principal investigator, is now nearing completion. The following preliminary results have been obtained from chronic and acute exposures of three fish and three invertebrate species to parathion.

Adult bluegills were crippled (scoliosis) or developed tumors in the throat area after several months exposure to 0.34 µg/1, and accumulated 30 to 60 times this amount in their tissues. In a test still in progress, fathead minnow growth was reduced at 3.2 µg/1, and crippling occurred at 15 µg/1. Brook trout were less sensitive, being unaffected after several months exposure to 470 µg/1, although egg hatchability was reduced at 32 µg/1. Surprisingly, the trout accumulated 200 times this amount in blood and 400 times this amount in their muscle tissue. Ninety-six hr. TL50 values for the three species were 0.51 mg/1 for bluegills, 2.5 mg/1 for fatheads, and 1.8 mg/1 for brook trout.

Only TL50 data are available for the invertebrates, and some of these are reported below:

Daphnia	4 day	0.63	µg/l parathion
	7 day	0.4	µg/l parathion
	3 wk.	0.08	µg/l parathion
Gammarus	4 day	0.4	µg/l parathion
Midge	4 day	31	µg/l parathion
	7 day	3.1	µg/l parathion

(John G. Eaton)

V. The atrazine and lindane research contracts with Bionomics, Inc. under the direction of Dr. Ken Macek are also nearing completion. These contracts, like the one with parathion, involve exposure of three fishes and three invertebrates in order to determine acute and chronic toxicity.

Considering atrazine first, there was no observable adverse effects of 0.25 mg/l on fathead minnow adults after several months or on their progeny after 30 days. The acute TL50 concentration was 0.83 mg/l. Bluegills were unaffected by chronic exposure to 0.25 mg/l atrazine but suffered 15% mortality acutely at 0.36 mg/l. Brook trout experienced reductions in growth of adults and fry at concentrations of 0.25 mg/l and above. The atrazine median tolerance limit of brook trout was 4.9 mg/l, so application factors for fatheads and bluegills would appear to be similar to one another but different from the one for trout. Among the invertebrates, the chronic safe concentration of atrazine for the midge was 0.12 mg/l with adult emergence being decreased at 0.25 mg/l. Fecundity was reduced in Daphnia at 0.3 mg/l and in Gammarus at 0.14 mg/l. Acute TL50's were 6.9 mg/l for Daphnia magna (96 hr), 5.7 mg/l for Gammarus (48 hr), and 0.72 mg/l for midges (48 hr).

On the lindane contract, 25 µg/l was found to be safe chronically for bluegills, 49 µg/l for fatheads, and 16 µg/l for brook trout. Acute TL50's for the three were found to be 30 µg/l, 54.5 µg/l, and 25.8 µg/l indicating application factors are similar for all three species. For the invertebrates when exposed to lindane, the lowest chronic effect levels were 6.2 µg/l for the midge, 25 µg/l for Daphnia magna, and 2.2 µg/l for Gammarus. Forty-eight hour TL50 concentrations for the three invertebrates were 1.6 mg/l, 48 µg/l, and 31 µg/l, respectively. (John G. Eaton)

VI. A contract with Envirogenics Co., El Monte, California, Rick D. Cardwell as principal investigator, is also desinged to investigate the acute and chronic toxicity of chlordane to fathead minnows, bluegills, brook trout and three aquatic invertebrates (Daphnia magna, Gammarus sp., and a midge). Acute and chronic tests of the fish are almost completed and testing of the invertebrates has just begun.

Estimated 96-hr LC50's for fathead minnow, bluegill, and brook trout are 40, 65, and 100 µg/l, respectively. Chronic mortality of fathead minnows exposed from the larval stage was not greatly affected by chlordane concentrations ranging from 6 µg/l to zero until the fish began to spawn. Subsequently, mortality was high in concentrations of 1.4 µg/l and above. Mortality of adult brook trout during an exposure lasting six months prior to spawning was as follows: 6.7 µg/l (100%), 3.3 µg/l (50%), 1.7 µg/l (21%), 0.8 µg/l (12%), 0.4 µg/l (12%), control (4%). Control trout were transferred to the 6.7 µg/l concentration at the beginning of the spawning period. To date, after two weeks of spawning activity in the lower concentrations, no fish have spawned in chlordane levels of 6.7 or 3.3 µg/l. In the partial chronic using adult bluegills the only excessive mortality prior to spawning occurred in the highest concentration (6.7 µg/l) where 45% of the fish died. However, during spawning bluegill mortality was as follows: 6.7 µg/l (30%), 3.3 µg/l (15%), 1.7 µg/l (5%), 0.8 µg/l (5%), 0.4 µg/l (0%), control (0%). (Donald T. Allison)

VII. Another contract with Bionomics, Inc. is designed to provide information on the chronic and acute effects of treflan, heptachlor, endosulfan, and acrolein on fathead minnows and Daphnia magna. The fathead minnow chronics with treflan and endosulfan will be terminated this month. The heptachlor and acrolein fathead chronics are at the spawning stage. A 6-month time extension with no additional funding was approved for this contract in order to complete the last 2 pesticide chronics and remaining Daphnia work. Seven days after starting, 65-80% mortality and 20-35% loss of equilibrium was observed in the fish at 40 µg/l of treflan. All fish chronically exposed to 20 µg/l died within the first 49 days and all of the fish at 10 µg/l died within 174 days. These fish exhibited hemorrhaging, scoliosis, or loss of equilibrium. Some mortalities occurred at 5 µg/l between 174 and 348 days exposure indicating an accumulative toxic effect that eliminated only the least resistant fish at that concentration. Daphnia magna acute values (mg/l) for treflan are: 24-hour TL50 0.320 (range 0.211-0.411); 48-hour TL50 0.193 (range 0.110-0.321). A treflan Daphnia magna chronic test was completed with production of young being reduced at 0.10, 0.05, and 0.025 mg/l.

The endosulfan fathead minnow acute incipient TL50 was 0.86 µg/l. High mortality was observed at .60 µg/l and all fish died within 236 days. Control eggs transferred to this concentration died prior to or at hatch and transferred control fry died in one day. Daphnia magna acute values (mg/l) for endosulfan are: 24-hour TL50 0.213 (range 0.153-0.299); 48-hour TL50 0.156 (range 0.049-0.494).

A 10-day heptachlor acute TL50 for fathead minnows was 7.02 µg/l (range 5.76-8.54). During the heptachlor chronic all fish died at 4.0 µg/l between test days 34-38.

Preliminary static bioassays with acrolein indicate an acute toxicity of 0.05-0.10 mg/l for fathead minnows. Mean growth increments for 30 to 60 days chronic exposure were slightly greater in all experimental groups than controls. Daphnia magna acute values (mg/l) for acrolein are: 24-hour TL50 0.061 (range 0.021-0.260); 48-hour TL50 0.057 (range 0.012-0.210). (Alfred W. Jarvinen)

VIII. In a contract on the chronic and acute toxicities to baygon, baytex, propachlor, and methomyl to fathead minnows and Daphnia magna, with Kenneth Macek, Bionomics, as principal investigator, time independent TL50's and confidence limits have been established for propachlor at 0.52 mg/l (0.43-0.63) and methomyl at 1.75 mg/l (0.90-3.5). Chronic exposures will begin soon. (Roger O. Hermanutz)

IX. Yearling brook trout (Salvelinus fontinalis) were exposed through a reproductive period to five concentrations (39-502 ng/l) of the insecticide toxaphene for seven months on an Interagency Agreement with the Fish Pesticide Research Laboratory, Columbia, MO. All concentrations except the lowest reduced egg viability. The maximum acceptable toxicant concentration (MATC) for brook trout exposed to toxaphene in water lies between 39 and 68 µg/l which results in an application factor (MATC/96-hr LC50) of 0.0037-0.0065.

Analytical methodology has been developed to quantitatively identify toxaphene in water at concentrations as low as 0.025 µg/l. This sensitivity is achieved by extracting 20 liters of water with polyurethane foam.

Toxaphene residue levels in the brook trout exposed to 502 ng/l were 8 mg/kg, a concentration factor of 16,000. Elimination of one-half of the total toxaphene residues in adult brook trout removed from the high exposure occurred after about 50 days. The toxaphene components in the tissues during uptake were only 2 to 18% different from those in the standard. After elimination of half the residues, however, a 2 to 44% greater change was observed in early eluting peaks as compared to later ones. (Leonard H. Mueller)

X. Since 1962, mirex has been used extensively as a control agent for the imported fire ant (Solenopsis nevissiuma richteri Forel). The insecticide is usually distributed as a bait consisting of corncob grits, soybean oil, and mirex. The effect of mirex on marsh and estuary species has been established, but little work has been done with fresh-water animals until recently. During the last year tests with both the bait and technical mirex have been conducted using Daphnia magna and aquatic insects.

Daphnia magna were exposed to different levels of bait from very low to far in excess of what would be spread in the environment according to present Department of Agriculture regulations. Mirex leached from a 0.15% commercial bait formulation into the test water to concentrations of 0.34 µg/l to 1.21 µg/l. There were no significant effects on D. magna survival and reproduction at these levels.

Since mirex is soluble in water only to the above mentioned concentrations, acetone was used a s carrier to introduce mirex into test water at higher concentrations. Statistical analysis has yet to be completed on the results of three tests, but an apparent effect on both survival and reproduction occurs between nominal concentrations of 40 and 80 µg/l. Further tests are planned in order to more firmly establish this chronic effect level. Also, residue data will be determined from the exposed adult D. magna.

Experiments with the midge Tanytarsus dissimilis Joh. using bait as the insecticide source have shown no effect at water saturation concentrations of mirex. These experiments are being repeated for verification. Further tests are planned to determine the effects of 30-day exposure of mirex to stream insects and to determine the importance of ingestion to mirex toxicity. (Richard L. Anderson and Richard W. Carlson)

XI. The chronic toxicity of Sevin, Malathion and Diazinon to Daphnia magna was studied in three-week constant flow tests. Concentrations in µg/liter causing no significant impairment to reproduction were 8.7 for Sevin, 0.16 for Diazinon and 0.57 for Malathion. (Kenneth E. Biesinger)

XII. Continuous-flow and static bioassays were conducted at 18° C, with survival and reproduction as measures of relative toxicity of eight PCB's Aroclor 1221 (A-1221), 1232, 1242, 1248, 1254, 1260, 1262, and 1268. Three PCB-mixture bioassays were also conducted. Aroclor 1248 was the most toxic to Daphnia magna of the eight Aroclors tested in static tests; the 3-week LC50 was 25 µg/l. Aroclor 1254 was the most toxic PCB to Daphnia under continuous-flow conditions with a 3-wk LC50 of 1.3 µg/l. Ninety-six hour LC50 values for 1242 and 1248 on Gammarus pseudolimnaeus in continuous-flow tests were 73 and 20 µg/l. Survival after 60 days was 52% at 8.7 µg/liter 1242 and 53% at 5.1 µg/liter 1248. Reproduction and survival of young were normal at 2.8 µg/liter 1242 and 2.2 µg/liter 1248. The midge Tanytarsus dissimilis, in continuous-flow tests, did not emerge in abundance above 5.1 µg/liter 1248 or 3.5 µg/liter 1254. The 3-wk LC50 for Aroclor 1254 was 0.65 µg/liter for larvae and 0.45 µg/liter for pupae. Tissue residues in Gammarus pseudolimnaeus ranged from 4.0 µg/gram 1254 in control animals to 552 µg/gram 1248 in scuds held for 60 days in water containing 5.1 µg/liter 1248. Al Nebeker)

XIII. Two 9-month continuous-flow bioassays and several intermediate length continuous-flow tests were conducted to determine safe levels of Aroclor 1242, 1248, and 1954 for the fathead minnow Pimephales promelas) and Aroclor 1248 for the flagfish, Jordanella floridae. Calculated 96-hour LC50 values for newly hatched fathead minnows were 7.7 µg/liter for Aroclor 1254 and 15 µg/liter for 1242. Three-month old fatheads had a 96-hour LC50 of 300 µg/liter for 1242. Reproduction occurred at and below 1.8 µg/liter 1254 and at and below 5.4 µg/liter 1242. Newly hatched young were the most sensitive life stage. Growth of young fatheads was also affected above 2.2 µg/liter 1248, and none survived above 5.1 µg/liter after 30 days. Young flagfish did not survive at 1248 concentrations above 5.1 µg/liter and did not grow well above 2.2 µg/liter. (Al Nebeker)

Fathead minnow fry less than 24 hours old at the start of the test were exposed for 30 days to Aroclors 1248 and 1260. Median toxicity values of 4.35 µg/1 for 1248 and 2.5 µg/1 for 1260 were obtained. Total mortality was observed at 8.0 µg/1 and 7.0 µg/1, respectively. Chronic tests have recently been completed with these Aroclors at concentrations of 3 µg/1 and below. While the data have not been analyzed statistically, there were no readily apparent effects on survival, growth, or reproduction. (Dave DeFoe)

XIV. The fathead minnows exposed to Aroclor 1248 and 1260 accumulated about 80 percent of the residue present after the 250 day exposure within the first 90 days. Aroclor 1248 was accumulated in lipids up to concentrations approximately 1.3×10^5 times that in the water. Previous work at NWQL measured bioaccumulation factors for Aroclor 1242 and 1254, and the data from these PCB studies indicate a consistent direct relationship between the percentage of chlorine in the PCB and the bioaccumulation factor (inverse relationship with water solubility).

The female fathead minnows in both exposures contained about two times more PCB/s on wet weight basis than the males and were more variable. Terminal males exposed to 3.5 µg/1 Aroclor 1248 contained 194 $\pm$ 12 µg/gm on a wet weight basis. Since these variations can be accounted for largely by the greater percentage lipids in the females, the data show that the residues cannot be adequately described throughout the bioassay unless the sex of the fish is included in the analysis.

The fathead minnows which were thinned after 66 days of exposure were transferred to Lake Superior water, and the residues and growth in composite samples were measured four times over a 60-day period. Approximately 30 percent of the PCB residue (as total µg) was eliminated and/or metabolized in 60 days. (Gilman D. Veith)

XV. Brook trout were exposed to sublethal water concentrations (1.0, 0.33, 0.11, 0.04, 0.01, and 0.0 µg/l, nominal concentration) of a commercial polychlorinated biphenyl, Aroclor 1254, to determine its effect on growth and reproduction and to determine the uptake of PCB's by the trout. The adult fish were terminated after 71 weeks of exposure with no effects noted on growth or survival. Reproduction occurred successfully in October and November at all toxicant concentrations. Exposure of the eggs and fry produced will continue until April 1974.

Residue samples were taken from fish after 14, 27, 36, 41, 48, 55, 60, and 71 weeks exposure. Analyses completed thus far indicate that after 55 weeks of exposure the brook trout accumulate from 0.5 to 12.0 µg/l PCB (on a wet weight basis) in the whole body and from less than detectable (<0.20 mg/l) to 2.0 mg/l in the fillet from nominal water concentrations of 0.01 to 1.0 µg/l. (Virginia M. Snarski)

Oceans Systems, Tarrytown, New York, was awarded a contract which now has been underway for just over a year and deals with the acute and chronic toxicity and tissue accumulation of the two most common phthalate esters found in the environment--di-n-butyl phthalate (DBP) and di-2-ethyl-hexanoyl phthalate (DEHP)--in both fish and invertebrate animals. Three phthalate chronic exposures of fish are underway at this time. One involves the exposure of bluegills to DEHP at concentrations of 2.09, 0.77, 0.38, 0.16, 0.07 mg/l and controls. The other two chronics are both with brook trout, one involving exposure to DEHP (4.21, 1.48, 0.53, 0.11, 0.03 mg/l and controls), and the other to DBP (2.28, 0.68, 0.18, 0.05, 0.01 mg/l and controls). No DEHP effects were noted on bluegill or brook trout survival, growth or reproduction at any of the concentrations tested. All brook trout exposed to 2.28 mg/l DBP died after one month, and all trout exposed to 0.68 mg/l DBP died following four months exposure. Studies on spawning success, and growth and survival of young, for both DEHP and DBP are underway and will be complete in four to five months. (James M. McKim)

XVI. In a contract with Envirogenics Company of El Monte, California, to determine the acute toxicity of acetone, triethylene glycol (TEG), dimethylformamide (DMF), and Triton X-100 (a product of Rohm and Haas Company) to three species of fish and the chronic toxicity of the last three to fathead minnows and brook trout, the following approximate 7-day LC50 values (in mg/per liter) and have been determined:

Chemical	Fathead Minnow	Bluegill	Brook trout
TEG	92,500	56,500	67,000
Acetone	7,100	7,500	6,000
DMF	7,000	6,200	8,400
Triton X-100	5	14	11

In the chronic tests with brook trout, the fish are beginning to spawn, and so far individual levels of up to 2 mg Triton X-100, 100 mg DMF, and 640 mg TEG per liter have produced no significant effects. In the first month of the chronic tests with fathead minnows, 2 mg Triton X-100 per liter killed 95% of the fry exposed to it, 640 mg TEG per liter killed 70% of the fry exposed to it, but 100 mg DMF per liter had no significant effect. (Charles E. Stephan)

XVII. In a malathion degradation study at room temperature in Lake Superior water, the half-life at pH 6 was found to be 3 days, as compared to 9 weeks in distilled water. The solubility of endrin in Lake Superior water was found to be near 90 µg/l at room temperature. A total of about 3,300 water and tissue analyses were run in direct support of the fish acute and chronic pesticide bioassays in the past 12 months. The acquisition of a new GLC system, including automatic sampler, integrator, and recorder, along with a programmable calculator and plotter, has reduced by about one-half the time spent by three persons on gas chromatographic determinations and calculations. (Leonard H. Mueller)

Lake Michigan fishes representing 13 species from 11 regions were captured in the fall of 1971 and analyzed for chlorobiphenyls (PCB's) and DDT and analogs. The major PCB's in Lake Michigan fishes are the tetra-, penta-, hexa-, and heptachlorobiphenyls, although the lesser chlorinated PCB's present in fishes (expressed as Aroclor 1254 - wet weight) ranged from 2.7 µg/gm in smelt to 15 µg/gm in lake trout. Essentially all trout and salmon greater than 12 inches in length contained PCB's of concentrations greater than the FDA tolerance level of 5 µg/gm. The concentrations of total DDT (sum of analogs) ranged from less than 1 µg/gm in suckers to approximately 16 µg/gm in large lake trout.

The presence of PCB's was routinely confirmed by perchlorination to decachlorobiphenyl. Composite samples from selected regions of the lake were also qualitatively analyzed by GC/MS. The major components (based on weight) identified include C_{12}, C_{13}, and C_{17} hydrocarbons in addition to hydrocarbon mixtures resembling fuel oil;

the six p,p´ and o,p´ isomers of DDT, DDD, and DDE; approximately 15 PCB's containing 2 to 7 chlroine atoms per molecule; nonochlor; diotyladipate, dibutyl-, dihexyl-, and dioctylphthlates; hexachlorobenzene, and dieldrin. The "heptachlor epoxide" commonly reported in Lake Michigan was identified as dichlorobenzophenone and no evidence of heptachlor epoxide was found. Other components of the Lake Michigan fishes included a series of polychlorinated cyclodiene compounds, DD MU, cis- and trans-chlordane, kelthane, polynuclear aromatic hydrocarbons, and chlorinated polynuclear compounds. (Gilman D. Veith and Douglas W. Kuehl)

XVIII. The need to characterize complex mixtures of potentially hazardous organic chemicals and their metabolites in fish tissue relies heavily on gas chromatography/mass spectrometry as a primary analytical tool. An evaluation of present residue cleanup techniques has demonstrated that the development of cleanup techniques for GC/MS analyses have not kept pace with developments in instrumentation. Techniques which are suitable for quantitative analyses via GLC generally do not provide adequate cleanup capabilities for explicit confirmation via GC/MS when the concentration of the chemicals is less than 0.5 µg/gm in the wet tissue. Moreover, attempts to scale-up procedures increase the likelihood of contaminating the sample with reagent impurities.

A procedure for isolating greater quantities of pesticides and related chemicals which may be present at concentrations less than 0.1 µg/gm for subsequent GC/MS analysis has been evaluated and submitted for publication. After exhaustive extraction of the trace chemicals and lipids from the tissue, the trace chemicals are removed from the bulk of the co-extracted lipids through preferential desorption from Micro Cel-E, a sorbant with a 490 weight-percent capacity for oil. Briefly, large quantities of lipid residue are mixed with Micro Cel-E to form a dry powder from which the pesticides and related chemicals are preferentially partitioned into an organic solvent mixture. The trace chemicals are subsequently isolated from the remaining co-extractants by gel permeation chromatography. Although the basic principles of the individual steps are methods existing in the literature, neither is sufficient when used alone, and the outlined procedure combines the high capacity of Micro Cel-E with the high efficiency of gel permeation chromatography. The procedure permits the semi-quantitative isolation of trace chemicals from 0.5 Kg of fish tissue into 100 µl of hexane, sample preparation. (Gilman D. Veith and Douglas W. Kuehl)

XIX. The need for identification of specific chemical compounds responsible for environmental pollution has resulted in the development of new instrumental techniques which are much more sensitive and specific than nuclear magnetic resonance or infrared spectroscopy. Because of the complexity and trace amounts in environmental samples, the most valuable technique to date has been shown to be computerized gas chromatography-mass spectrometry. The GC/MS computer system now in use at the National Water Quality Laboratory consists of a Varian 1700 gas chromatograph interfaced to a Varian CH-5 series III single focusing mass spectrometer and equipped with a Varian Spectro System 100 MS an on-line 12K computer. The computer makes possible efficient use of the data generated by the mass spectrometer. The computer peripherals include a 240 thousand word magnetic drum and two magnetic tape drives for program and data storage, a display scope, line printer, punch and plotter for output devices and a data phone coupler for library search.

During the first eleven months of 1973, 120 GC/MS or direct probe/MS run samples were analyzed at NWQL for a total of approximately 36,000 spectra. Sample types include standards, environmental extracts of water, fish and lake sediment, extracts from laboratory bioassays, extracts of waste oil samples, new potentially hazardous synthetic organic compounds, and special samples done at the request of laboratories other than NWQL. (Gary E. Glass)

XX. Recently total DDT analogs in our fish food supply were observed to be approximately twice what they had been previously. It was found that rising costs necessitated a reduction in the amount of fish meal and the subsequent switch from soy bean oil to fish oil to satisfy fatty-acid requirements. Determinations of pesticide concentrations in other commercially available fish foods have revealed total DDT analogs to routinely run from about 0.2 to 0.3 mg/KG. Because of the desire to hold pesticide residues resulting from the food to a minimum in fish used for toxicity research, negotiations are underway to have a private source prepare fish food to our specifications using ingredients known to have low pesticide content. (Virginia M. Snarski)

ROAP 16ABI - Temperature and Dissolved Oxygen Requirements for Freshwater Organisms

Oxygen

I. Effects of reduced concentrations on embryos and larvae of freshwater fishes.

Tests on the effects of reduced dissolved oxygen concentrations on the embryos and larvae of the mountain whitefish, largemouth bass, white bass, and lake trout were completed.

Mountain whitefish were tested at oxygen concentrations of 100, 50, 35, 25, 20, and 12.5% saturation at 4 C, and 100, 50, and 25% saturation at 7 C. A reduction of the oxygen concentration to 50% saturation at both 4 C and 7 C did not affect survival. At 4 C a moderate reduction in survival occurred at 35% saturation, and marked reductions at saturations of 25% and below. No hatching occurred at 12.5% saturation. At 7 C survival was similar between the controls and 50% saturation, and this survival was also similar to those at 4 C with the same oxygen concentration. Few fish hatched at 25% saturation and 7 C, and those died within 24 hours after hatch. Development (incubation time, growth, and first-feeding) was inhibited with each successive lowering of the dissolved oxygen at both temperatures.

Largemouth bass were tested at oxygen concentrations of 100, 70, 50, 35, and 20% saturation at 20 C and 23 C. At oxygen concentrations as low as 35% saturation, survival of largemouth bass was adequate. However, even at the highest reduced oxygen saturation (70%) growth was inhibited, and at saturations of 50% and below, hatching was premature and first feeding was delayed.

White bass were tested at oxygen concentrations of 100, 70, 50, 35, and 20% saturation at 16 C and 19 C. At 16 C white bass showed a decrease in survival with each consecutive reduction in the oxygen concentration at 7 days after hatching. This trend was only slight from 100% saturation to 35%, but a marked reduction in survival took place at 20%. There were no differences in average fish length at different oxygen concentrations at this time. At 35% saturation and above, larvae were first observed in a horizontal sustained swimming position on the fourth day after hatching; at 20% on the following day. At 19 C survival at hatch was similar to and development faster than at 16 C and the same respective oxygen concentration. All larvae at 20% saturation and 19 C died before reaching the horizontal swimming stage, and after 10 days of exposure a high mortality was observed at all of the higher oxygen concentrations. This mortality was probably due to starvation, although plankton was offered to the fish. It is apparent that 20% saturation of dissolved oxygen is not adequate for young white bass survival, and concentrations of 35% saturation inhibit yolk sac absorption.

Lake trout were tested at oxygen concentrations of 100, 50, 35, 20, and 15% saturation at 7 C, and 100, 50, 40, 30, and 20% saturation at 10 C Lake trout development (at both temperatures) from fertilization through first feeding was inhibited at all reduced oxygen saturations tested (50% and lower). Survival was adversely affected at all reduced concentrations although only slightly at 50% saturation at 7 C.

A test to determine the effects of reduced dissolved oxygen concentration on the spawning behavior of the largemouth bass is underway. Mature fish are being exposed to oxygen concentrations of 100, 80, 65, 50, and 35% saturation and a "natural" temperature regime and photoperiod.

Bluegills are being held in 3-foot and 4-foot circular tanks and are being exposed to a "natural" temperature regime and photoperiod. This test will determine if the bluegill will successfully spawn in these tanks, and will be suitable for spawning behavior testing at reduced oxygen concentrations. (Richard E. Siefert and Anthony R. Carlson)

Consumption during embryonic and larval stages

II. Measurements of the oxygen consumption during embryonic and larval stages of brook trout, lake trout and coho salmon were made at 7 to 8 C. Brook trout and lake trout were similar in rate of oxygen consumption; the coho salmon consumed oxygen at a much higher rate. In each species the consumption rate increased markedly after hatching, and again when feeding was started. By feeding some and witholding food from others of the same age, it was proved that the second abrupt increase was caused by feeding and not by the developmental stage.

Oxygen consumption rates increased with the age of the embryo. The mean rates at hatch in µg per fish per minute were: brook trout, .075; lake trout, .072 and coho salmon, .124. The rates at first feeding were brook trout, .160; lake trout, .235 and coho salmon, .707. The rates two weeks after first feeding were brook trout, .281; lake trout, .429 (one week after); and coho salmon, .891.

The oxygen consumption of brook trout embryos and larvae during their first 13 weeks of incubation was measured at dissolved oxygen concentrations of 20, 35, 50, and 80% saturation. The rates of oxygen consumption and time of hatching were practically the same at 50 and 80% saturation; the consumption rate was less and hatching was delayed by a day at 35%. At 20% saturation the consumption rate was about a fifth of those at 50 and 80%, hatching was delayed by 5 days, and nearly 90% had died by the twelfth week of incubation. (William A. Spoor)

Effects of low concentrations for periods varying in time of onset and duration on embryonic and larval stages

III. Studies to learn the effects on respiration and survival of exposing embryonic and larval stages of freshwater fish to low concentrations of oxygen for periods varying in time of onset and in duration were conducted with northern pike and largemouth bass.

It seems well established (to a considerable extent by work done at the NWQL) that embryos and larvae can develop and survive through the start of feeding during continuous exposure to oxygen concentrations as low as 25% saturation, but the literature contains very little useful information about the effects of temporary exposure to reduced oxygen concentrations, especially to concentration ranges likely to be encountered by the fish. This lack of information is unfortunate because the oxygen concentrations during the spawning seasons of warmwater fish seem more likely to undergo temporary depressions lasting for a few hours (e.g., a pre-dawn sag) or for a few days than to remain at low concentrations throughout the entire embryonic and larval periods. Further, judging by the results of experiments conducted so far, if the oxygen concentration is adequate for normal development most of the time, but subject to a sag, the fish are in greater danger than they would be at a permanent low concentration.

The study with northern pike was a preliminary study undertaken primarily to test the equipment and experimental design. This work showed that a reduction in the dissolved oxygen concentration from near saturation to 25% saturation two or three days before hatching, and during hatching, is more damaging than a change from high to low several days after hatching or remaining at 25% saturation throughout the embryonic and larval periods. Change from low to high concentrations improved survival, and embryos changed from low to high two days before hatching developed as well as those kept at the higher concentrations throughout.

The work on largemouth bass was the first stage of the study of this species. In this work one set of 4 groups of embryos (each group starting with 200 fertilized eggs) was started at 90 to 100% oxygen saturation and another set of 4 groups was started at 25% saturation. One group of each set was changed to the alternative concentration on the second day of incubation, another group on the fourth, and a third group on the sixth (hatching occurred on the 4th or 5th day). The fourth group of each set was maintained at the original oxygen concentration throughout. At 25% saturation 47% survived through the start of feeding and, as with the pike, changing from low saturation to high improved survival. The reciprocal change was detrimental, that from high to low on the sixth day causing total mortality within 24 hours. At 90% saturation 86% survived through the start of feeding.

The information obtained from the study of northern pike and largemouth bass suggests the importance of such variables as (1) the degree to which the oxygen concentration is lowered, (2) the duration of exposure to the reduced concentration, and (3) the developmental stage at which the reduction is made. Earlier work at this laboratory

has shown that the (4) temperature and (5) water flow rate must be taken into account. Other variables that may be significant are (6) the rate of change in concentration and (7) the effect of changing from an intermediate to a low concentration. Because the flagfish, green sunfish and fathead minnow will spawn in the laboratory throughout the year, they were tested as substitutes for use in studying these variables when embryos from seasonally spawning fish are not available. The fathead minnow looks the most promising because it provides large numbers of fertilized eggs of known age. (William A. Spoor)

IV. A research grant under the guidance of John J. Peterka, North Dakota State University, Fargo, North Dakota, was awarded to determine the dissolved oxygen concentrations in natural spawning habitats of selected freshwater fishes.

Major effort from February to April was spent toward developing and assembling equipment for measuring dissolved oxygen in the field. From April to May measurements of dissolved oxygen, water, and air to temperatures were made in a marsh used by spawning northern pike. From May to July measurements of dissolved oxygen, water, and air temperatures were made in spawning beds of largemouth bass, pumpkinseeds, and bluegills.

Large fluctuations in dissolved oxygen were found in the northern pike spawning site at a depth of .01 m from the bottom (near the embryos). For example, from April 18 to May 9, the maximum recorded dissolved oxygen concentration was 16.4 mg/liter and the minimum was 1.9 mg/liter. Temperatures at this depth ranged from 12.4 C to 5.0 C. Bluegill embryos in a natural nest were exposed to an average daily change of 4.8 mg/liter of dissolved oxygen and a 3.3 C temperature change. (Richard E. Siefert)

Temperature

I. Largemouth Bass Spawning. Largemouth bass were tested to quantify reproductive success at constant winter temperatures. The test is one of a series to determine if the usual length winter chill period and the associated low temperatures at the latitude at which a species is abundant are needed for successful reproduction. The data add to temperature criteria information for the species and broaden the data base for estimating criteria for other species.

Information from field studies indicates that largemouth bass reproduce successfully when exposed to winter temperature regimens ranging from (1) annual temperatures in Silver Springs, Florida, which are constant the year around at 22.8 C, with spawning occurring

primarily in April through June, to (2) a few to several months at temperatures under ice in northern areas with spring rise in temperature triggering spawning in April through June when water temperature has exceeded 15.6 C, to (3) high seasonal temperatures in subtropical areas which decrease to about the minimum spawning temperature in fall, and then increase to trigger spawning between November and May at 15.6 - 26.7 C with the peak in February at 21.1 C. Reproductive success under these temperature regimens has not been determined quantitatively.

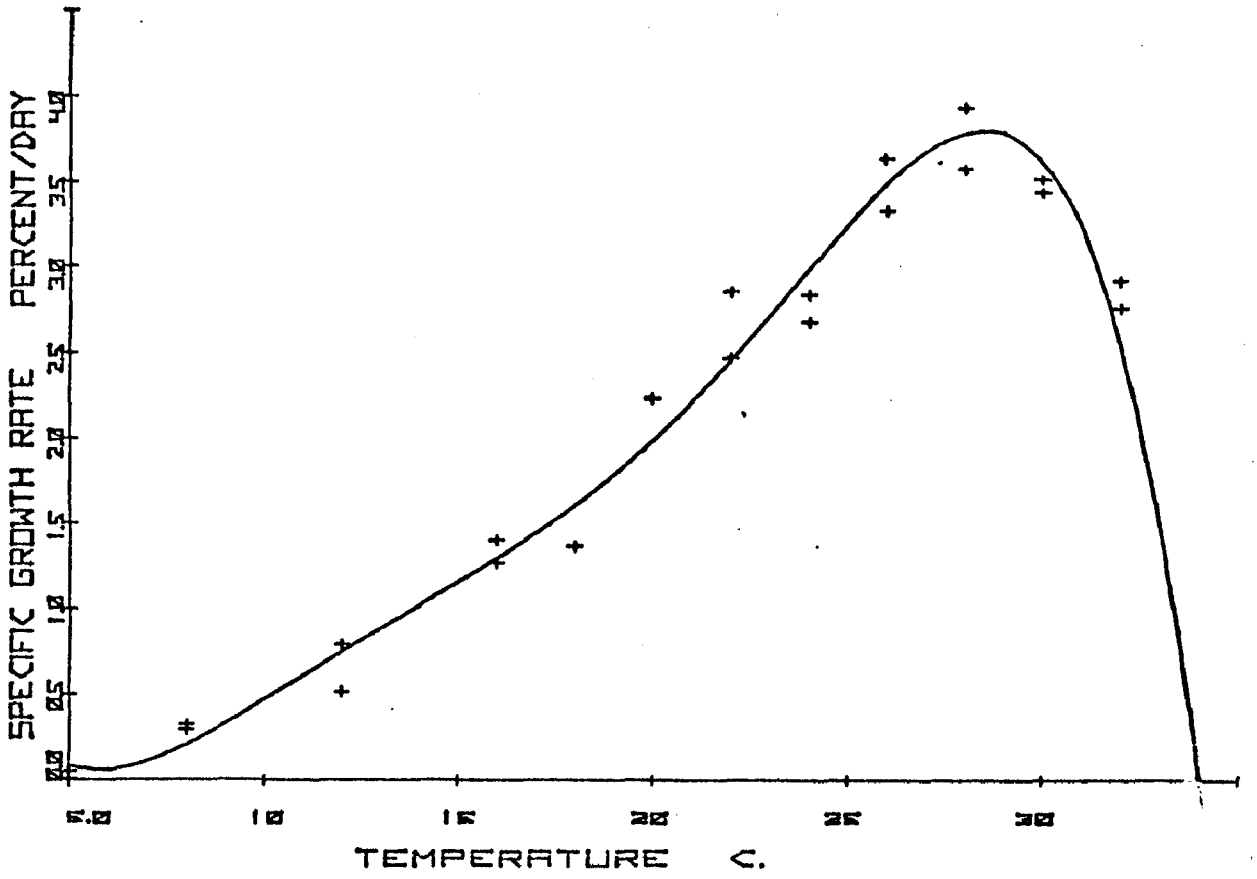
Largemouth bass were exposed to nominal constant temperatures of 15, 18, 21, 24, and 27 C. Twelve fish were held at these temperatures from October 27 to March 22 when four fish (two pairs) were placed in duplicate 4-foot diameter tanks for spawning. Two spawning substrates were placed in each tank. These consisted of gravel-covered nylon mesh mats in 14 x 17" plastic pans.

Nesting activity, as indicated by gravel swept from the center of the mat, was highest at 18 and 21 C, but occurred to some extent in all tanks.

Spawning occurred only at 21 C between April 18 and June 10. Eleven spawnings from both duplicates produced a mean number of eggs per spawning of 7,000 and 2.5 spawnings per female. The mean fertility of the spawnings at 21 C, measured at formation of the neural keel, was 96%. (Bernard R. Jones)

II. Yellow Perch Growth. Growth of juvenile yellow perch was determined in an eight-week study conducted at constant temperatures at 2 C intervals from 8 - 34 C. Growth was maximum at 28 C with no statistically distinguishable reduction at 26 or 30 C.

Rate of growth increased with increase in temperature up to 30 C. Rates at 26, 28, and 30 C, at which best growth occurred, were 3.5, 3.8, and 3.5 percent increase in weight per day, respectively. At 32 C the rate dropped to 2.8 percent per day and was accompanied by a high percentage of deformed individuals. Little or no growth occurred at 8 C and complete mortality occurred in less than 7 days at 34 C. These relationships, particularly the closeness of the temperature of optimum growth to the lethal temperature, are illustrated in the following figure.



Rainbow Trout Behavior. A study of the behavior of 3-4 inch rainbow trout in relation to different thermal barriers is being conducted in cooperation with the University of Minnesota, Duluth. The work, which is being conducted by B. Monson a graduate student, is designed to determine the thermal barriers that fish acclimated to temperatures of 5, 10, and 15 C will penetrate to obtain food and the effect on the fish of penetrating high temperature barriers. The laboratory work is scheduled for completion in January 1974. (J. Howard McCormick)

III. A contract was awarded to Bayshore Systems Corporation, Springfield, Virginia. Under the direction of Joseph R. Jahoda, to develop two types of remote sensing prototypes: an acoustic tag to identify position and temperature of individual large fish and an activity detector to locate offspring from natural reproduction. Effort has been concentrated on finishing the design of the acoustic fish tag to be field tested at the Monticello Field Station. The Bayshore Systems approach is to use a sufficient number of hydrophones to decrease the range of the fish tag transmitters and thereby increase the life of the batteries. By limiting the effective range of the fish tag to about 75 feet and receiving a signal once an hour, a life of 1 year and a size of 0.60 X 1.7 inches long (7 grams in water) is feasible. To obtain the temperature of each fish, two possible techniques are available (a) a thermistor is included in the fish tag and it controls the repetition rate of the pulsed signal, (b) thermistor elements are placed in the hydrophones and the temperature of the immediate area is monitored. One correlates the temperature of the hydrophone with the closest fish. A tag approximately 1.3" long and 5 grams in water can be achieved with this approach. A broadband receiver and amplifier system will locate up to 20 fish tags per channel operating at individual frequencies of 50-250 KH_2.

Field tests have been delayed because of delays in installation of conduit, however one test was made on site in November. This test identified significant interference problems with the transducer and receiver in the water which will require some form of electromagnetic shielding to reduce the background "noise level". This problem has not yet been solved and may result in further cost over runs to complete this contract. A time over run to September 1974 was requested during the past quarter. (Kenneth E. F. Hokanson)

IV. A grant was awarded to the University of Minnesota, St. Paul, Minnesota, with Lloyd L. Smith, Principal Investigator, to determine temperature requirements of egg, larvae, and juvenile walleye and sauger. A grant extension was awarded this year to complete work on the sauger which have been difficult to collect for experimentation. Juvenile sauger were collected from the Mississippi River and growth tests were initiated this summer. The optimum temperature for growth, fed excessive amounts of minnows, was 22° C. The upper incipient lethal temperature of juvenile sauger was 27° C when acclimated to 10° C and reached a maximum of 31° C when acclimated to 26° C. Sauger thermal requirements are very similar to walleye. These data support the assumptions used in establishing temperature criteria for the Ohio River. All experimental work has been completed and the final report will be submitted next quarter on schedule. (Kenneth E. F. Hokanson)

V. A contract was awarded to Eco-Logic Analytical Consultants, San Diego, California with C. R. Bernick as Program Manager, to complete work started by Plessey Environmental Systems involving historical temperature records as related to fish population dynamics. The data have been encoded on computer tapes and catalogued as to the type and amount of information available. All information with the exception of the computer tapes and a conversion program has been received. Analyses of the data base are now being made to determine the best way of presenting the encoded information. Planned use of the encoded data includes analyses of the data and making the data base available to federal. state, academic and industrial personnel. (Kenneth E. Biesinger)

VI. A grant was awarded to Waterloo Lutheran University, Waterloo, Ontario, Canada, with Robert McCauley as Principal Investigator, to describe the seasonal changes in preferred temperatures of adult yellow perch. Initial efforts concentrated on a literature review of publications in the field of temperature preference and correspondence and consultation with workers active in the field. Consideration of the types of temperature selection devices suitable for yellow perch resulted in the decision to work with horizontal gradient tanks following the design of Zahn, an eight-foot deep vertical gradient tank, and a behavioral thermoregulatory device modified after the original design of Neal and Magnuson. Three rooms allotted by their university were painted, electrical circuits and fish holding tanks were installed. Problems were encountered in establishing a stable horizontal gradient, but this has been solved. Adult perch have been collected from Lake Erie and Lake St. Clair and will be acclimated to temperatures 5-20° C prior to temperature selection studies.

The principal investigator has received another grant to study the effects of the immediate thermal history on temperature selection of rainbow trout. This grant has provided an additional technician part-time for the perch grant. Rainbow trout are fed a small radio tag with a thermistor the size of a pill. Preliminary results suggest that rainbow trout maintain a constant internal body temperature while they move over a wider range of external water temperature. (Kenneth E. F. Hokanson)

VII. In order to determine the influence of turbidity on fish species abundance in the western arm of Lake Superior, a grant was awarded to the University of Wisconsin, Superior, Wisconsin, under the direction of William A. Swenson.

Hours to Median Mortality at 10° C in Supersaturated Water [1/]

Species	1.30 atm	1.25 atm	1.20 atm	1.15 atm
Coho salmon (Oncorhynchus kisutch)				
a. juvenile (parr)	-	43; 73	131; 157; 264	0 in 13 days
b. hold-over smolts	-	11.5; 13.0	29.5; 38.5 40; 45	303
c. jacks (premature adult males)	- -	19	56; 63	15% in 13 days
d. adult males	-	21	45; 51	166
e. adult males	-	20	74; 86	264
Chinook Salmon, spring (Oncorhynchus tshawytscha)				
a. juvenile (parr)	-	-	-	-
b. smolts	-	-	36	No mort. in 268
c. adult females	7.8	17.5	46; 80	526
d. adult males	8.8	16.3	40; 68.5	

Species	1.30 atm	1.25 atm	1.20 atm	1.15 atm
Sockeye salmon (Oncorhynchus nerka)				
a. juvenile (parr)	-	37.5	63; 127	303
b. smolts	-	21; 23	58; 58	
c. adults	-	≤ 18	82	30% in 8 days
Steelhead trout, summer (Salmo gairdneri)				
a. yearlings	-	18.5; 18.5	46	0% in 268 hours.
b. adults	-	31	118	-
Steelhead, winter (Salmo gairdneri)				
a. parr	-	32; 38	78; 87	280
b. adult females	-	-	92	10% in 350 hrs.
c. adult males	-	-	76	30% in 350 hrs.
Rainbow trout (Salmo gairdneri)				
a. parr	-	26.5	51	-
b. 1.5 year old	-	31.5	46	30% in 120 hrs.
Largemouth bass (Micropterus salmoides)				
a. Juvenile	193	5% in 10 days	0% in 10 days	
b. adults	138	10% in 10 days	0% in 10 days	

[1/] Sample size was at least 10 fish per level except for adult summer steelhead

Preliminary analysis of data from the first field collecting season indicates that temperatures during the warm water period tend to separate fish into different size groups and predator from prey species. A second factor, turbidity, tends to continue this process by driving trout farther offshore out of the turbid zone, leaving walleye pike as the prime large predator in the in-shore zone during turbid periods. The walleye does not feed as actively at Lake Superior temperatures as do trout. Turbidity is believed, through a species specific avoidance mechanism, to reduce predation on smelt below that expected in clear water. On the other hand, smelt under turbid conditions tend to move in-shore and off the bottom, where they enter the habitat of mid-water larval lake herring.

It is believed that during these periods, when the predacious smelt move into the water column position occupied by the herring, that they exert their most adverse influence on the year-class-strength of the herring stocks.

The fact that smelt yearling feed avidly on newly hatched lake herring has been confirmed by laboratory observation at the NWQL. Field evidence of this predator-prey relationship is, however, only circumstantial in that smelt feeding on larval fish was confirmed.

The implications of these interactions between environmental factors and interspecific relationships between fishes suggest that turbidity is an important factor contributing to the decline of the lake herring of Western Lake Superior. However, this decline is not independent or separate from the introduction of the smelt or over-exploitation by the fishery. (J. Howard McCormick)

RAOP 21AKG - Effects of Nitrogen (Air) Supersaturation on Columbia River Fishes

A system has been developed to supply water supersaturated with air to fish testing chambers by injecting air into water under pressure. The system is inexpensive, easy to control, and provides water of uniform quality. Further, a simple method for measuring the total dissolved air pressure was devised and used in all testing to monitor the levels of supersaturation. Tests were conducted at several levels of supersaturation ranging from 1.10 to 1.30 atmospheres of total dissolved gas pressure and the time to 50 percent mortality was determined for several life stages of coho salmon (Oncorhynchus kisutch), spring chinook salmon (Oncorhynchus tshawytscha), sockeye salmon (Oncorhynchus nerka), summer and winter steelhead (Salmo gairdneri), and largemouth bass (Micropterus salmoides). These are listed below:

Considerable differences were noted in survival time and at least eight factors were identified which influence the tolerance of fishes to air supersaturated water. These are species, subspecies or race, sex, life stage, acclimation and test temperatures, swimming activity and solar radiation.

Spring chinook smolts, yearling summer steelhead, yearling rainbow trout and adult female coho were the four most sensitive animals tested; largemouth bass and carp were the least sensitive fish when tested at 10° C and judged by time to median mortality.

Aquatic insects and crayfish were more resistant than fish to supersaturation, but did develop gas emboli in their blood, gills, and tissues at higher levels of supersaturation. Supersaturated water is acutely lethal to at least one life stage of several Pacific salmonids at levels at or above 1.15 atm TDGP (115 mm Hg hyperbaric). A level of 1.10 atm total dissolved gas pressure (TDGP 110 percent of atmospheric pressure or ca 76 mm Hg hyperbaric) was not acutely lethal and is probably safe for most aquatic environments except in fish farms, hatcheries, or possible heated effluents where prolonged exposure to 1.10 atm TDGP would cause mortality and ill health.

The Weiss Saturometer as modified by Western Fish Toxicology Station staff is a suitable means of measuring total dissolved gas pressure and has an accuracy of $\pm$ 1 mm Hg.

The external signs of gas bubble disease vary between species and become most severe at moderate to low levels of supersaturation. Positive identification of air emboli in the blood is necessary to confirm gas bubble disease. Gross pathobiology of gas bubble disease has been described relative to these experiments. These results provide a baseline for developing a rank order of susceptibility to gas bubble disease and for developing application factors for estimating effects between species in later chronic tests. Smolts of spring chinook salmon were found to be most sensitive and largemouth bass were least sensitive to the test conditions. It is recommended that the maximum allowable level of total dissolved gas pressure be limited on an interim basis to 1.10 atm (110 percent of atmospheric pressure). (Ronald R. Garton)

ROAP 25AIC - Effect of Oil(s) on Freshwater Fish, Other Freshwater Life, and Wildlife

Research undertaken in 1973 on the effects of oils on the freshwater environment concentrated on meeting the requirements of the Federal Water Pollution Control Act of 1972. This legislation

required that EPA determine the long-term chronic biological effects of the disposal of waste oil. A number of acute and chronic exposures of various forms of used crankcase oil to selected freshwater organisms were therefore conducted. In addition, extensiv e chemical analyses were undertaken to characterize the various chemical components and structure of the oils used.

Biological Tests

I. Flow-through 7-day acute toxicity tests were performed by exposing a top minnow, Jordanella floridae, and the fathead minnow, Pimephales promelas, to soluble components of waste oil. The stock toxicant solutions were prepared by pumping 238 liters of Lake Superior water through 26 liters of oil for 18 hours and then allowing the mixture to separate for 6 hours. The aqueous phase was removed and used as 100% toxicant. This stock solution was diluted with a proportional diluter to the desired concentrations. Twenty 30 day-old fish in 40 liters of water at 25° C, and with a turnover rate of 6 tanks/day, were used on the Jordanella tests. Fathead tests were run at 20° C with fifteen 45 day-old fish in 14 liters of water and with a turnover rate of 10 tank volumes per day. The 96-hr LC50's were estimated by graphical interpolation to be 1.0% by volume dissolved oil for Jordanella and 1.6% for fatheads. Additional flow-through tests with these species are presently underway.

Static tests with oil layers were performed with fatheads. Fifteen fish were placed in tanks containing 20 liters of aerated Lake Superior water at a temperature of 18° C. The 96-hr LC50 was determined by graphical interpolation to be 1.1% by volume.

Preliminary tests with oil-water emulsions indicate that the LC50 for fatheads is approximately 0.04% by volume oil.

A test to determine the effects of chronic exposures of up to 1% dissolved waste oil on the growth, survival, and reproduction of Jordanella floridae is presently underway. This test began with eggs which subsequently hatched and survived in all tested concentrations. However, 1 day-old fry hatched in control water and transferred to 1% and 0.4% dissolved oil were dead in 48 hours. In addition, 1 day-old fry transferred from 0.064% to 1.00% and from 0.026% to 0.40% did not survive. Apparently some acclimation to the toxicant occurs during the egg stage which allows the fry to survive in concentrations which are toxic to fry unexposed as eggs. (Steve F. Hedtke)

Chemical Analysis of Waste Oil Toxicants

II. Chemical studies at the National Water Quality Laboratory have centered on characterizing the waste oil and the dissolved oil stock solutions. Studies of the residues found in the exposed fish will be completed in the near future.

The waste oil used in this study was analyzed and found to consist of 76 percent saturated hydrocarbons; 4 percent aromatic hydrocarbons including C_2 to C_6 alkylbenzenes, C_1 to C_3 alkylnapthalenes, C_1 and C_2 alkylindenes, methylbiphenyl, C_1 and C_2 alkylfluorenes and alkylphenonthrenes; 3 percent polar compounds; 15 percent solid material; 2 percent volatile components, predominantly C_7 through C_{10} hydrocarbons; and the following metals:

Al = 15 mg/1, Cu = 18 mg/1, Fe = 220 mg/1, Pb = 18,500 mg/1, Si = 17 mg/1, Sn = 6 mg/1, Na = 59 mg/1, Ca = 688 mg/1, Zn = 1359 mg/1, and Mg = 410 mg/1.

The dissolved oil toxicant is being characterized by atomic absorption spectroscopy and through the use of XAD nonionic exchange resins in conjunction with GC/MS. The stock solution of water has been shown to contain the following metals: Pb = 4.4 $\pm$ 0.2 mg/1, Zn = 16 $\pm$ 1 mg/1, Cd* = 60 $\pm$ 2 µg/1, Hg <0.1 µg/1, Ag* <0.08 µg/1, Mo* <0.2 mg/1, Co $\leq$0.8 µg/1, As* $\leq$0.3 mg/1, Cr <0.14 mg/1, Se* <0.1 mg/1, Ni <0.1 mg/1, Fe <0.1 mg/1, Mn <0.1 mg/1, and Cu <0.15 mg/1. Particulate Pb or Zn did not exist in any significant amounts. Rather, studies with XAD-2 resins indicate that most of the Pb in the water is in an absorbable form, possibly an organometallic form which may be more readily accumulated by fish. Fish tissue samples have been collected in an effort to determine the metal residues and relate the observed levels of those from other bioassays with metals.

Studies have shown that the hydrocarbon fractions of the stock toxicant are similar to those in the waste oil, with the exception of a decrease in the polynuclear hydrocarbons on a relative bases. More detailed studies of the water, as well as the exposed fish tissue, are underway (Gilman D. Veith, Douglas W. Kuehl, and John E. Poldoski)

*Excluding possible volatile forms.

ROAP 16AAJ - Short Cut Methods to Determine Toxicant Criteria

I. Investigations were carried out to gather more information on the validity of the application factor approach (ratio between the "no effect" toxicant concentration and the 96-hour TL50) used to predict the chronic toxicity of pollutants in varying environmental situations. This experiment consisted of investigating the effect of water quality and spieces type on the level of the mortality found with zinc. If the level of death response could be predicted for a species in a given water from results obtained on another species in a different water, then more confidence could be placed in the predictive value of this approach.

To test this hypothesis, rainbow and brook trout were exposed simultaneously to zinc in two different water hardnesses. In order for the prediction to be valid, it was a necessary condition that the slope of the response curves be the same. The results of these experiments are shown in the following table.

Table 1. Number of Observed Deaths Out of 50 Fish Exposed for Ten Days to Specified Measured Average Total Zinc Concentrations

	Hard Water (150 mg/l $CaCO_3$)				Soft Water (45 mg/l $CaCO_3$)			
	Brook Trout		Rainbow Trout		Brook Trout		Rainbow Trout	
	Measured Average Zn Concentration	Number Dead	Measured Average Zn Concentration	Number Dead	Measured Average Zn Concentration	Number Dead	Measured Average Zn Concentration	Number Dead
Run 1	7.69	39	6.87	50	2.58	42	2.81	50
	4.79	6	4.50	50	1.73	36	1.88	49
	3.09	5	3.14	45	1.14	23	1.23	50
	2.27	1	2.00	8	.77	8	.85	44
	1.44	1	1.42	5	.54	1	.61	38
	Control	1	Control	0	Control	0	Control	1
Run 2	9.06	48	8.63	50	2.50	27	2.38	50
	5.84	15	5.91	50	1.64	23	1.60	50
	3.65	2	3.54	49	1.04	3	1.05	45
	2.33	0	2.20	4	.69	4	.65	34
	1.49	0	1.49	0	.42	0	.44	17
	Control	0	Control	0	Control	0	Control	0

The preliminary analysis of these data indicated that the assumption of equal slopes held between species but not for waters at different hardness. In addition, on the basis of the components of zinc present that were measured, it was not possible to explain the difference in the waters to a changing ratio of "toxic metal (Zn) species". Essentially, all zinc in the bioassay solutions appears to be "dissolved". The observed differences of the toxicity of zinc have not yet been fully explained. More chemical experiments are required to determine the extent of complexation. Additional bioassays are required to determine the effect of ions such as calcium on the organism. (Todd W. Thorslund, John E. Poldoski, and James M. McKim)

II. The importance of early detection of lead poisoning for the prevention of death and serious morbidity has been demonstrated. The enzyme, Δamino levulinic acid dehydratase (ALAD), is a component in the biosynthesis of heme, where it catalyzes the formation of porphobilinogen.

Lead, even at very low concentrations, severely inhibits this enzyme. The present study of the activity of this enzyme in the red blood cells of brook trout was initiated to investigate the use of this enzyme in predicting chronic lead poisoning long before other physiological and biochemical changes might occur.

An improved method for the assay of ALAD (Burch & Siegal, Clin. Chem. 17: (10) 1971) was adapted for use with fish. Accuracy and reproducibility of the procedure was checked on human blood samples. The values fell within the stated normal limits for humans not exposed to lead. These results are presented in Table 2, along with the corresponding blood lead levels as assayed by the Delves Micro Cup Method (H. Delves, Analyst, 95; 431, 1970).

Table 2. Control ALAD and Blood Lead Values

	Number Sampled	Units ALAD (mean)	Pb# (μg/ml) (mean)
Human	5	120	15.3
Brook Trout	19	333	0.06
Catfish	5	246	--
Rainbow Trout	1	307	--

As seen in Table 2, brook trout ALAD was found to be higher than human controls. A mean value of 19 brook trout controls was 333 units of ALAD. A rainbow trout control value was 307 and the catfish control value was 246 units. Enzyme analyses were performed on brook trout exposed to sublethal concentrations of cadmium and mercury for one month, and no effect on ALAD activity was seen. A two-month exposure of brook trout to lead at water concentrations of 250 and 500 μg lead/liter caused a marked decrease in enzyme activity. Blood lead levels ranged from 6-8 μg/g at the two water concentrations tested. In trout exposed continuously for 22 months to water lead concentrations of 0, 30, 60, and 125 μg lead/liter, marked decreases in enzyme activity occurred even at the lowest Pb levels.

These preliminary data show a uniform decrease in enzyme activity as the lead concentration increases and suggests that this enzyme may be of importance in detecting subtle Pb poisoning in fish populations. (E. Hunt and J. McKim).

III. Carbonic anhydrase (CA, carbonate hydro-lyase, E.C. 4.2.1.1) is of special concern in environmental biochemical studies because it is active in gill tissue and therefore is particularly vulnerable to water soluble toxicants. This enzyme is important both in ion regulation and respiration process in fish, and plays an important role in the function of many organs in addition to the gill. A primary function of this enzyme, in gill tissue is to catalyze the interaction of water and carbon dioxide with carbonic acid, which evidently is the rate limiting sequence for the several interrelated reactions involving H_2O, CO_2, H_2CO_3, H^+, HCO_3^-, and $CO_3^=$.

In the current investigation 55 compounds and 2 mixtures, which generally have relevance to water pollution and water quality problems, were studied for their effect on CA from red blood cells of the catfish, Ictalurus punctatus. This enzyme was inhibited strongly by heavy metal cations, less strongly by metallo-organic ionic compounds, some anions and some pesticides, and weakly inhibited by light metal cations, metallo-organic non-ionic compounds and some test organic compounds.

Certain physical-chemical properties of this enzyme were also determined: activity vs/pH, activity vs/incubation temperature (incubation temperature of enzyme varied; reaction carried out at 1° C), activity vs/reaction temperature, activity vs/enzyme concentration, and activity vs/substrate concentration. (James H. Tucker and Glenn M. Christensen)

IV. Many ions and complexes of heavy metals are significant water pollutants, with compounds of mercury and cadmium being among the most toxic agents to aquatic animals.

Most biochemical oriented studies of water toxicants are carried out with mature animals although there is a clear need also to study pre-adult life stages. This has a particular concern in environmental protection activities because some research evidence indicates that specific pre-adult life stages may be more affected by some water contaminants and other environmental stressors than adults. This evidence indicates that permissible concentrations of water contaminants must be based upon experiments which include exposure studies of pre-adult stages.

In the current study embryos and alevins from control and exposed brook trout were homogenized and subjected to analysis for ATP (adenosine-5'-triphosphate), protein (total), GOT (L-aspartate: 2-oxoglutanate aminotransferase, E.C. 2.6.1.1), ACH (acetylcholine acetyl-hydrolase, E.C. 3.1.1.7), and ALP (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.1). In addition, the weight of each organism and the thickness of the chorion of each embryo were determined.

ATP, being a very ubiquitous reactant in living matter, was studied as it seems likely that most metabolic malfunctions would affect the biochemistry of this tissue component. Also, it has been shown that oxidative-metabolism increases in fish embryos after fertilization, which would involve ATP mobilization and reactivity. Prior investigations at this laboratory showed that the total protein level and GOT activity changed in the blood plasma of adult fish upon exposure to metal cations. It has also been reported that certain metal cations affect the activity of fish brain ACH. Cholinesterase activity can first be detected in eggs of Salmo gairdnerii ten days after fertilization, which indicates an important stage in the development of the nervous system. In another study it was found that the activity of ALP (with acid, phosphatase and pyrophosphatase) increased during development of the embryos of rainbow trout, particularly during the "eyed" stage.

The results of this study showed that the enzymes ACH and ALP in brook trout embryos are either absent or are very inactive. Therefore, it is likely that a toxicant which impairs the activity of these enzymes--as is the case with chlorinated hydrocarbons and carbamates--would not show a high toxicity during this stage of development. Methylmercury(II) appeared to have some effect upon these factors in embryos at 0.1 to 1.0 µg/liter; Cd (II) may have a slight effect upon the parameters measured, whereas Pb (II) has no significant effect. With regard to fry, the three ions all appear to elicit biochemical changes. The weight of the animals decreased; there were only subtle effects upon the protein content; the activity of GOT and ALP increased. The activity of ACH increased for Pb (II) and decreased for MeHg (II) and Cd (II). (Glenn M. Christensen).

V. The uptake and distribution of Hg, Cd, and Pb in 8 tissues (gill, liver, kidney, blood, muscle, spleen, gonad, and brain) of the brook trout were measured over a 38-week exposure period.

After 38 weeks exposure of first generation trout to Cd, tissue cadmium residues in the kidney showed the greatest increase, followed by the gill and liver (Table 1). Slight increases were also noted in the spleen and gonads; however, no increase was detected in the muscle tissue. The average percent recovery of cadmium from spiked samples was 99.6 ± 6.5.

All brook trout tissues sampled showed increases in lead residues (Table 2) following 38 weeks of Pb exposure. The kidney showed the greatest increase, followed by the gill and liver. Preliminary results of the lead residues in tissues of 44-week old second generation offspring showed a similar relationship (Table 3). The gill, liver, and kidney of second generation trout at 500 µg Pb/liter, however, contained much larger concentrations of lead than the first generation fish. The average percent recovery of lead from spiked samples was 95.0 ± 14.4. (Duane A. Benoit, Edward N. Leonard, and James T. Fiandt)

Table 1. First Generation Brook Trout After 38 Weeks Of Exposure to Cadmium (10 fish sampled per concentration)

Mean Cadmium Residues µg/gram of Brook Trout Tissues (Dry Wt.)						
Cadmium Exposure Conc. µg/liter	∿ 0.04	0.3	0.7	1.5	3.0	6.0
Gill	0.5	2.5	3.2	3.7	5.0	9.3[a/]
Liver	0.2	2.4	4.1	8.2	9.8	5.9
Kidney	1.6	11.4	21.4	28.4	49.4	45.2
Muscle	0.1	0.2	0.2	0.2	0.1	0.1
Spleen	0.6	0.8	1.0	1.4	1.8	1.6
Gonad	0.3	1.4	2.6	3.8	3.8	2.8

a/ Since there were no fish alive in the 6.0 µg/liter concentration after 38 weeks, the data for the "20 weeks" fish was included in the Table.

Table 2. First Generation Yearling Brook Trout After 38 Weeks of Exposure to Lead (10 Fish Sampled Per Concentration)

Lead Residues µg/gram of Brook Trout Tissues (Dry Wt.)						
Lead Exposure Conc. µg/liter	0.4	31	62	125	250	500
Gill	5.7	34.8	48.6	117.0	206.9	326.0
Liver	1.7	20.8	32.2	67.8	73.2	83.4
Kidney	4.5	43.6	66.5	216.2	332.8	476.6
Muscle	3.4	5.1	8.0	6.6	3.4	4.4
Spleen	3.6	12.4	8.2	18.0	29.0	59.6
Gonad	3.0	9.5	4.4	12.8	16.3	13.9

Table 3. Second Generation Brook Trout Offspring After 44 Weeks of Exposure to Lead[a/]

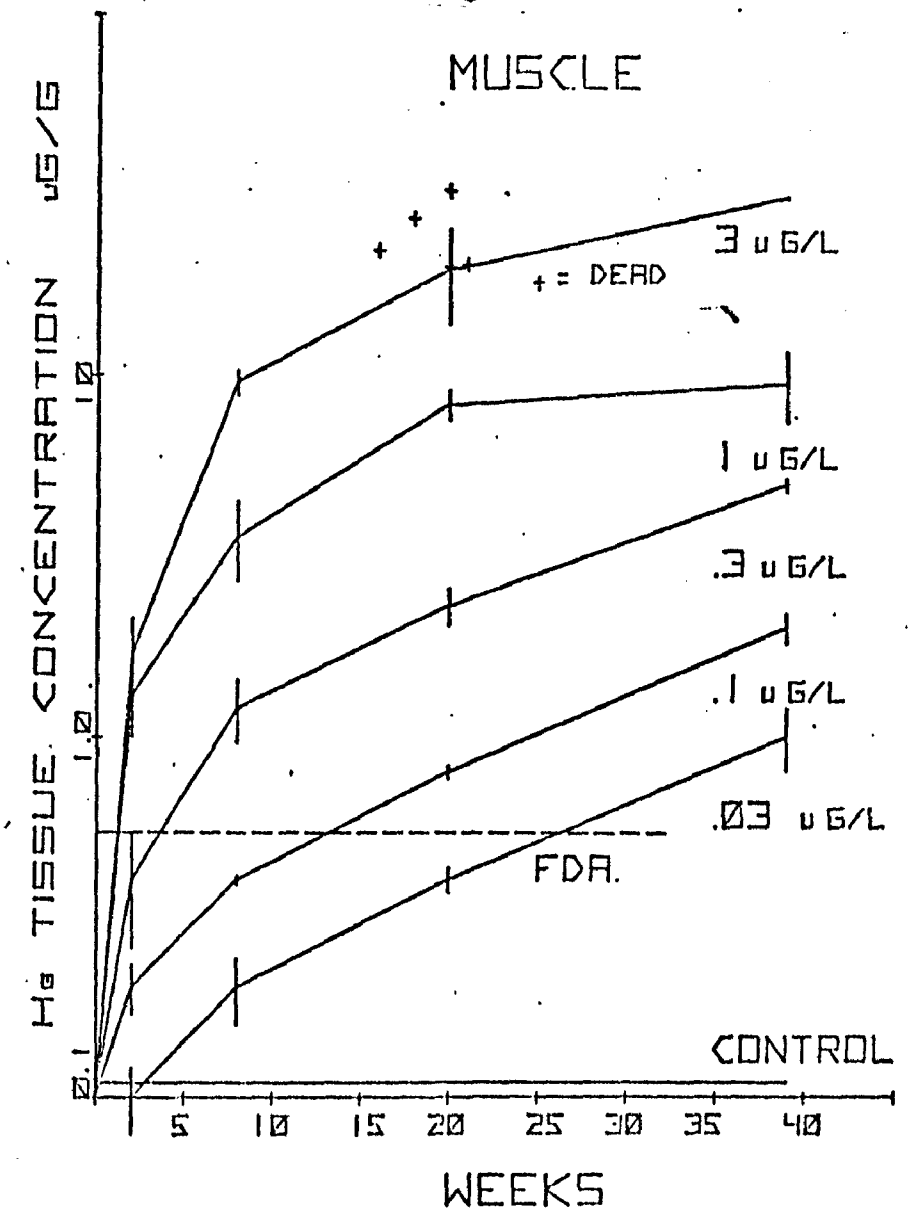
Lead Residues µg/gram of Brook Trout Tissues (Dry Wt.)						
Lead Exposure Conc. µg/liter	0.4	31	62	125	250	500
Gill	6.2	54.5	47.0	89.2	266.0	499.4
Liver	3.1	20.7	14.4	35.5	134.0	235.4
Kidney	2.0	17.0	22.7	101.8	326.2	824.4
Muscle	1.8	3.4	2.4	3.1	4.8	6.6
Spleen	2.0	1.2	(7.3)	(5.2)	(32.2)	(30.3)
Gonad	2.4	2.4	3.4	5.6	7.2	13.1
Brain	1.6	3.4	(3.8)	(1.0)	(10.1)	(13.0)

[a/] All numbers are the average of ten (10) fish sampled. Exceptions are the numbers in parentheses which are the average of 2-5 fish sampled.

The concentration of mercury in the tissues of brook trout exposed to methylmercury chloride in the water for 39 weeks is presented in Figure 1. The uptake from the water was extremely rapid, and even at the lowest water concentrations tested, the residues exceeded FDA's limit of 0.5 ppm for mercury in foods for human consumption. Half-life studies were run on fish removed from all the mercury water concentrations tested. After 4 months in mercury-free water there was no significant drop in the total mercury levels in these previously exposed animals.

These tissue data indicate that, depending upon the length of exposure, mercury may eventually accumulate to toxic levels when they are exposed to low levels of methylmercury chloride. With regard to human consumption of mercury-exposed fish, none of the water concentrations tested produced fish acceptable for human consumption. (James M. McKim).

Figure 1



VI. Channel catfish, Ictalurus punctatus, were exposed to methylmercuric chloride at nominal water concentrations of 4.0, 1.0, 0.25, and 0.06 µg/liter for six months in a flow-through system at 25° C.

The experimental findings indicate that (1) at 25° C methylmercuric chloride at water concentrations of 4 and 1 µg/liter is lethal to the channel catfish in thirty and ninety days, respectively, with obvious histopathologic changes to the gill, kidney, gut, and liver; (2) tissue uptake data for the lower non-lethal concentrations indicated toxicant accumulation which may reach toxic levels in the tissues upon longer exposure; (3) based on the FDA's "interim guideline" level of 0.5 ppm in food, none of the concentrations tested resulted in muscle residue levels acceptable for human consumption. (James H. Tucker)

VII. The purpose of this study was to evaluate changes in cough frequency as a possible short-term indicator of the long-term effects of various toxicants on brook trout. All tests were conducted using Lake Superior water and the fish were studied individually in electrode chambers. (William A. Spoor, Timothy M. Neiheisel, Robert A. Drummond 1971).

Changes in cough frequency, locomotor activity, and feeding behavior of yearling brook trout appeared within 2 - 24 hours at copper concentrations as low as 6-15 µg/liter. Each of these responses appears to be useful for predicting the concentration range of copper likely to have no long-term effects on the species. Cough frequency was the best of the three indicators for showing stress according to the copper concentrations tested. (Robert A. Drummond, William A. Spoor, and Gayle F. Olson 1973).

The lowest concentration of methylmercuric chloride (MMC) and mercuric chloride added to Lake Superior water that caused a significant increase in cough frequency in brook trout was 3 µg/liter. Cough frequency is a good short-term indicator of the long-term effects of MMC. Increases in cough frequency were proportional to the concentration (from 3 to 12 µg Hg/liter) of both compounds at pH 7.5. The fish were more responsive to MMC when the pH of the test water was lowered to 6.0; response to mercuric chloride was not changed by lowered pH. Fish exposed to MMC at pH 6.0 contained more total mercury in their gills and red blood cells than fish tested at pH 9.0. The uptake of mercury by brook trout exposed to mercuric chloride did not differ significantly at pH 6.0 and 9.0 (Robert A. Drummond, Gayle F. Olson, and Allen R. Batterman 1974).

The lowest concentration of cadmium to cause a significant increase in cough frequency was 6 µg Cd/liter. No additional tests are planned. A manuscript--some factors which affect the toxicity of cadmium to brook trout--is currently being prepared for possible publication.

Lead nitrate at a concentration of 125 µg Pb/liter was the lowest concentration of lead tested thus far to cause a significant increase in cough frequency. Additional tests are planned to determine if brook trout will respond to concentrations less than 125 µg Pb/liter.

A definitive increase in cough frequency has not been demonstrated when brook trout are exposed to sodium dichromate and zinc sulfate for periods lasting several days. Although some fish exhibited an increase in cough frequency, the results are considered inconclusive.

The following concentrations of malathion, lindane, and diazinon were found to cause a significant increase in cough frequency among brook trout:

Compound tested	No. fish tested	No. fish exhibiting a significant increase in cough frequency	Lowest concentration tested thus far
Malathion	8	8	12.2±1.4 µg/l
Lindane	8	8	5.3±1.0 µg/l
Diazinon	8*	4	24.6+4.4 µg/l
Atrazine and/or Parathion	Not tested yet		

*All eight fish lost virtually all of their natural body coloration. They were light-tan in color when examined at the end of the test.

All compounds will be retested at concentrations equal, or less than, the above reported values because several control fish responded to the acetone (260 ppm) carrier. We have modified our delivery system so that brook trout are not subjected to a concentration of acetone greater than 125 ppm. Preliminary tests indicate that brook trout do not respond to 128 ppm of acetone by increasing their cough frequency.

Several pilot tests were conducted to determine if fish other than trout and salmon would respond to toxicants by increasing their cough frequency. Largemouth bass, northern pike, bluegill sunfish, black crappie and channel catfish were found to respond to copper by increasing their cough frequency. Yellow perch, black crappie and channel catfish (other species not tested) exhibited an increase in cough frequency when exposed to methylmercuric chloride. In general, the increase in cough frequency was directly proportional to the concentration of copper and methylmercuric chloride as found for brook trout.

An instrument for indirectly monitoring specific movements or physiological events of fish, such as the frequency of opercular movements, "coughing", locomotor activity, and heart rate, was developed at the National Water Quality Laboratory. The previous instrumentation for indirectly monitoring these movements (locomotor activity excepted) required a set of inexpensive electrodes and an expensive EKG-EEG-EMG polygraph, consisting of a preamplifier, amplifier, and strip-chart recorder. We have now developed (Drummond and Dawson 1974) an inexpensive solid-state amplifier that can replace the expensive preamplifier and amplifier and is compatible for use with a variety of recording equipment. The solid-state amplifier should be particularly useful to researchers and students interested in instrumentation of this type but who cannot afford the necessary equipment costing several thousands of dollars. (Robert A. Drummond)

VIII. Concomitant with exposure of aquatic organisms to toxic chemicals is the need to measure the toxicant in the test chamber. For many discrete organic chemicals, the present analytical methodology is adequate to measure the chemicals at concentrations of a few ng/l. However, for toxicants which are complex mixtures such as the PCB mixtures, the determinable limit of present techniques is often 20 to 50 ng/l which precludes analytical support data for exposures at 10 ng/l.

To increase the sensitivity of PCB analyses in chronic exposures of trout to Aroclor 1254 and to assess the possibility of PCBs in Lake Superior water, a perchlorination technique using $SbCl_5$ was refined and evaluated. The greater sensitivity is realized due to the conversion of all chlorinated biphenyls to a single chlorocarbon, decachlorobiphenyl, and to the inherent greater electron capture response of the more highly chlorinated product. Equally important is the fact that the water extract can be taken to dryness and perchlorinated directly allowing the decachlorobiphenyl to be cleaned up for GLC analysis without interference from trace PCB contamination by reagents and glassware.

Analyses of PCBs in Lake Superior water and water from control tanks in the exposure laboratory were conducted by passing 20 ℓ of water through a column of polyurethane foam, extracting the adsorbed PCBs from the column with hexane and acetone, perchlorinating the extract residue, and quantitating by GLC. Confirmations of the perchlorination products were made using direct-probe mass spectrometry. The concentration of PCBs in the control tank water was approximately 3.4 ng/l (as Aroclor 1254 determined by analysis of fish residues) and the lake water contained less than 1.5 ng/l PCBs. The detection limit of the perchlorination method for PCB analysis is approximately 1 ng/l in water, and is largely dependent on contaminants in the $SbCl_5$ and the hexane extraction solvent. The technique also provides a confirmation technique for PCB in tissue residue analyses where GC/MS confirmation is not available. (Gilman D. Veith)

ROAP 16AAI - Develpment of Biological Test Methods

I. Research emphasis for this ROAP is placed on the development of techniques and procedures which will provide a broader base of test organisms for both acute and chronic bioassay testing. Laboratory procedures for successful rearing and propagation of a species must be developed prior to ascertaining if the species can be "stressed". If this cannot be accomplished, then the organism cannot be classified as a candidate for use in future exposures to develop water quality criteria for aquatic life.

Bioassay techniques for the parthenogenic chironomid, Tanytarsus dissimilis Joh. were developed. These techniques include both static and flowing full life cycle exposures and acute bioassays of selected stages of the life cycles. The procedures are now being prepared for presentation to the National Water Quality Laboratory Aquatic Bioassay Committee for their adoption as recommended procedures. Copies should be available in early 1974. (Richard L. Anderson)

Art Buikema, Virginia Polytechnic Institute has established laboratory colonies of the planktonic rotifer Polyarthra vulgaris using natural water and a food consisting of mixed protozoans enriched with a vitamin and trace mineral mixture. Colony densities of up to 10,000 rotifers per liter have been achieved.

First experiments were directed toward establishing basic biological information and were centered on determining photoperiod and temperature requirements. Other experiments are examining the population dynamics of this rotifer. A bioassay procedure is being developed. (Richard L. Anderson)

II. Acute toxicity work with salmon in FY 73 was directed toward determination of the effects of various experimental conditions on the LC50 data produced from the standard 96-hour flow-through bioassay.

A continuous flow serial dilution apparatus was designed and two preliminary tests were run at five concentration levels of zinc to determine toxicity of zinc to juvenile sockeye salmon. The 96-hour LC50 was determined to be approximately 1000 µg/l for fish averaging 6 cm in length and weighing 1.8 gms. The diluter system was then modified to deliver three concentrations of zinc solution, one at the 96-hour LC50 level, one at 130% of that and another at 65% of that level. Subsequent experiments were conducted at these concentrations with sockeye to determine the effect of changing experimental variables, such as acclimation time and feeding.

Two sets of experiments were run in which one-half of the test fish were fed Oregon moist pellets and the other fish were not fed. For the first 48 hours, the fed fish were more sensitive to zinc poisoning, but after about 72 hours, the fed fish were less sensitive than those which were not fed. This phenomenon has not been adequately explained, but it does demonstrate that bioassay results between fed and non-fed fish are not directly comparable, and this factor can be an important source of variability in toxicity data.

Three sets of experiments were run to compare the effect of acclimation time upon LC50 data. The experiments compared one week acclimation to none, two weeks versus three days, and one week versus two weeks. In each case, acclimation was done in the test aquaria with test dilution water. In each set of experiments, the fish with the longest acclimation time were more resistant to zinc poisoning.

At lethal concentrations of zinc, sockeye become very skittish after about 48 hours of exposure. When disturbed, they rush about the aquaria in a panic which results in the fish rolling over to belly-up position, where it remains quivering and often with gills and gill covers flared and extended. This sometimes leads to death, but the fish often recover to die later in the experiment. Since some fail to recover, any disturbance may cause a change in death rate of the fish, and thus a change in LC50.

Experimental results to date emphasize that toxicity data are strongly influenced by conditions of the test. The final report of this research project will be a proposal for a standard bioassay technique to be used in toxicity tests with juvenile Pacific salmon. (Ronald R. Garton)

III. The National Water Quality Laboratory has continued to support the Committee on Methods for Toxicity Tests with Aquatic Organisms in its effort to obtain uniformity in methodology and nomenclature. Other organizations cooperating in the work of the committee include two Department of the Interior laboratories, EPA Office of Pesticides Programs, two other EPA research laboratories, three industry associations, some EPA enforcement offices, several individual companies, and some state and university programs. Over 200 other interested people are being contacted through a mailing list. The committee plans to publish four methods for acute tests with fish and macroinvertebrates, encourage support of activities of the American Society for Testing and Materials in the area of toxicity tests with aquatic organisms, and disband in 1974. NWQL's input to such activities is funneled through the National Water Quality Laboratory Committee on Aquatic Bioassays, which is responsible for establishing methods for acute and chronic tests with fish and macroinvertebrates. (Charles E. Stephan)

IV. A series of observations were made in the lbaoratory on seven small native fish species to determine the advisability of their use in chronic (life-cycle) toxicity studies. Specific requirements essential for completing a life cycle in the laboratory were studied for each species.

Notropis lutrensis, the red shiner, has matured and spawned in the laboratory in 16 weeks from hatch at 22-25 C and a 16-hour light photoperiod. Under these conditions they have continued spawning semi-continuously for more than one year. The species is easily maintained. Adult fish are fed trout food granules and frozen brine shrimp while larval fish are started readily with brine shrimp nauplii.

Notropis hypselopterus, the sailfin shiner, has matured and spawned in the laboratory in 20 weeks from hatch at 25 C and 16-hour light photoperiod. This species spawns semi-continuously for more than one year under these conditions. These fish require a water depth of about 14 inches for spawning. Feeding is as reported for the red shiner.

Johnny darters were satisfactorily reared from eggs but although various combinations of temperature and photoperiod manipulation were used, no spawning occurred in 17 months from hatch. It is believed that this species is probably a second year spawner even under optimum conditions, and work was suspended on it for that reason.

Brook stickleback. This species was obtained from the Duluth area as adults. Spawnings were obtained readily from each of five tanks containing two males and three females. No problem was experienced in hatching the eggs, and young were raised easily on brine shrimp nauplii. It was discovered, however, that the young fish would not tolerate any handling of any kind, apparently dying from shock. This means that it would be a practical impossibility to use the fish as a test animal in the laboratory, so work on the species was discontinued.

Jordanella florida. Procedures and test methods involving the use of this species as a test organism have been published. The species shows tremendous promise as a test animal because of the relatively short period from egg to egg (approximately 8 weeks). It is a prolific egg producer with numerous spawnings, and is quite easy to rear and maintain under laboratory conditions.

Green sunfish. Some work with this species has been done. They have been spawned and the F_1 fish matured and spawned in one year. From hatched eggs F_1 fish are being raised in an attempt to speed growth and reduce the age of maturity. At present, the prospects are encouraging and it is believed that this species would make a satisfactory laboratory test fish.

Longear sunfish. Work with this fish is in progress. There is no problem in spawning the species, and feeding of adults and young is accomplished with ease. Current efforts include an attempt to grow and mature the F_1 fish in the least possible time. (Wesley E. Smith)

V. A rearing study with Gammarus pseudolimnaeus, a freshwater amphipod, was completed this year. Objectives were to determine the effects of food, light, and test duration on the fecundity of this animal. Results showed that this species feeds equally well on soaked aspen or birch leaves, but does poorly with maple leaves. A light intensity of greater than 20-foot candles is needed for satisfactory reproduction. Light intensities of 500-foot candles visually increased the algal growths in the test tank waters and appeared to hinder the swimming of the amphipods. General Electric daylight fluorescent bulbs were apparently as satisfactory for rearing this species as a combination of Durotest Optima and wide-spectrum Grolux bulbs. No marked differences were found in the reproductive behavior between animals in tanks under a constant photoperiod and with a variable photoperiod. After 13 weeks at 17° C the production of young started. Young production continued throughout the duration of the test (a period of 38 weeks). (Jack W. Arthur)

VI. The copepod, Eucyclops agilis, was cultured in 1 L. battery jars at a temperature of 18 ± 1° C on a cycle of 12 hours darkness/12 hours light of an intensity of ∿ 13-foot candles at the water surface. Cultures were initiated by the introduction of ten gravid female copepods into a battery jar nearly filled with lake water filtered through #250 mesh screening. To this is added 3 ml of prepared copepod food*. It is possible to maintain a population adequate for the initiation of a flow-through bioassay by initiating at least two cultures per week. A flow-through chronic bioassay involving survival, growth and reproduction has been developed for these copepods and is being evaluated prior to a formal methods write-up.

*Food preparation: Blend for five minutes at high speed 5 grams of trout starter granules, 225 grams of Cerophyll, and 125 ml of lake water. Pour blended mixture through #250 mesh screening, rinsing the remaining portion from the blender with 25 ml lake water and also pour this rinse through the screening. Store in the refrigerator. (Rita R. Le Duc)

VII. Dan Martin, Bureau of Sport Fisheries and Wildlife, Yankton, South Dakota, has maintained stock cultures of Cyclops in Missouri River water and Lake Superior water for eight months, and it appears that permanent cultures have now been established. No differences between populations of each culture were noted. Observations on life history using live food cultures have been completed, and observations on life history using dried foods are in progress. Stock cultures of Cyclops using dried foods are underway.

All efforts to culture Bosmina under controlled, reproducible conditions have failed. "Air-locking" appears to be the principal cause of mortality and no method of preventing this has been found. Consequently, cultures of Chydorus, a similar sized cladoceran, are being maintained and future emphasis will be placed on the study of this organism. (Richard E. Seifert)

Grant 802210 - An Evaluation of the Sensitivity of Cladocerans to Copper, Chromium and Zinc. Miami University, Miami, Ohio. Charles W. Winner - Project Investigator. Timothy W. Niehiesel - Project Officer.

This research grant awarded to Miami University, Miami, under the direction of Charles W. Winner is designed (1) to devise acute and chronic bioassay techniques for use with cladocerans, taking into account adequate nutrition, the effects of various food types, and the size of containers in which bioassays are conducted; (2) to determine how much variation exists between various species of cladocerans in their sensitivity to heavy metals; and (3) to evaluate the application-factor-concept as it applies to acute and chronic toxicities of heavy metals to cladocerans.

Two quarters of work have been completed, with most of the work being directed toward the development of a rearing technique that will ensure reproducible results in both short- and long-term toxicity testing. It was found that: (1) vitamin-enriched algal food was superior to either yeast or fish pellets, particularly in the standard reconstituted water, (2) standard soft reconstituted water reduced longevity when compared to pond water, however, longevity and reproduction seem to be consistent for animals reared in it; (3) that increasing the salt concentration of the standard soft water to produce a medium hard water increases the longevity beyond that obtained in pond water; (4) daily transfer of animals to new media reduced longevity when compared to transfer every three days; and (5) species sensitivity to toxicants with short-term tests indicate D. amibgua was more sensitive to copper than D. magna.

Several acute toxicity tests have been completed with Daphnia magna using standard soft water, and algae as food. The LC50 values for Zn and Cu are intermediate between the fed and unfed LC50 values found by Biesinger and Christensen (1972) in Lake Superior water. (Timothy M. Neiheisel)

VIII. Under the direction of Lloyd L. Smith, Jr., University of Minnesota, St. Paul, Minnesota, five lines of experiments are incorporated in the project plans to evaluate the goldfish and fathead minnows as possible standard bioassay animals for both acute and chronic testing: (1) acute lethal bioassays; (2) non-toxic chronic bioassays (for development of methodology); (3) toxic chronic bioassays; (4) investigation of a reference toxicant; (5) tests to determine the effects of temperature and temperature acclimation on acute bioassays.

Four and one-half of the planned nine series of acute bioassays have been completed.

Table 1. Mean LC50 Values for Replicate (N=5) Acute Bioassays of Four Toxicants to Goldfish and Fathead Minnows (in mg/1).

LC50	Toxicant			
	NaCl	PCP*	Cr^{+6}	Guthion
Fathead Minnow				
24-hr	7711	.201	136	5.45
96-hr	7547	.197	51	1.81
Asymptotic	7538	.197	17.5	0.72
Goldfish				
24-hr	9613	.220	265	7.59
96-hr	7086	.201	118	2.35
Asymptotic	7086	.201	30.1	0.95

*Pentachlorophenol.

The pattern of mortality differs widely among the different toxicants. The onset of mortality is rapid with both sodium chloride and penta-chlorophenol (PCP). By 48 hours, mortality usually ceases and the defined asymptotic LC50 is attained by 96 hours. With hexavalent chromium, the onset of mortality is slow, particularly with goldfish where there is frequently insufficient mortality by 24 hours to report an LC50. However, mortality then continues to occur for the entire 11-day test. Mortality from Guthion is somewhat unusual. The fish react immediately to the addition of Guthion, and some mortality occurs from 2 minutes to a few hours after introduction of the toxicant. This mortality appears relatively independent of toxicant concentration such that equal percentages of fish may die at low, intermediate, or high concentrations. There is then a period of approximately 12 to 24 hours when no mortality occurs, and then fish begin to die again. This resumption of mortality does correspond to toxicant concentrations. Like chromium, the defined asymptotic LC50 is not attained by 11 days, although this level is approached.

An abnormal stock of fish was detected by either a different pattern of response as with PCP or by decreased 96-hr and asymptotic LC50's as with sodium chloride and Guthion. Only the hexavalent chromium did not reveal a difference in response.

A 2^3 factorial chronic bioassay of temperature, tank size, and food was set up with goldfish. The purpose of this experiment is to develop techniques for chronic bioassays of goldfish, particularly with the intent of obtaining reproduction in less than one year. The bioassay has been running for approximately one year.

Table 2. Mean Weight (g) of Goldfish After 11 Months Growth Under Given Experimental Conditions

20° C				25° C			
Oregon moist diet		Mixed diet		Oregon moist diet		Mixed diet	
large tank	small tank	large tank	small tank	large tank	small tank	large tank	small tank
82.51	78.01	72.14	1/	112.67	93.72	89.62	80.62
90.07	79.09	87.56	66.27	114.67	97.96	81.48	82.46

Although all fish in the experiment have attained a size and reached an age at which spawning is known to occur, no reproductive behavior or secondary sexual characteristics have appeared. Gonads of fish that have died during the past few months have been undeveloped. Since it is not desirable for such a chronic bioassay to be conducted for more than one year, this experiment has not revealed satisfactory conditions to achieve reproduction.

A chronic exposure of fathead minnows to Guthion has also been initiated. Initial results indicate that eggs and fry survival is satisfactory at concentrations below approximately 2.5 µg/l. (Robert W. Andrew)

ROAP 16AAO - Complex Effluent Criteria.

The contract to determine suitable sites for evaluating the biological testing of aqueous effluents was completed. The final report for this contract listed eighteen possible industrial or municipal sites acceptable for testing. (Charles E. Stephan)

ROAP 16AAF - The Evaluation of Laboratory Chronic Bioassay Methodology by Continuous Chronic Exposure of a Natural Ecosystem.

Construction and completion of the test facility on Shayler Run to validate laboratory findings in a natural stream were completed in 1968. The first year of testing was designed to accumulate baseline data prior to beginning a two-year exposure to Cu^{++} at a constant concentration of 120 ppb. The Cu^{++} exposure was continued for 35 months with data accumulated over the entire exposure period. This five-year study was completed in mid 1973, with the details of the study being written in five parts.

Part I is the introduction to the project dealing with the background and rationale, the physical aspects of the project, copper data analyses and the stream water quality. Part II encompasses the biological aspects of the study, handling the fish species native to the stream and the effects of the Cu^{++} exposure to these fishes. Part III encompasses the effects of Cu^{++} on the invertebrates of the stream. Part IV addresses itself to the acute and chronic toxicity tests conducted throughout the study. Finally, Part V will be a Summary Report for the completed study. Preliminary results from the data accumulated during this study can be obtained from the Chief of the Newtown Fish Toxicology Station. (William B. Horning)

ROAP 05ADD - Tolerance Levels of Ammonia for Aquatic Life

This research grant was awarded to Dr. C. J. D. Brown, Montana State University, in the fall of 1970 to determine the acute and chronic toxicity of ammonia to the rainbow trout and the fathead minnow. During the first year grant period, because of several set-backs not directly related to the exposure research, very little was accomplished. However, upon final completion of the physical testing facility, research was started and is now progressing quite well. Dr. Brown has retired since the grant was initiated and Dr. Vance Thurston is now the principal investigator.

To date, 38 rainbow trout bioassays have been completed using ammonia, nitrite, copper or ammonia/copper complexes as the toxicant. The water quality conditions for these bioassays were pH 7.9, temperature 9° C and total alkalinity of 180 mg/1 expressed as $CaCO_3$. Results from these bioassays show that the lethal threshold concentration for ammonia using adult rainbows (340 gram average weight) is 13.5 mg/1; that the lethal threshold concentration for ammonia to juvenile rainbows (17 gram average weight) is 26.8 mg/1; that the lethal threshold concentration for rainbow fry is between the values for adults and juveniles; that the lethal threshold concentration for copper to rainbows in the 1-4 gram range averaged 0.16 mg/1; that the lethal threshold concentration for nitrite to rainbows is 0.14 to 0.22 mg/1 (expressed as total nitrogen). When bioassays with copper and ammonia combined were conducted, the toxicity of the mixture was greater than the toxicity of copper and ammonia separately. Field studies are now being conducted to verify these results under natural conditions. Present projections indicate that the grant will be successfully completed by December 1974. (Kenneth E. F. Hokanson)

ROAP 21AKH - Tolerance Limits of Hydrogen Sulfide for Aquatic Life

Grant R800992 - Influence of Hydrogen Sulfide on Fish and Arthropods. This grant was awarded to the University of Minnesota, St. Paul, Minnesota, under the direction ot Lloyd L. Smith to continue work on chronic effects of hydrogen sulfide on several fishes and important fish food organisms. Experimental work continues only with Hexagenia and Louisiana crayfish as this 5-year grant nears completion. "No effect" levels for growth of juvenile bluegill and brook trout were 0.002 mg/1 undissociated hydrogen sulfide in recently completed bioassays. Progress continues on development of an analytical technique for measurement of undissociated hydrogen sulfide in aqueous solutions. A vapor phase equilibration technique has been developed in which sulfide in water is displaced by nitrogen. Recovery of 100% sulfide has been achieved by collection on glass bead columns coated with 0.1 N zinc acetate. Calibration curves are being developed in order to determine ionization constants at several temperatures and pH conditions. This procedure is being developed to improve the accuracy of the current standard methods for total sulfide measurements and to correct the reported bioassay results if necessary. The principal investigator has requested a time extension to prepare the final report since project personnel have now been shifted to a new Environmental Protection Agency grant to develop hydrogen cyanide criteria. (Kenneth E. F. Hokanson)

ROAP 16AAE - Bioassay Technical Support

I. Organic Compounds in Industrial Wastewaters. In response to a memorandum from William Donaldson last December, a work plan was developed to assess the short-term toxicity of approximately 30 organic compounds identified in industrial wastewaters. Static 96-hour tests have now been completed for nine compounds (Styrene, acetophenone, furfural, cresol, camphor, ethanol, benzyl alcohol, eugenol and butanol) using one to two month old fathead minnow larvae at a test temperature of 20° C. Preliminary LC50 values showed that P-cresol was the most toxic (19.8 mg/l) with ethanol the least toxic (13,480 mg/l). All tests were run in natural Lake Superior water without the addition of any other solvent to aid in solubilization of the test compound. Two compounds were also tested in soft reconstituted water according to a formula recommended by the Committee on Methods for Toxicity Tests for Aquatic Organisms. Almost identical toxicity results were obtained with this diluent water when compared to that obtained in Lake Superior water. (Vincent R. Mattson and Charles T. Walbridge)

II. Toxicity of a Disinfected Sewage Effluent. The purpose of this study was to establish short-term lethal levels and no effect concentrations of a chlorinated, dechlorinated, ozonated and nondisinfected secondary sewage effluent to selected macroinvertebrates and fish. A draft of the report has been completed and is undergoing internal review. This report will be submitted for publication in the Environmental Protection Agency's Ecological Research Series. Source of the secondary sewage effluent was from Silver Bay, Minnesota. Lake Superior water served as the diluent source for the tests. The 7-day exposure tests were conducted with 7 fish and 6 invertebrate species. The chlorinated effluent was appreciably more toxic than the other three effluents with short-term animal survival affected in chlorinated sewage concentrations as low as 6-12 percent. The corresponding 7-day TL50 values of total residual chlorine to the fish and invertebrates ranged from 0.08 to 0.26 and 0.21 to >0.81 mg/l, respectively. More than 50 percent of all the animals tested survived in 100 percent concentrations of dechlorinated and ozonated effluent and 11 of 13 species in 100 percent concentration of nondisinfected effluent. With the chlorinated effluent most fish mortality occurred in the first 24 hours whereas longer exposure periods were needed to cause invertebrate mortality.

The three species used for the long-term generation cycle tests were fathead minnows, the amphipod, Gammarus psuedolimnaeus, and the water flea, Daphnia magna. For these tests the highest nominal sewage concentrations were 20 percent. Survival and reproduction of these animals were affected mainly in the chlorinated effluent. For the fathead minnows, survival and spawning was affected in mean

total residual chlorine levels of 110 and 42 µg/l. Survival of the next generation progeny after 30 days was reduced in mean residual chlorine concentrations of >21 µg/l. The amphipods failed to survive in the highest mean test chlorine level (123 µ gg/l) and did not reproduce in mean residual chlorine concentrations ≥ 53 µg/l. Reproduction at mean residual chlorine concentrations of 19 µg/l were lower than the corresponding controls. For the daphnia tests, yeast and trout chowcerophyll was continuously added to the diluent water to maintain satisfactory survival and reproduction in the control tanks. Approximately 10 µg/l total residual chlorine eliminated their survival and reproduction. Statistical analysis of all chronic data will be performed to further elucidate the levels of residual chlorine having no-effect for all three test species. (John W. Arthur)

III. Acute Toxicity of Selected Toxicants to Six Fish Species - Envirogneics Company. Purpose is to determine the acute toxicity of seven compounds to six fish species. The following is a summary of the results gathered so far and reported in terms of measured concentrations. The threshold LC50 values for selenium dioxide to bluegills, goldfish, and fathead minnows were about 18, 9, and 3 mg/l, respectively. The threshold LC50 values for sodium cyanide to goldfish, fathead minnows, and brook trout were about 0.5, 0.2, and 0.2 mg/l, respectively. The threshold LC50 values for sodium arsenite to bluegills and goldfish were about 32 and 33 mg/l, respectively. The threshold LC50 values for beryllium sulfate to goldfish was about 60 mg/l and that for sodium pentachlorophenate to goldfish about 0.2 mg/l. However, 500 mg/l lead chloride did not kill fathead minnows in a four-day static exposure. A range finding test has been conducted with goldfish and O-xylene. (Charles E. Stephan)

IV. Literature Research on Water Quality Requirements and Toxicology of Fishes (18050 FQW) - Oregon State University, Peter Doudoroff. The purpose of this project is to prepare critical reviews of the world's literature on the toxicology of cyanides, sulfides, ammonia, and ammonia salts, carbon dioxide, and dissolved oxygen to fish. In addition, Soviet literature has been collected dealing with fish toxicology and lengthy English summaries have been prepared.

To date 24 Russian articles on fish toxicology have been translated into English with lengthy summaries and comments. (Mary J. Lewis)

V. Effects of Chlorinated Effluents in Lake Michigan (18050 HPM) - University of Wisconsin, Alfred Beeton. The purpose is to determine the effects of chlorinating a large municipal effluent on Lake Michigan biota and possible corrective measures. Chemical surveys of the Milwaukee harbor around the Jones Island sewage outfall showed that active chlorine species were limited to a small area within 100 M of the outfall. Monochloramine concentrations of 0.133 mg/1 were measured at the outfall, 0.03 mg/1 70 M from the outfall and no chloramines measurable 200 M from the outfall in the surface waters. At a depth of 6 M, no chloramines were detectable at sampling points surrounding the outfall. The 96-hour TL50 values of sodium hypochlorite and monochloramine to Cyclops bicuspedata was determined to be 0.005 - 0.1 and approximately 0.09 mg/1, respectively. (Donald T. Olson)

VI. Environmental Impact of Products from the Chlorination and Ozonation of Municipal and Industrial Waste (R800675), University of Minnesota, Carlson. The purpose of this grant has been the determination of chemical and toxicological changes in organic compounds due to disinfection practices such as chlorination and ozonation.

It has been shown that chlorine is incorporated into organic molecules by lowering the pH. Determinations are also being made of the structural components necessary for toxicity using the Hansch approach. With chlorinated phenols, toxicity increased to Daphnia magna with increasing molecular chlorine content. All compounds that have been investigated have been oxidizable with ozone without significant toxicity. During this oxidation with ozone, hydrogen peroxide was produced and is of concern because of its known high toxicity of 182 compounds to Daphnia magna. (Gary E. Glass)

VII. Effect of Cyanide on Freshwater Fish and Invertebrates, University of Minnesota, Smith. Purpose of this project is to determine the effects of temperature, pH, and oxygen on cyanide toxicity, the additive or synergistic relationships between heavy metals and cyanide, evaluate the life history stages most sensitive, and determine application factors that might predict safe levels of cyanide or cyanide complexes. This project is scheduled to start this month. (Robert A. Drummond)

ROAP 1BA021 - Technical Assistance Plan

In 1973 the National Water Quality Laboratory responded to requests by various organizations to such an extent that 1,979 man-days

(7.6 man-years) of effort were expended. The following Table demonstrates the relative distribution of these requests in man-days:

USEPA Office of Enforcement (Reserve Mining Company Case)	1,449
USEPA Regions	94
Other USEPA (mostly Headquarters)	213
States, Municipalities and Universities	45
Other Federal Agencies	15
Literature Review for Journal of the Water Pollution Control Federation	105
Miscellaneous	58
Total	1,979

The most significant responses involved the Reserve Mining Company litigation, preparation of the freshwater temperature criteria for aquatic life, and the annual literature review, "Effects of Pollution on Freshwater Fish", published in the Journal of the Water Pollution Control Federation (June 1973, 45:1370-1407).

The USEPA's Division of Water Quality and Non-Point Source Control requested that the staff of NWQL prepare a document for temperature criteria to be used as their response to Section 304(a)(1) of PL92-500. The NWQL staff, utilizing the National Academy of Science revision of Water Quality Criteria, 1968, developed maximum weekly average temperatures for growth and reproduction, for heated plume limitations to protect against low temperature shock, and short-term maximum temperatures to protect against mortality during the seasons of growth and reproduction for 24 fish species. These criteria were included in the Proposed Criteria for Water Quality, Volume I, USEPA, October 1973.

The annual literature review on pollution effects on freshwater fish involved the acquisition, evaluation, and summarization of 375 publications. This effort will be repeated for the 1973 literature and is an excellent means for the staff to be aware of current research being conducted on pollution effects to the aquatic environment.

The Reserve Mining Company case is currently under litigation with the effort now being expanded to provide technical case coordination for the Justice Department, USEPA and State attorneys who are prosecuting the case. During 1973, field and laboratory studies were completed in order to assess the effects of the Reserve Mining Company discharge into Lake Superior at Silver Bay, Minnesota. Reports on the results of these studies were compiled and submitted to the U. S. District Court, Minneapolis, Minnesota. The summary given to the Courts of each study in which staff at the NWQL played a major role is given below.

Characterization of the North Shore Surface Waters of Lake Superior.

The taconite processing operations at Silver Bay, Minnesota, are contributing to an increase in turbidity in Lake Superior, particularly in the area southwest of Silver Bay, but also to a lesser extent in other nearby areas. Suspended solids in the same area have been increased as the result of the ore-processing operations.

Material from the tailings discharge is being transported to all parts of the study area, and current data indicate that this being the case, the tailings fines are at least being distributed throughout the Duluth arm of Lake Superior.

Many episodes of green water seen along the North Shore of Lake Superior are caused by particulate matter from the tailings operation.

A Study of Western Lake Superior: Surface Sediments, Interstitial Water and Exchange of Dissolved Components Across the Water-Sediment Interface

A study of western Lake Superior during the summer of 1972 has demonstrated that lake sediments contribute dissolved silica, calcium, magnesium, potassium, manganese and ortho phosphate to the overlying lake water.

The surface sediments in the area of Silver Bay, Minnesota, were found to be mainly composed of taconite tailings covering an area greater than 110 square miles. This layer was found to be more than 8 cm thick 1-3 miles off shore at Beaver Bay and Split Rock sampling stations.

The composition of lake water in the area of Silver Bay, Minnesota, when compared to an area at Hovland, Minnesota (70 miles up current, NE), showed higher concentrations of potassium (+10%), manganese (+800%), suspended solids (+800%), and turbidity (+500%). Suspended solids in the Silver Bay area are mainly composed of taconite tailings.

During the second cruise period, September 15-24, 1972, current meter data showed a large river of water (3.6 trillion gallons/day) flowing past RMC's delta in a southwesterly direction toward Duluth. Calculations based on Lake current data and increases in concentrations of measured components show that 4,500 tons of suspended taconite tailings, 5,100 pounds of dissolved manganese, and 200,000 pounds of dissolved potassium were being transported "downstream" in the bottom 30 meters of Lake water per day.

Interstitial water of lake sediments is much higher in concentrations of dissolved components. Higher concentrations of silica (+30%), magnesium (+50%), and copper (+200%) were found to occur in taconite tailings sediment compared to lake sediments.

Samples of lake water collected at different depths indicate the lake was generally well mixed. However, measurable concentration gradients of silica, potassium, manganese, copper and oxygen were observed at several sampling stations, with metals increasing and oxygen decreasing close to the water-sediment interface.

Any material which is deposited on the lake bottom and is subsequently dissolved in the interstitial water is available for further chemical and biological interaction in the lake system. Lake currents and turbulence effectively mix the bulk water, eliminating extensive concentration gradients. Undisturbed lake water-sediment cores stored for two months at lake conditions, showed distinct concentration profiles of dissolved substances exchanging from higher concentrations in the interstitial water to the interface bulk water. Earlier studies demonstrated that taconite tailings dissolved under lake conditions in the laboratory. These findings have now been documented in Lake Superior. Lake sedimented taconite tailings continue to contribute dissolved components to the interstitial and bulk water of the lake.

Distribution of Taconite Tailings in Lake Superior and Public Water Supplies

This data report includes results of the analysis of Lake Superior water used in the drinking water supplies of Duluth, Two Harbors, Beaver Bay, Silver Bay, and Cloquet, Minnesota, where tailings were found to be present. Grand Marais, Minnesota, Superior, Wisconsin, wellwater and Ashland, Wisconsin, water samples did not show tailings by X-ray diffraction. Analysis of sediment from all streams and rivers tributary to Western Lake Superior showed only one sample with a trace of cummingtonite-grunerite. Water samples saved at the Lakewood Pumping Station of the Duluth water supply from the periods 1939-1940 and 1949-1950 did not contain cummingtonite-grunerite. Samples from 1964-1965, after the Reserve Mining Co. discharge had begun, contained large amounts of this mineral. These results confirm the validity of using the amphibole mineral cummingtonite-grunerite, which makes up a large % of the Reserve Mining Company discharge, as a valid tracer for the discharge. The asbestiform morphology of taconite tailings amphibole minerals is described and date are presented showing the characteristic acicular or fibrous habit.

Stomach and Residue Analysis of Fourhorn and Slimy Sculpins from Two Areas Along the North Shore of Lake Superior

1. Sculpins living in the vicinity of the taconite plant at Silver Bay eat fewer Pontoporeia than sculpins living away from it, indicating a reduction in Pontoporeia in the areas sampled near the plant.

2. At least at certain times, sculpins living near the taconite plant eat more fish eggs, perhaps due to a reduced abundance of Pontoporeia.

3. The results from the first cruise, while less informative, support the results of the second cruise in regard to the regional abundances of Pontoporeia.

This part of the study was initiated to determine if measurable changes in the concentrations of trace metals and organic residues occur in sculpins which live in the areas of Lake Superior that are covered with taconite tailings. The work was undertaken with full realization that the content of some metals such as mercury in fish may not be determined by the concentration of the metal in the habitat. Rather, the observed residue may be the result of more subtle transport interactions of the animal with the environment and food supply. Moreover, whereas mercury concentrations within a population may be correlated with size, the concentration of other metals, such as Fe, Cu, Mn, and Zn, appear to be regulated by species-specific physiological processes. Furthermore, it was found that there was no accumulation of copper in the opercle, red blood cells, and blood plasma in fish exposed to lethal and sublethal concentrations of copper. Increases in the concentration of copper in the liver and gill tissue were not observed at low concentrations of copper until the fish were exposed to 27 µg/l in water. Thus, the lack of residues does not preclude a stress on the animal.

The data have shown that discernible trends do not exist in the concentrations of metals in sculpins along the north shore of Lake Superior. The absence of any pronounced differences may be due to insufficient sample size from the population, analytical variations which are significant at the low concentrations encluntered, unelucidated physiological processes alluded to above, similarities in the composition and availability of the metals in the two environments, or a combination of these effects. Of particular interest is the fact that, although trends are not apparent along the north shore, the data presented consistently show that both fourhorn and slimy sculpins from the north shore contain approximately 1.5 to 2 times more mercury than do the sculpins from the Apostle Islands or Keweenaw Bay area. It will be important to determine whether this variation is a result of different geochemical environments in the respective watersheds, or if the Apostle Islands and Keweenaw Bay area are more representative of the natural Lake Superior environment and the entire north shore region of Lake Superior has been contaminated.

Periphyton Growth on Artificial Substrates in Lake Superior

1. Mean water transparency measurements at two transects southwest from Reserve Mining Company were less than transects sampled northeast from the plant.

2. The predominant current direction measured in the surface water layers in the study area was in a southwesterly direction.

3. Diatoms comprised the largest portion of the periphyton forms counted from both substrates. Summarizing the periphyton forms by phyla showed no consistent differences at the transect locations used during each sampling phase.

4. Significant correlations were found at each depth between total cells and chlorophyll, and dry weight and solids from the two-week nylon net determinations.

5. Higher periphyton growth and solids concentrations were determined from the most inshore nylon nets as opposed to those suspended offshore. Mean tailings levels were more equally distributed except at Split Rock where higher levels were found closer inshore.

6. Detectable levels of tailings were measured from nets suspended in the North Shore waters as far as 61 miles northeast and 22 miles southwest from the plant.

7. Statistical considerations in this report neither confirm nor deny an association between periphyton growth and taconite tailings.

Effect of Taconite Tailings Upon Lake Superior Periphyton Under Controlled Conditions

Periphyton growth was monitored during this study by chlorophyll and cell count measurements and indirectly by oxidizable organic material. Growth was found to be significantly stimulated in tanks having tailings concentrations of greater than or equal to 5 mg/l.

Transfer of Elements Associated with Taconite Tailings to the Liver and Kidney of Rainbow Trout

Liver and kidney of rainbow trout accumulated several radioactive elements previously associated with neutron-activated taconite tailings. Livers contained gamma-emitting radionuclides of sodium (^{24}Na), potassium (^{42}K), bromine (^{82}Br), iron (^{59}Fe), cesium (^{134}Cs), cobalt (^{60}Co), and rubidium (^{86}Rb) from the tailings; the kidneys were not analyzed for the short-lived materials (^{24}Na, ^{42}K, and ^{82}Br) but did contain the other radioactive elements found in the livers.

Since contamination of the biological samples was avoided, the transfer of these elements from taconite tailings to internal organs of fish indicates that at least these elements in tailings are biologically available.

The Toxicity of Two Flocculants and One Flotation Reagent to Selected Aquatic Animals in Lake Superior Water

Superfloc 330, Calgon M-500, and Azamine A-3 were found to be toxic to aquatic life. Twelve-day constant flow tests with lake and rainbow trout showed that 0.3 mg/liter Superfloc 330 killed half of the animals tested. Mysis relicta were killed at concentrations of <0.06 mg/l Superfloc 330 and a concentration of 0.9 mg/l Azamine A-3 in 14 days.

Analyses and Laboratory Experiments with Taconite Tailings

The chemical characteristics of taconite tailings are complex and vary with the physical characteristics of the sample, such as particle size distribution, as well as chemical conditions. Tailings have been shown by spark source mass spectrometry, neutron activations, and atomic absorption and emission spectroscopic techniques, to contain a large number of the elements. Many of these metals can also be found in water associated with tailings and in larger concentrations in acid leachates from tailings. Among these elements are Hg, Cu, Mn, As, Zn, Mg, Se, and Co.

Gas chromatographic and mass spectrometric analysis of organic residues extracted from composite tailings samples demonstrates the presence of hydrocarbon oils associated with the tailings patticles.

X-ray diffraction and electron micrographs of RMC taconite tailings show the presence of asbestiform minerals. Preliminary data show 1 x 10^8 (100 million) fibers of asbestiform minerals (cummingtonite-grunerite) per milligram of <2 micron size fraction. The total number of particles per milligram of <5μ size composite of taconite tailings was found to be 8.8 X 10^8 (800 million) by Coulter Counter.

The presence of an organic complexing agent in an aqueous suspension of taconite tailings gives the predictable increase in dissolution of tailings due to the stabilization of metal ions going into solution. Such complex formation is known to influence metals concentrations in natural waters.

Analysis of the overlying and interstitial waters from 64-day-old settled tailings samples, kept cold and dark, shows large increases in concentrations of SiO_2, Ca, Mg, Na, K, Cu, Mn, and Fe from the original lake water values. These increases are also evident in measurements of the water just over the sediment interface, indicating the transport of these dissolved elements into the water column. A large increase in specific conductance is observed as the interface is approached and oxygen levels are depressed.

No differences in solubility of taconite tailings were found due to neutron-activation followed by leaching for the measured parameters SiO_2, Ca Mg, Na, K, Ca, Mn, Fe, pH, and conductance.

A Summary of the Studies Regarding the Effect of the Reserve Mining Company Discharge on Lake Superior

1. Cummingtonite is an accurate tracer for measuring tailings in Lake Superior. It is not found in tributaries.

2. Tailings are deposited on the bottom over most of the western part of Lake Superior.

3. Tailings are a major component of the suspended solids of the western part of the Lake.

4. Tailings are a major factor causing green water at least along the Minnesota shore.

5. Tailings reduce water clarity 25% or more over an area of at least 600 square miles.

6. Tailings have been found as a major component of the suspended solids in the City of Duluth and National Water Quality Laboratory intakes in every sample that has been analyzed during 1972-1973.

7. In 500 days, tailings dissolved approximately twice as fast as a natural lake sediment and to the extent of .8 to 1.0%.

8. Calcium, magnesium, sodium, alkalinity, manganese and silica are major constituents comprising the soluble portion.

9. The discharge contributes at least 160,000 pounds per day of dissolved solids to the Lake water, not including contribution from suspended tailings, tailings on the Lake bottom, and those in contact with the interstitial water.

10. A 100 square mile test area of the lake having high tailings deposition contained higher potassium and manganese concentrations compared to a similar area of low tailings deposition. The interstitial water had higher silica, magnesium, copper, calcium, and manganese, but lower organic carbon and hydrogen and reactive phosphate concentrations.

11. The discharge usually has higher counts of bacteria than the intake, but coliform counts are relatively low.

12. Bacteria associated with the tailings are stimulated to grow or survive longer in lake water with tailings present.

13. Tailings as low as 4 ppm have a mild stimulatory effect on phytoplankton growth under some conditions. The manganese content of tailings may be an important contributing factor.

14. Results from incomplete experiments suggest tailings may have a strong stimulatory effect on algal periphyton.

15. Pontoporeia, an important food species of lake trout and herring, limited to a few lakes in the U. S., are reduced in numbers over an area at least some 30-40 miles southwest of the plant. There is an increase in midges and oligochaetes.

16. This reduction in Pontoporeia is reflected in altered food habits of a fish, the sculpin, living in the area of reduced Pontoporeia populations.

17. Tailings do not appear to be directly toxic to most organisms.

18. Changes in organism populations would have to approach 50% before they would be detected in the Lake.

19. Tailings are chemically and biologically active.

The final summary was prepared by Dr. Donald I. Mount, Director, NWQL as an overview for all the summary reports.

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