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ACUTE TOXICITY OF RESIDUAL CHLORINE ON BLUEGILL AND CHANNEL CATFISH IN ILLINOIS

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ABSTRACT

Ninety-six hour residual chlorine bioassays were conducted on bluegill and channel catfish with a modified Mount and Brungs proportional dilutor. The 96-hour TL_{50}s for bluegill and catfish were 0.33 and 0.09 mg/l, respectively, and the environmental effects of water temperature and fish weight were quantified through linear regression analyses. The product of the 96-hour TL_{50} and the 0.1 Illinois safety factor places the allowable residual chlorine at the limit of detection for the amperometric titrator and essentially means no detectable residual chlorine is allowed in Illinois waters.

INTRODUCTION

The proper management and surveillance of water quality is predicated on the need to maintain suitable quality for each use that is made or will be made of Illinois' lakes and streams. Thus it is necessary to know the water quality criteria for each use, if effective rules are to be applied to sources of waste discharging into the water bodies of Illinois. Water quality criteria for potable water, recreation, agriculture, and industrial use are fairly well defined. On the other hand, there remains considerable doubt on the water quality standards required to sustain an adequate fishery. This is particularly true with regard to heavy metals, ammonia, residual chlorine, and organochemicals.

The current regulations in Illinois, where numerical values have been developed limiting presumably toxic substances in waste discharges and natural water bodies, are based upon studies using, as test specimens, aquatic organisms not native to Illinois waters. In addition to a list of chemical constituents for which maximum permissible concentrations have been determined, Rule 203(h) of the Water Pollution Regulations of Illinois states:

> Any substance toxic to aquatic life shall not exceed one-tenth of the 48-hour median tolerance limit (48-hr TLm) for native fish or essential fish food organisms.

It is within the purview of this rule that this investigation regarding the effects of residual chlorine upon some native fishes of Illinois was undertaken.

Since the adoption of the rule, changes of the 48-hr TLm to 96-hr TLm have been considered and will most likely be made. Here the median tolerance limit (TLm) is the concentration at which 50% of the test specimens survive. It is also referred to as LC_{50} (lethal concentration) and TL_{50}.

RESIDUAL CHLORINE

The Illinois Environmental Protection Agency has established a limit of 400 fecal coliform per 100 ml in all effluents. This requires disinfection procedures; and the most common disinfection practice in Illinois is chlorination.

When chlorine is introduced into water either in gaseous form (Cl_2) or as hypochlorite ($Ca(OCl)_2$), the same end products are produced. These are hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-). Chlorine, hypochlorous acid, and hypochlorite ions in water are considered free residual chlorine. If ammonia is present, its reaction with chlorine and hypochlorous acid produces chloramines. Chloramines in water are referred to as combined residual chlorine. The total residual chlorine in water is the sum of the free and combined residual chlorine. Residual chlorine is primarily introduced into the aquatic environment from the disinfection of sewage plant effluents. The other major source of residual chlorine is cooling water discharges from electrical generating plants where chlorine is frequently used for minimizing the fouling of heat transfer surfaces. For Illinois conditions, the exposure of fish to residual chlorine is more likely in the case of waste effluents than in the case of cooling water discharges.

In the reaction of chlorine or hypochlorous acid with ammonia, three chloramines may be produced. They include monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine (NCl_3). The relative amounts of each are dependent upon the molar ratio of chlorine to ammonia, pH, temperature, and length of reaction time. Monochloramine is the predominant form developed in treated sewage effluents during chlorination. It is the chloramine likely to occur in Illinois surface waters.

LITERATURE REVIEW

Rosenberger (1971) and Merkens (1958) concluded that free chlorine is slightly more toxic to fish than dichloramine and that dichloramine is more toxic than monochloramine. Thus total residual chlorine will be more toxic at pH values below 7 because the proportion of free chlorine and dichloramine is greater. Brungs (1973) states that the toxicity of

the principal components of total residual chlorine is not sufficiently different as to preclude using total residual chlorine to define acute toxicity.

Rosenberger (1971) used regression analysis in finding that larger fish died faster than smaller fish. He attributed this to a smaller ratio of gill surface area to body weight for larger fish. He assumed the gill to be the principal site of chlorine toxicity; however, Fobes (1971) found that the respiration rate of gill tissues from white suckers, exposed to lethal concentrations of chlorine, did not change. Dandy (1969) found "in all cases where the fish were exposed to chlorine until the first signs of disequilibrium occurred, and then immediately transferred to fresh water, death invariably ensued even though life expectancy in the chlorine solution at the time of transfer was several hours."

In standard laboratory bioassays, intolerant cold water fish, especially salmon and trout species, have been very sensitive to residual chlorine. Seven-day TL_{50}s of 0.01 to 0.08 mg/l total residual chlorine have been reported (Merkens, 1958; Arthur, 1971; and Holland et al., 1960). For warm water fish, there is a narrow range of acute lethal chlorine concentrations. According to Arthur and Eaton (1971), all fathead minnows died within 72 hrs at 0.154 mg/l but none died for 7 days at 0.085 mg/l.

Coventry (1935) stated that 0.4 mg/l was lethal to sunfish and some bullheads. The 96-hr TL_{50} for black bullhead is 0.099 mg/l according to Arthur (1971). A 15-hr TL_{50} for smallmouth bass was reported to be 0.5 mg/l by Pyle (1960). Ninety-six hr TL_{50} values for largemouth bass, yellow perch, and white sucker are, respectively, 0.261, 0.205, and 0.132 mg/l residual chlorine as reported by Arthur (1971).

The on-site testing of chlorine toxicity in sewage plant effluents by Basch et al. (1971) found 50% of rainbow trout dying in 96 hours at residual chlorine concentrations of 0.014 to 0.029 mg/l. When the effluents were not chlorinated, mortality did not occur.

In Maryland, northern Virginia, and southeastern Pennsylvania, Tsai (1968, 1970, and 1971) found that fish populations in small streams below 156 sewage treatment plants decreased in number of species toward more pollution-tolerant fish. The species diversity index was reduced 50% at 0.10 mg/l residual chlorine. Fish were not found in water with a total residual chlorine of 0.37 mg/l.

Dickson et al. (1974) found that ammonia concentrations in the Clinch River, Virginia, had reduced most of the free chlorine to residual chlorine about 23 feet below the powerhouse discharge and that much of the residual chlorine was reduced in the first 500 feet. No bluegill mortality could be attributed to chlorine toxicity at any station, although a maximum of 0.55 mg/l residual chlorine and 0.07 mg/l of free chlorine was attained at one station.

METHODS AND EQUIPMENT

A modification of a proportional dilutor by Mount and Brungs (1967) was used whereby continuous water flow was provided through 12 glass test chambers. Each chamber has a volume of 22 liters, and the flow rate, 250 ml/min, produces a 95% volume displacement every 9 hours. The apparatus permits the continuous flow of 5 different concentrations of toxicant into duplicative test chambers with 2 chambers being available for control purposes.

Dilution water originates from municipal wells. Although residual chlorine has not been detected in the water, an activated charcoal filter has been installed on the main supply line prior to the introduction of water to the header boxes serving the dilutor.

Two header boxes are used. The first one, consisting of a fiberglassed steel barrel, permits regulation of water temperature. In it is a thermoregulator which can be set at a desired temperature. Significant cooling from the preset water temperature energizes a relay which activates a solenoid-controlled valve on a hot water line. Water flows from the steel barrel to a polyethylene plastic header box where air agitation keeps the contents mixed and provides a sustained dissolved oxygen level.

Stock solutions of residual chlorine were formulated by mixing 0.375 molar solutions of ammonia (from NH_4Cl ACS reagent grade) and HOCl (from 70% $Ca(OCl)_2$ technical grade) after adjustment to pH 8.4. The pH of the hypochlorous acid solution was adjusted with HCl; the pH of the ammonia solution was adjusted by the addition of NH_4OH, so that excess ammonia was present. By adding the HOCl solution slowly to the ammonia solution, the ratio of NH_3 to HOCl was always greater than one and thus insured the formation of monochloramine alone at pH 8.4 (Palin, 1950; Morris, 1975; and White, 1972). This form of residual chlorine is stable in the laboratory and environment and is the form most often found in natural waters. It is also the only form of residual chlorine found in the test chambers under conditions of analysis described by Wallace and Tiernan, Inc. (1971) and Johnson (1975). All forms of residual chlorine are quantified in terms of chlorine equivalent weights.

Hardness, alkalinity, and pH were determined in the control chambers and two other test chambers twice a day. Dissolved oxygen levels were measured at 0, 48, and 96 hrs, and water temperature was monitored continuously. Hardness determinations were by EDTA titrametric method with Eriochrome Black T as an indicator. Alkalinity and pH were determined by a Leeds and Northrup meter, using 0.02N H_2SO_4 as a titrant for alkalinity. Dissolved oxygen concentrations were measured by a Yellow Springs Instrument Model 54 oxygen meter; and temperature was monitored by a Yellow Springs Instrument Model 46 Tele-Thermometer with output recorded on a Cole-Parmer Mark VII recorder. Illumination for the 16-hr photoperiod was furnished by a combination of Duro-Test and Wide Spectrum Gro-lux fluorescent lighting in circuit with a timer.

The major modification in the dilutor apparatus was a syringe style pipettor with a two-way check valve from Manostat, which was fed from a container of toxicant. A normally open four-way Skinner air solenoid valve was placed into the circuit of the electrical switch, which operates the water solenoid valve in the standard Mount and Brungs dilutor. During cycling of the dilutor, the water bucket arm descends to engage the switch and breaks the electrical circuit. This shuts off the water solenoid valve and opens the air solenoid valve causing the arm of the air cylinder to be extended. The extended arm depresses the plunger of the pipettor to inject an exact amount of toxicant from the syringe into the mixing bowl. When the bucket arm rises to complete the electrical circuit again, the water solenoid valve opens and the air solenoid valve causes the air cylinder arm to retract. Two external springs return the plunger of the syringe to the locked position of the pipettor necessary for the intake of desired syringe volume through the two-way check valve. The original internal spring was replaced by two external springs to ensure the reliability necessary for the very frequent and long term cycling in bioassays. The advantages of this system are an easily adjustable volume of toxicant, a fail-safe design directly timed by dilutor function, an ability to dispense solutions with suspended particles as in the residual chlorine solution, and a relatively low price for a system comprised of an air solenoid valve, air cylinder, and pipettor.

Other than those chemical constituents periodically monitored, as mentioned earlier, the following characterize the water used in each bioassay run:

Chemical Oxygen Demand	ND	Fluoride	1.1 mg/l
Ammonia-N	0.08 mg/l	Silica	31.8 mg/l
Nitrate-N	5.18 mg/l	Calcium	123 mg/l
Phosphate-P	0.02 mg/l	Magnesium	42 mg/l
Sulfate	168 mg/l	Iron	0.03 mg/l
Chloride	34 mg/l	Mercury	$<2 \times 10^{-5}$ mg/l

CHARACTERISTICS AND REACTIONS OF FISHES

The bluegill (*Lepomis macrochirus*) used in this investigation were native fish removed from an area pond and separated into three weight groups. The channel catfish (*Ictalurus punctatus*) were obtained from the Illinois Department of Conservation after shipment from a hatchery in Senecaville, Ohio. In all instances, both bluegill and channel catfish were conditioned to the dilution water for a minimum of 10 days. When necessary, the temperature was raised 1°C/day and maintained at the desired temperature for 10 days.

At the beginning of each bioassay, the temperature and toxicant concentration for each test chamber were determined. One fish at a time was randomly placed in the different aquaria until each of the twelve chambers held 10 fish. Therefore each bioassay used 120 fish, including 20 control fish, giving a total of 600 bluegill and 280 channel catfish for the entire study.

Because of the rapid mortality at high concentrations, each bioassay was continuously monitored the first 32 hours. The time of each mortality could then be recorded. After death, the fish were thoroughly blotted to remove excess moisture, their lengths were measured, and they were weighed.

At the higher concentrations, the bluegill exhibited erratic swimming within 2 hours. From a resting position, the fish made a short, rapid movement forward, rested, then swam rapidly forward again. When the fish were near the bottom of the tank, they would dart upward, in a straight, diagonal line. This short, erratic swimming might occur 5 to 10 times in an hour.

As the bluegill's equilibrium was lost, it hovered near the surface, attempting to remain upright by using the caudal fin. Prodding produced a weak, lateral movement, completely different from the rapid, short, and straight swimming noted earlier. At this stage, removal of the bluegill from the toxicant concentration did not revive it.

Before death occurred, the fish rested ventral-side up on the bottom of the tank. Respiration was slow and erratic, with gill pouches spread open. Some fish had hemorrhaged pectoral and caudal fins. Death was determined by lack of reaction to prodding and the cessation of gill movement.

The stress patterns of the catfish and bluegill differed. The catfish were more listless, had increased ventilation rates and muscle contractions, and produced a mucous film on the body. They also became rigid, maintaining a perpendicular position to the bottom of the tank. Sometimes death would occur in this position. Otherwise the fish died dorsal-side up, giving the appearance of resting. As with the bluegill, some pectoral and caudal fins were hemorrhaged.

In the catfish and bluegill, the time span between the first signs of stress and death was directly proportional to the toxicant concentrations. At high concentrations, death occurred after 2 hours of stress; however at low concentrations, stress might last as long as 12 hours.

RESULTS

The test conditions for the five bioassays using bluegill are summarized in Table 1. For estimating the median lethal time, i.e., that time at which 50% mortality will occur at a particular residual chlorine concentration, the percent mortality was plotted for each concentration on log-probability paper. This procedure is demonstrated in Figures 1a and 1b for bluegill runs on 9/30/74 at 0.79 mg/l and on 10/7/74 at 0.49 mg/l, respectively. The estimated median lethal time is that time at which 50% of the fishes died. Acute toxicity curves were developed for each bioassay run by plotting the median lethal times on the vertical axis versus corresponding concentrations on the horizontal axis, all on log-log paper. When less than 50% mortality occurred for a particular concentration, the point was plotted on the 96-hr line. The resultant curves, as shown in Figures 2a, 2b, and 3b, permit the determination of

TABLE 1. Test Conditions during Bioassay Runs on Bluegills

Date	Toxicant range (*mg/l*)	Temperature (*°C*)	Avg. fish weight (*grams*)	pH	Alkalinity (*mg/l*)	Hardness (*mg/l*)	Dissolved oxygen (*mg/l*)
9/30/74	0.18-0.79	20	1.85	8.13	317	451	7.5-8.6
10/7/74	0.24-0.79	20	1.85	8.02	310	452	7.5-8.6
10/14/74	0.23-0.77	21	0.31	8.19	313	443	7.5-8.6
10/21/74	0.15-0.51	21	0.31	8.00	306	467	7.5-8.6
11/4/74	0.15-0.51	30	1.24	7.95	316	398	6.4-7.0

median tolerance limits, TL_{50}. The TL_{50} is that concentration at which the toxicity curve becomes asymptotic to the time axis.

As shown in Figures 2a through 3b, median lethal times in all cases for bluegills were less than 96 hours. In most cases, under the conditions of the tests, the median lethal time was 24 hours or less. Nevertheless, the bioassays were continued for at least 96 hours, and the data obtained are reported as the 96-hr TL_{50}.

In the two bioassays using channel catfish, it is probable that concentrations lower than 0.10 mg/1 should have been employed. At 30°C, 60 to 90% of the fishes died at the lower concentration of toxicant used (0.11 mg/1); at 20°C, 100% of the fishes died within 96 hours for all concentrations. Under these circumstances the median tolerance limit is not so clearcut as that determined for the bluegills. A separate bioassay using total residual chlorine concentrations at 0.05 mg/1 and 20°C did not produce any mortality in channel catfish over a 96-hr span. From this it is concluded that a very narrow range of tolerance, from 0.05 to 0.10 mg/1, exists for channel catfish.

FIGURE 1.--Percent mortality for bluegill.

FIGURE 2.--Acute toxicity curves for bluegill showing weight comparisons.

FIGURE 3.--Acute toxicity curves for bluegill showing temperature comparisons.

FIGURE 4.--Acute toxicity curve for channel catfish.

Use of procedures as discussed earlier and as shown in Figure 4 gave an estimated median tolerance limit of 0.09 mg/l total residual chlorine. Also, as shown in Figure 4, there is very little difference in plotting the data for 20°C temperature conditions versus 30°C temperature conditions. Therefore, the data were not considered separately.

STATISTICAL METHODS

The assessment of median tolerance limits by graphical methods is an acceptable procedure and where statistical methods are limited, it is the only practicable means for determining limitations on aquatic toxicants. Graphical procedures, however, do not permit quantitative evaluation of the environmental factors that may influence tolerance limits in bioassays. In an effort to determine whether or not significant relationships existed, major portions of the data for bluegill and channel catfish bioassays were subjected to multiple and stepwise linear regression analyses. For this purpose, the dependent variable was the toxicant concentration in mg/l, here designated as TC. The independent variables were (t), time in minutes from the beginning of a bioassay for a change in percent mortality (M) to occur in each tank of water at a temperature (T) in degrees Celsius.

The average weight (W) of all dead fish occurring in a tank was calculated at every change in percent mortality. A single equation was developed in the following form:

$$TC = a - b (\log t) + c (M) - d (T) + e \log W$$

where a, b, c, d, and e are computed coefficients. Data for time (t) and accumulated average fish weight (W) were found to be geometrically distributed and were normalized by use of logarithms.

The three variables, TC, t, and M, used in plotting graphic 96-hour TL_{50} are the first three variables programmed for linear regression analysis. Selection of residual chlorine concentrations in the 0.10 to 0.45 mg/l range corresponds to that section of the toxicity curve (Figures 2 and 3) which becomes more linear and asymtotic to the time axis in graphic analysis. The best fit of a linear projection across the curve of data points in the graphic toxicity curve (Figures 2 and 3) predicts higher concentration than the observed value around the 0.3 mg/l residual chlorine interval. It predicts lower residual chlorine concentrations than observed values in the 0.5 to 0.8 mg/l range. Also, the 96-hour TL_{50} would be predicted lower as evidenced by the curvature of the toxicity curve in graphic analysis. In linear regression analysis, precisely the same predictive results occur when projected from all of the 0.10 to 0.79 mg/l residual chlorine data.

The inclusion of water temperatures and logs of accumulated average fish weights as independent variables in the equation increases the correlation coefficient and decreases the standard error as evidenced in Table 2. The form of the final equation for bluegill is:

$$TC = 1.1804 - 0.1834 \log t + 0.00147 (M) - 0.01428 T + 0.1560 \log W$$

It should be noted that although there are three average weight groups of 0.31, 1.24, and 1.85 grams (g), the range of fish weight is from 0.10 to 2.9 g. In regression analysis there was a computed average weight of all dead fish for every change in mortality in each of the 27 tanks in 0.10 to 0.45 mg/l residual chlorine range. Often at high toxicant concentrations, several fish would die at one time, and cause one large change in percent mortality. There were 148 computed average fish weights for the 148 observations of percent mortality change during which 182 of 270 fish died. Therefore, the number of observations for each variable was 148 for time, percent mortality, and accumulated average fish weight and 3 for water temperature. Although the range of fish weight was limited to under 3 g, the smallest fish had the greatest sensitivity to residual chlorine. Thus extension of fish weight range

TABLE 2. Statistical Characteristics of Predictive Equation Bluegill Bioassay

Parameter	F values	Multiple correlation coefficient (r)	Standard error of estimate (SE)
Log time (t)	14.9	0.3045	0.0729
% Mortality (M)	30.9	0.5463	0.0643
Temperature (T)	34.4	0.6460	0.0588
Log fish weight (W)	113.9	0.8725	0.0379

TABLE 3. Comparison of Graphic TL_{50} to Predicted TL_{50} Bluegill Bioassay

Bioassay	Graphic 96-hour TL_{50} (*mg/l*)	Predicted 96-hour TL_{50}* (*mg/l*)
1.85 g @ 20°C	0.33	0.31
0.30 g @ 21°C	0.25	0.26
1.24 g @ 30°C	0.18	0.15

**Based on concentration range 0.144 to 0.440 mg/l*

in that direction is limited since the average weight of all fish in two bioassays was 0.31 g with minimum fish weights of 0.10 g. Similarly, the greatest sensitivity of bluegill was in 30°C water so that extension of water temperature is limited if held to those temperature maximums observed in native Illinois streams and lakes. Linear regression analysis insures that eye-hand coordination used in graphic 96-hour TL_{50} determinations is understood in terms of effect on the desired determination of toxicant levels.

The predicted residual chlorine values were calculated on the basis of 148 times at which 148 changes in percent mortality occurred in fish with 148 computed average weights at 3 water temperatures. The comparison of these predicted residual chlorine values and the observed residual chlorine level at the time of each change of percent mortality is illustrated in Figure 5. Table 3 compares the similarity of 96-hour TL_{50} values as predicted by linear regression analysis and graphic analysis.

Table 2 shows the increase in F factor and correlation coefficients as time, percent mortality, accumulated average fish weight, and water temperature were included. The ranking of parameters on the basis of toxicant level effect was 1) log time, 2) percent mortality, 3) log of accumulated average fish weight, and 4) water temperature. When each of

FIGURE 5. Predicted residual chlorine from all percent mortality changes from all bioassays versus observed residual chlorine.

the 3 fish weight and water temperature groups were processed separately by linear regression analysis, only log time and percent mortality significantly increased r in the 1.85 g average weight fish at 21°C and the 1.24 g average weight fish at 30°C. In fish of average weight 0.31 g at 21°C, the log of average fish weight increased r by 0.10 so that the 96-hour TL_{50} of that group was dependent upon log time, percent mortality, and log of average fish weight in that order of importance.

Similar analyses of the channel catfish data indicate that the toxicant concentration relationships with t, M, T, and W are different from that determined by bluegill bioassays. The order of importance is 1) time, 2) mortality, 3) weight, and 4) temperature. With channel catfish, water temperature appeared to be only marginally significant as shown in Table 4. It was not included in the equation.

The predictive equation developed for channel catfish bioassays is:

$$TC = 0.8753 - 0.264 \log t - 1.00103 M + 0.2133 \log W$$

The channel catfish, reflecting a 96-hour TL_{50} of about 0.09 mg/l residual chlorine in contrast to a 96-hour TL_{50} range for bluegill of 0.18 to 0.33 mg/l residual chlorine, is obviously the more sensitive fish. The water pollution regulations require an application factor of one-tenth in establishing a maximum permissible concentration. The permissible concentration of residual chlorine for channel catfish protection would be about 9 micrograms per liter, and that for the protection of bluegill would range from 18 to 33 micrograms per liter in Illinois streams. Thus, for all practical purposes, residual chlorine should not be detectable in any stream in Illinois.

TABLE 4. Statistical Characteristics of Predictive Equation Channel Catfish Bioassay

Parameter	F values	Multiple correlation coefficient (r)	Standard error of estimate (SE)
Log time (t)	470.9	0.9019	0.0492
Percent mortality (M)	69.8	0.9418	0.0385
Log fish weight (W)	46.2	0.9598	0.0324
Temperature (T)	6.3	0.9622	0.0315

SUMMARY

1) Bluegill and channel catfish were subjected to varying concentrations of residual chlorine in waters relatively high in alkalinity and the salts of calcium and magnesium.

2) Acute toxicity curves were developed for each species permitting assessment for 96-hour TL_{50}.

3) In the case of bluegills, the 96-hour TL_{50} ranged from 0.18 to 0.33 mg/l and was dependent upon water temperature and fish weight.

4) In the case of channel catfish, a more sensitive fish to residual chlorine, the 96-hour TL_{50} was about 0.09 mg/l. Temperature was not a factor.

5) For each type of fish species, predictive equations were developed that permitted the quantitative evaluation of environmental factors within the experimental boundaries of the 96-hour TL_{50} bioassays.

6) For the protection of the fishes investigated and consistent with the water pollution regulation of Illinois, residual chlorine in Illinois streams should not be detectable.

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