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Evaluation of Factors Affecting the Toxicity of Chlorine to Aquatic Organisms

Prepared by S. C. Crumley, Q. J. Stober, P. A. Dinnel

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University of Washington

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ABSTRACT

This report provides a state-of-the-art review of 111 of the most recent available references on the interactive effects of chlorine and various environmental factors on saltwater and freshwater aquatic organisms. The chemistry of chlorine in freshwater and seawater is discussed as it relates to the evaluation of toxicity studies. Those factors found to affect the toxicity of chlorine are concentration, exposure time, temperature, chemical species of chlorine and biotic factors such as species, life stage, and size of organism. Other factors such as pH and metal pollutants modify the toxicity of chlorine; however, the two factors of primary importance in the determination of toxicity are concentration and exposure time. Further research is needed on the reaction products of chlorine in both aquatic environments which will require development of new analytical methodology. Toxicity testing which simulates the transient low level exposures of organisms in cooling water mixing zones is needed to provide more accurate estimates of conditions in the receiving water. References defining the additive, synergistic or antagonistic effects of chlorine with one or more other chemical pollutants were very limited.

SUMMARY

This report was prepared for the Nuclear Regulatory Commission to provide a comprehensive review of recent available data on the interactive effects of chlorine and various environmental factors on aquatic organisms. The chemistry of chlorine in freshwater and seawater was discussed as it related to the evaluation of toxicity studies.

Several factors which were found to affect the toxicity of chlorine include concentration, exposure time, temperature, chemical species of chlorine and biotic factors such as species, life stage, and size of organism. In addition, other environmental factors such as pH and metal pollutants (copper and nickel) modify the toxicity. The two factors of primary importance in the determination of toxicity are concentration and exposure time. The concentration of chlorine and duration of exposure is dependent to a large degree on the chemical reactions of chlorine following addition to natural waters. Although the chemistry of chlorine in freshwater has been fairly well delineated, the relative toxicities of free chlorine and monochloramine for both intermittent and continuous exposure regimes remains in question and is in need of further experimentation. The types of reactions that occur in seawater probably are similar to those in freshwater, however; the reaction products differ due to the release of bromine from naturally occurring bromide salts. The halogenated compounds formed in seawater appear to be responsible for the toxicity, however, experimental data is lacking on their relative toxicities.

At present, there is inadequate analytical methodology for the estimation of low concentrations (μg/L) of chlorine and none of the present methods is specific for chlorine or even the halogens. Furthermore, it appears that fish may be affected by chlorine concentrations below the limits of detection. Consequently, it is imperative that appropriate techniques be developed that are specific for chlorine and its reaction products in both freshwater and seawater at μg/L concentrations.

The exposure of an organism to chlorine under natural conditions is dependent on where and how it interacts with a discharge plume. Difficulty arises when results from laboratory experiments in which organisms are exposed to a constant level of chlorine for a set time are extrapolated to real world conditions where organisms are exposed to varying concentrations for short time periods. Several approaches have been proposed to overcome this discrepancy in an attempt to establish reasonable limits for chlorine discharge. An area of research requiring further attention is the relationship between the area under the time concentration curve of toxicity studies and mortality. This may provide a more accurate assessment of the cumulative effects of successive doses of highly variable concentrations, durations, and frequencies that occur during intermittent chlorination. There is also a further need for establishing a time

period between doses which would allow for recovery. Another approach is based on a comparison of estimated exposures of an organism in a discharge area with previously derived toxicity thresholds.

Temperature, as an environmental factor, seems to modify chlorine toxicity in laboratory experiments employing wide temperature ranges. This effect appears to be species-specific. Little effect is seen at low temperatures, while at higher temperatures, the effects are increased. However, temperature may not be a critical factor with the relatively small temperature changes observed at most power plant discharges.

The role of chlorine-metal interactions has only recently been investigated and data are too limited to present any conclusions. There does appear, however, to be an interaction but the extent of its importance will require further research.

Avoidance behavior of motile organisms occurs in both the laboratory and in the field in freshwater and marine environments. Avoidance or preference appears to have a wider range of inter and/or intra specific variability and to assess the dose rates encountered by motile aquatic organisms in the vicinity of mixing zones additional behavioral studies are needed.

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PREFACE & ACKNOWLEDGMENTS

A dramatic increase in the amount of scientific investigation on the environmental effects of chlorine and chlorine reaction products on non-target aquatic organisms has occurred in the last few years. The objective of this report is to supplement existing literature reviews with a state-of-the-art analysis of the most recent available toxicity data emphasizing the interaction of chlorine with various environmental factors to determine whether any new insights into the impacts on aquatic systems is beginning to emerge.

This study was sponsored by the U. S. Nuclear Regulatory Commission through a contract with the College of Fisheries, University of Washington. Dr. D. G. Chapman, Dean, was principal investigator.

1.0 INTRODUCTION

Concern about the environmental effects of chlorine and chlorine by-products on non-target aquatic organisms has led to a dramatic increase in the amount of scientific investigation in this area. The toxicity of chlorine in freshwater has received extensive investigation, however, it has been only recently that the problem of chloro-organics with potential health and environmental effects has been identified. The chemistry of chlorine in seawater has recently been recognized as much more complex than that in freshwater and has stimulated new research projects to determine the chemical reaction rates and biological toxicity in estuarine and marine ecosystems.

The use of chlorination as a biocide in both on- and off-stream power plant cooling systems continues to increase as additional generating stations are sited on inland and coastal waters. The discharge of chlorinated sewage and related industrial wastes into these same waters compounds the impacts on aquatic ecosystems. The interactions of chlorine with receiving waters are dependent on such factors as temperature, salinity, pH, ammonia and organic content. These variables are in constant flux, complicating extrapolation of laboratory data to field situations which are usually site specific.

Several comprehensive reviews on the toxicity of chlorine to freshwater, estuarine and marine organisms have appeared in the last few years (1, 2, 3, 4).

The objective of this report is to present a state-of-the-art review of the most recent available literature emphasizing the interactions of chlorine with various environmental factors to determine whether new insight into the impacts on aquatic systems is beginning to emerge as well as to indicate the areas where additional research is needed.

2.0 CHEMISTRY OF CHLORINE

2.1 Freshwater

Chlorine can be added to water in several different forms, e.g. chlorine gas, calcium hypochlorite and sodium hypochlorite. When chlorine gas is used it undergoes rapid hydrolysis according to the following equation:

$$Cl_2 + H_2O \rightleftharpoons H^+ + Cl^- + HOCl \qquad (1)$$

Hydrolysis constants range from 1.46 to 4.48×10^{-4} $(mol/liter)^2$ for the temperature ranges 0 to 25°C (5, 6, 7). Equilibrium conditions are established within a few seconds or less (8, 7). The hydrolysis constant of $K_h = 4.48 \times 10^{-4}$ at 25°C, indicates that with a chlorine solution on the order of several ppm at natural pH and chloride concentration, the reaction goes virtually to completion. The hydrochloric and hydrochlorous acids formed during this reaction cause a slight drop in pH; however, the HCO_3^- present in natural waters is usually sufficient to neutralize most of the acid produced. The hypochlorous acid formed immediately dissociates in accordance with the equation:

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad (2)$$

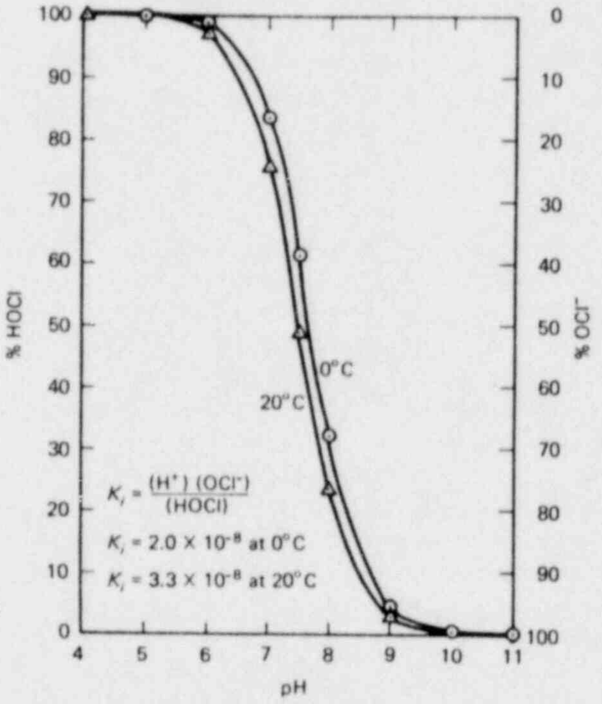
The dissociation constants range from $1.6\text{-}3.2 \times 10^{-8}$ for the temperature range 0 to 25°C (9). At pH values greater than 7.5 to 7.8 for 0 to 25°C hypochlorite ion becomes the dominant species in aqueous chlorine solution.

A distribution curve of the principal oxidizing species ($HOCl$ and OCl^-) for aqueous chlorine solutions of different pH values is shown in Fig. 1. However, Morris (11) points out that it is not the concentration of the predominant species alone that determines the contribution of a given mechanism, a pathway to the overall reaction, but rather the concentration of each constituent times its specific reactivity. Morris has estimated the specific reactivity of HOCl to be significantly greater than that of OCl^- such that the former must be regarded as the major reactive species for most oxidizing reactions of aqueous chlorine in diluted solutions ($<10^{-3}$m) at pH values between 5 and 9.

The chemistry of Cl_2 and the hypochlorites differ only in the initial hydrolysis step. For example, when $Ca(OCl)_2$ and NaOCl are added to water they ionize according to the following equations:

$$Ca(OCl)_2 \longrightarrow Ca^{+2} + 2\ OCL^- \qquad (3)$$

$$Na\ OCL \rightarrow Na+ + OCl^- \qquad (4)$$



	% HOCl	
pH	*0°C*	*20°C*
4	100	100
5	100	99.7
6	98.2	96.8
7	83.3	75.2
7.5	61.26	48.93
8	32.2	23.2
9	4.5	2.9
10	0.5	0.3
11	0.05	0.03

Figure 1. Disassociation of HOCl versus pH (taken from White (10))

According to the law of mass action, the OCl^- ion reacts with the H^+ ion in water as follows:

$$OCl + H^+ = HOCl \quad (5)$$

An equilibrium is then established for HOCl and OCl for the given temperature and pH.

Hypochlorous acid is capable of reacting with a number of inorganic reducing agents found in natural waters, e.g., Fe^{+2}, Mn, NH_3, WO_2^-, S^{-2}, SO_3^{-2}. It appears that electrophilic processes proceed by way of the chlorine action, in fact, the attraction of Cl for electrons is so great that it may split from the molecule directly as chloride ion as seen in the following reaction:

$$NO_2^- + HOCl \longrightarrow NO_2OH + Cl^- \quad (6)$$

with the nitrite displacing Cl^- (12, 13). The oxidation of sulfite may proceed similarly (14).

Several groups of organic compounds which are also subject to oxidation and substitution reactions include nitrites, amino acids, urea, phenols, and carbohydrates.

Any inorganic or organic compound which is capable of reducing the amount of chlorine added to a solution contributes to the phenomenon known as chlorine demand. The chlorine demand is defined as the difference between the amount of chlorine added and the amount remaining (residual) for a given reaction time.

The important role ammonia nitrogen plays in chlorine demand and formation of reaction products has been the subject of much attention (10). Ammonia nitrogen present in natural or wastewaters reacts rapidly with chlorine at pH 7 to 10 to form monochloramine (NH_2 Cl). The reaction may proceed further to replace another H^+ from the nitrogen of ammonia to give dichloramine (NH Cl_2) and trichloramine (NCl_3). The sequence of reactions can be viewed as follows:

$$HOCl + NH_3 = NH_2Cl + H_2O \quad (7)$$

$$HOCl + NH_2Cl = NHCl_2 + H_2O \quad (8)$$

$$HOCl + NHCl_2 = NCl_3 + H_2O \quad (9)$$

The more substituted chloramines are generally produced more slowly, at low pH and temperature, and at higher chlorine to ammonia ratio. Temperature, pH, time of reaction and the chlorine to ammonia ratio all need to be considered in determining product distribution from the reactions.

These reactions form the basis for understanding the breakpoint process which involves the oxidation of chloramine residuals and production of N_2 (Fig. 2). Several investigators have proposed reaction schemes to

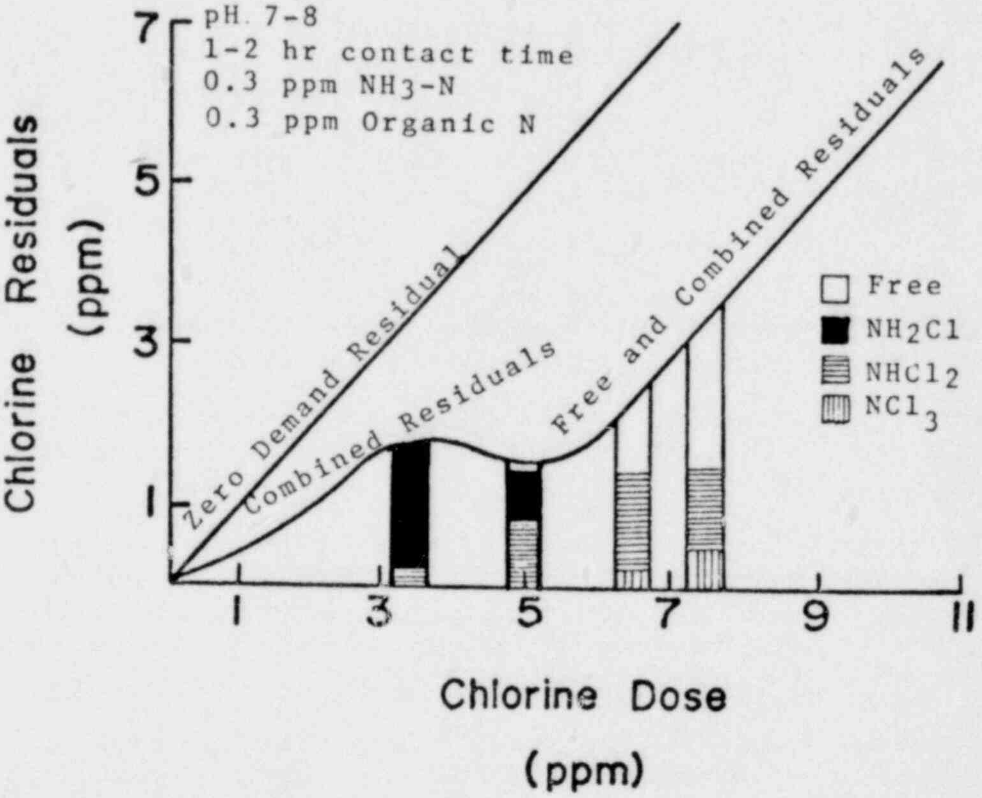


Figure 2. Breakpoint curve for ammonia and chlorine (taken from White (10)). The term free chlorine refers to the sum, $HOCl + OCl^-$. The term combined chlorine refers to the sum, $NH_2Cl + NHCl_2 + NCl_3$ + organic amine analogs of these species.

explain the chemical events occurring during this process (15, 16).

Organic nitrogen behaves differently than NH_3 in that it is slower reacting with HOCl. Whereas NH_3 and amino nitrogen can be oxidized within 1 minute, protein nitrogen does not appear to react significantly even with prolonged contact time (15, 17). The breakpoint curve produced from water containing 0.3 mg/L NH_3-N and 0.3 mg/L organic nitrogen, pH 7-8, and 1-2 hours contact time as shown in Fig. 3. Note that the breakpoint is less pronounced compared to the case of NH_3.

Several investigators have undertaken the seemingly impossible task of developing a model to predict the decay of chlorine in natural waters despite the diversity of organic material and lack of reaction rate constants for many of the compounds present (18, 19).

2.2 Seawater

The chemistry of chlorine in seawater is extremely complex and must be considered in light of the particular constituents present. Bromide ion, for example, has been shown to play a major role (20). In seawater the oxidative capacity of chlorine is transferred to bromide ion as well as various other byproducts (e.g. chlorinated hydrocarbons, chloramines, bromamines, etc.). Sugam and Helz (21) have recently proposed a sequence of reactions for chlorine degradation in a marine environment (Fig. 4). The actual reaction products formed during the conversion of one oxidant into another are dependent upon several variables among which are pH, salinity (amount of Br^-), ammonia nitrogen, chlorine dose and temperature (22).

The importance of ammonia nitrogen concentration on the chemistry of chlorinated seawater and its toxicological implications has been examined by Inman and Johnson (23). In full strength chlorinated seawater bromamines may be formed from the hypobromous acid resulting from bromide hydrolysis at low ammonia nitrogen concentrations. The degree of halogen substitution on nitrogen will be determined by pH and the halogen ammonia ratio (Fig. 5) (24). For ammonia nitrogen levels less than 0.4 mg/L, pH 8.1, and sufficiently large chlorine doses, tribromamine and hypobromous acid are the major products. When the ammonia nitrogen level is greater than 0.5 mg/L and the chlorine dose is less than 2.5 mg/L, monochloramine competes with bromide oxidation and a haloamine mixture of monochloramine and dibromamine results. At even greater ammonia concentrations and longer times monochloramine becomes the predominant oxidant species. Inman and Johnson (23) have also determined the critical ammonia nitrogen: bromide ratio where monochloramine formation begins to predominate over bromamine formation. This ratio reduces to 0.008 at pH 8.1. At higher ratios the authors feel that monochloramine should predominate after 30 minutes to 1 hour. At lower ratios, dibromamine would be the

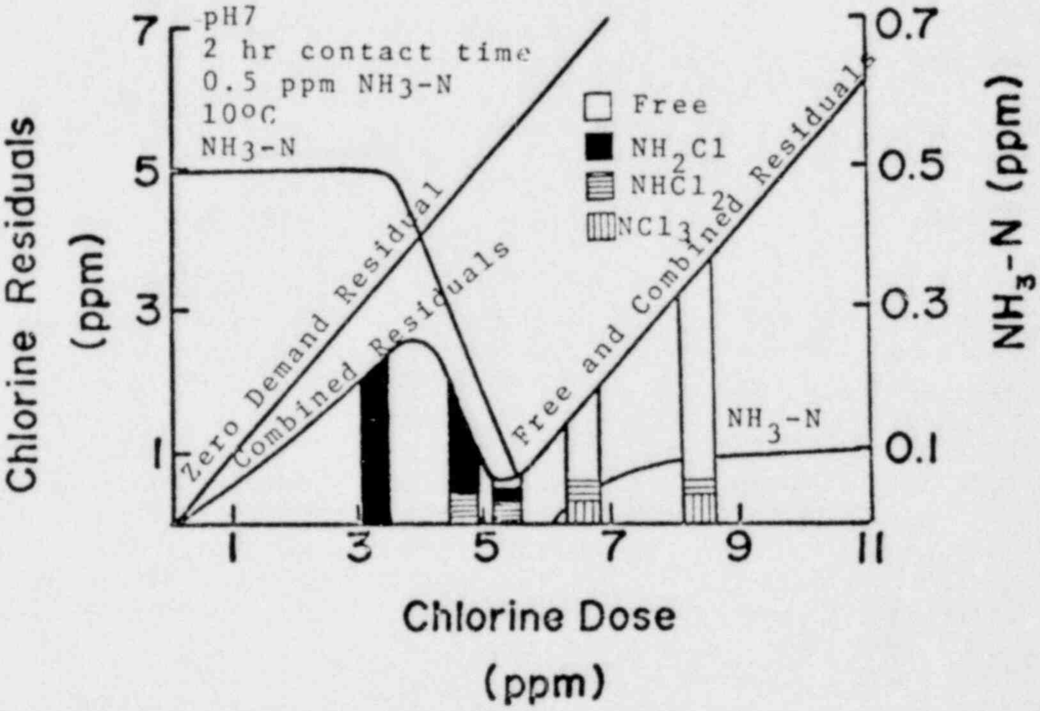


Figure 3. Breakpoint curve for ammonia, organic nitrogen and chlorine. (Taken from White (10)).

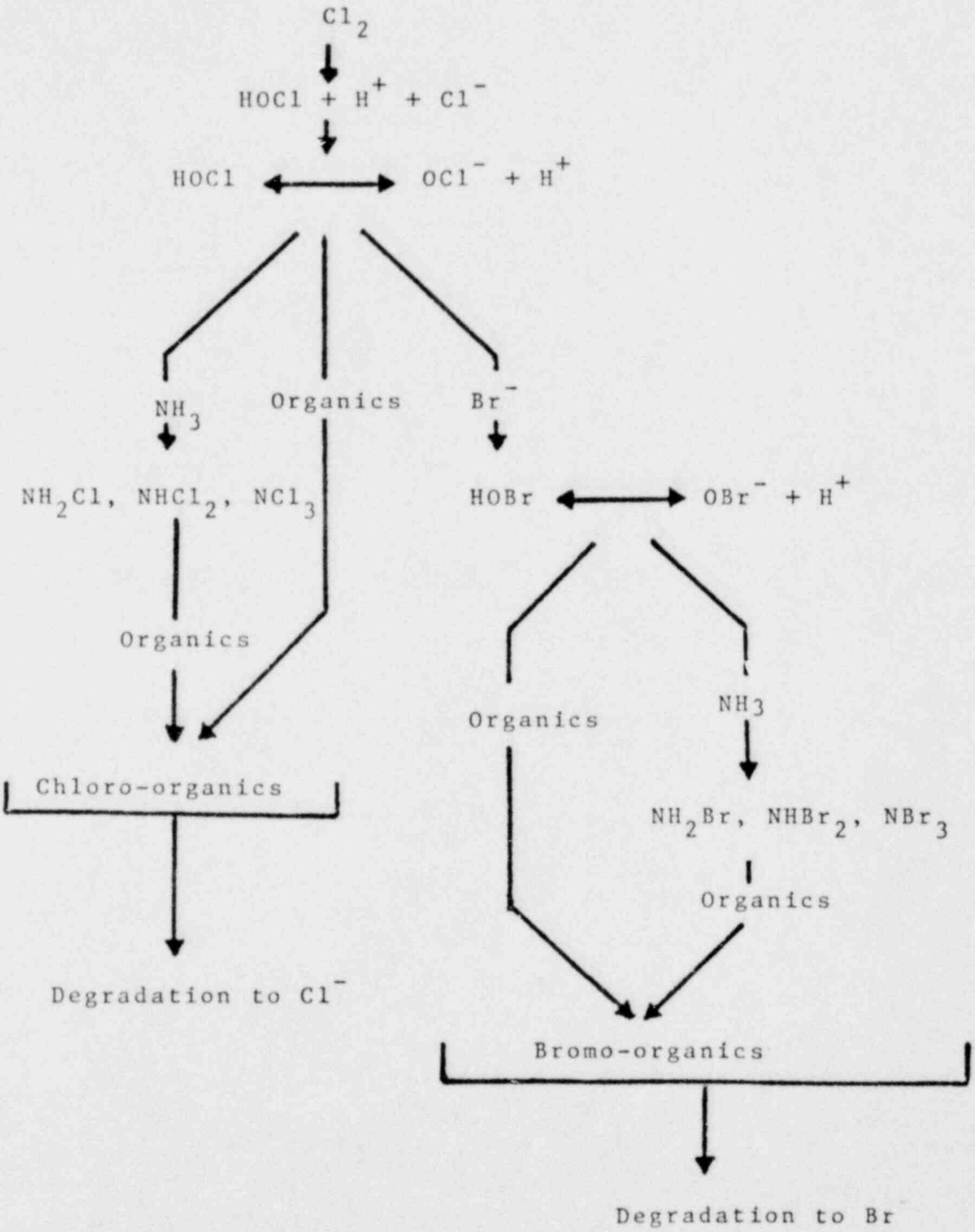


Figure 4. Proposed sequence of reactions for the degradation of chlorine in aquatic systems. Reactions of HOCl and HOBr with inorganic reducing agents have been omitted because they are probably of negligible importance in most cases. (Taken from Sugam and Helz (21)).

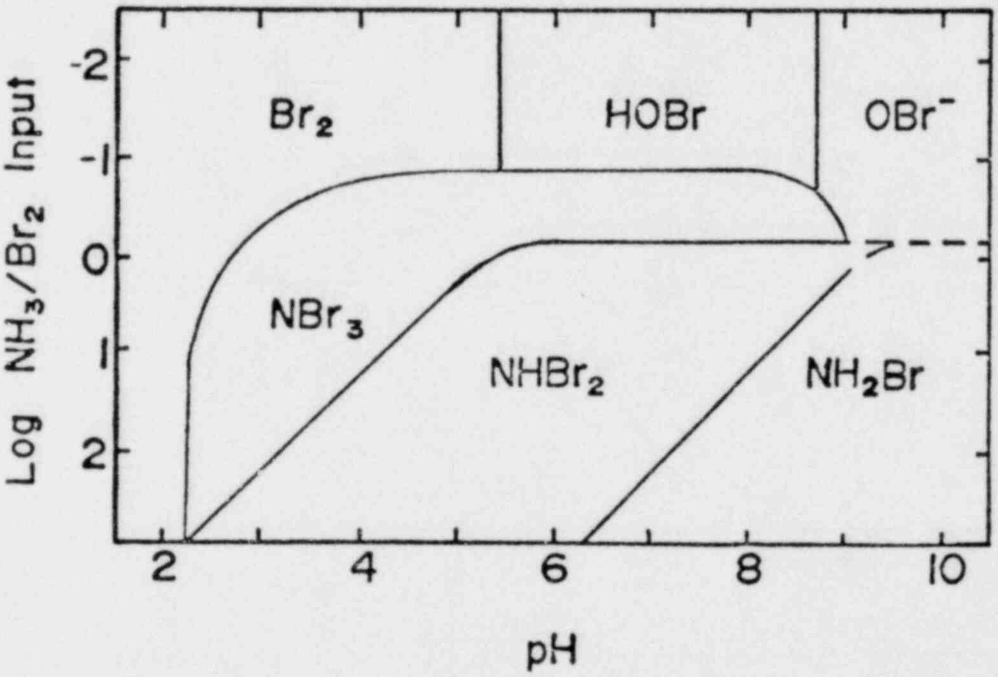


Fig. 5. Stability fields of bromine oxidants. (Taken from Johnson and Overby (24)).

major oxidant. However, they further point out that small amounts of monochloramine may be present as part of the total oxidant concentration in sufficient amounts to exert a toxic effect on various forms of marine life.

3.0 TOXICITY TO AQUATIC ORGANISMS

3.1 Toxicity Testing

The effects of chlorine on aquatic organisms in freshwater and seawater are addressed with particular emphasis on exposure time, concentration, and interactions of chlorine with environmental variables such as temperature and water quality.

Prior to discussing the effects of chlorine and its reaction products on aquatic organisms, it is necessary to define the terminology, test conditions, and procedures commonly used in toxicity tests.

Toxic effects may be divided into several overlapping categories which are shown in Table 1. These may also be used in some instances to describe the type of toxicity study being conducted. In general terms, however, toxic effects fall into two broad categories: 1) acute toxicity which is usually lethal and 2) chronic toxicity which may be lethal or sublethal.

The four techniques generally used for conducting toxicity tests are described in Table 2. Of these, the continuous flow or flow through technique in which test solutions are prepared continuously or every few minutes is the technique of choice for chlorine toxicity studies with macroinvertebrates and fishes.

Table 3 presents the terminology most commonly used for expressing results of toxicity tests. The importance of time-concentration relationships in toxicity tests necessitates that time should always be stated in the term (e.g. 96-h LC50), which is the concentration of a toxicant in solution that is lethal to 50% of the test organisms in 96 hours. The term ILC50, which is the intermittent lethal concentration of a toxicant to 50% of the test organisms during intermittent exposure tests, is ambiguous and should incorporate the number of exposures (4) and the total minutes of exposure time (160) in a 96-h observation period, e.g., 96-h ILC50 (4,160).

A comprehensive compilation of recent toxicity data (1969 to present) is presented in tabular form for plants (Table 4), invertebrates (Table 5) and fish (Table 6). The data have been grouped phylogenetically and the species with each group listed alphabetically. Saltwater and freshwater studies are indicated. The concentration of chlorine and time of exposure which produced a median response (e.g. LC50, EC50) was provided when available. Environmental factors which are known to influence toxicity (e.g. pH, temperature, salinity, life history stage) were included to aid in the interpretation of the data. Data involving wastewater chlorination were excluded because of the likely possibility of interactions between chlorine and various organic chemicals.

TABLE 1. GENERAL CATEGORIES OF TOXIC EFFECTS[*]

Acute - involving a stimulus severe enough to bring about a response speedily, usually within two to seven days for fish.

Subacute - involving a stimulus which is less severe than an acute stimulus, which produces a response in a longer time, and may become chronic.

Chronic - involving a stimulus which is lingering or continues for a long time, often used for periods of about one tenth of the life span or more.

Lethal - causing death, or sufficient time to cause it, by direct action.

Sublethal - below the level which directly causes death.

Cumulative - brought about, or increased in strength, by successive additions at different times or in different ways.

Delayed - symptoms do not appear until an appreciable time after exposure; often the response is triggered by occurrence of some other stress.

Short-term - acute but more indefinite

Long-term - chronic but more indefinite.

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*(Taken from Sprague 1969 (25)).

TABLE 2. TECHNIQUES GENERALLY USED FOR CONDUCTING* TOXICITY TESTS

Static Technique - test solutions and test organisms are placed in test chambers and kept there for the duration of the test.

Static Recirculation Technique - similar to the static technique except that each test solution is continuously circulated through an apparatus to maintain water quality by such means as filtration, aeration, sterilization and returned to the test chamber.

Static Renewal Technique - similar to the static technique except that the test organisms are periodically exposed to fresh test solution of the same composition usually once every 24 hours by transferring the test organisms from one test chamber to another.

Continuous Flow or Flow-Through Technique - test solutions flow into and out of the test chambers on a once-through basis for the duration of the test. Two procedures can be used: (1) large volumes of the test solutions are prepared before the beginning of the test and these flow through the test chambers and (2) fresh test solutions are prepared continuously or every few minutes in a toxicant delivery system.

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*(Taken from Committee on Methods (26)).

TABLE 3. TERMINOLOGY USED FOR EXPRESSING RESULTS OF TOXICITY TESTS[*]

LC50 - median lethal concentration of a toxicant in solution which is lethal to 50% of the test organisms.

EC50 - median effective concentration of a toxicant in solution at which a response other than death occurs to 50% of the test organisms.

LD50 - median lethal dose of a toxicant within the organism which is lethal to 50% of the test organisms.

ED50 - median effective dose of a toxicant within the organism at which a response other than death occurs to 50% of the test organisms.

LT50 - median lethal time. Used for mortality time in fixed concentrations.

ET50 - median effective time. Used for response time other than death in fixed concentrations.

TL_m, TLm, TL_{50}, TL50 - median tolerance limit. Term used primarily by U. S. pollution biologist. Equivalent numerically to LC50.

LL50 - median lethal level. For tests which yield mortality data where neither concentration or dose applies, e.g. tests with temperature.

EL50 - median effective level. For tests which use a response other than death where neither concentration or dose applies, e.g., tests with temperature.

ILC50 - intermittent lethal concentration of a toxicant in solution which is lethal to 50% of the test organisms during intermittent exposure tests.

Other terminology - usually used to describe the concentration at which toxicity ceases or the point beyond which 50% of the population can live for indefinite time:

- Incipient lethal level
- Ultimate median tolerance limit
- Lethal threshold concentration
- Median threshold concentration
- Asymptotic lethal concentration
- Asymptotic threshold concentration
- Others

[*] (Taken from Burton (27)).

Table 4. Toxicity of Chlorine to Aquatic Organisms: Plants

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	Reference
Chlorine	"phytoplankton"	SB, FW, LS	0.32(OT)	50% reduction in photosynthesis and respiration.	(28) Brook & Baker (1972)
Chlorine	"phytoplankton"	SB, SW, LS	<0.1(OT)	71% decrease in productivity after 4 hr exposure.	(29) Carpenter et al. (1972)
	CHRYSOPHYTA				
Chlorine	*Asterionella japonica*	SB, SW, LS	0.250(Iod)	24 hr IL_{50}; 10 C	(30) Gentile et al. (1976)
Chlorine	*Chaetoceros decipiens*	SB, SW, LS	0.140(Iod)	24 hr IL_{50}; 10 C	(30) Gentile et al. (1976)
Chlorine	*Chaetoceros didymum*	SB, SW, LS	0.125(Iod)	24 hr IL_{50}; 10 C	(30) Gentile et al. (1976)
Chlorine	*Detonula confervacea*	SB, SW, LS	0.20(Iod)	24 hr IL_{50}; 10 C	(30) Gentile et al. (1976)
Chlorine	*Monocrysis lutheri*	SB, SW, LS	0.20(Iod)	24 hr IL_{50}; 20 C	(30) Gentile et al. (1976)
Chlorine	*Rhodomenas baltica*	SB, SW, LS	0.11(Iod)	24 hr IL_{50}; 20 C	(30) Gentile et al. (1976)

Table 4. Toxicity of Chlorine to Aquatic Organisms: Plants

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	Reference
Chlorine	*Skeletonema costatum*	SB, SW, LS	0.095(Iod)	24 hr IL_{50}; 20 C	(30) Gentile et al. (1976)
Chlorine	*Skeletonema costatum*	SB, SW, LS	0.40-0.65 (Iod)	Adverse effect on growth, 5 min exposure.	(31) Hirayama & Hirano (1970)
			2.0	No growth 30 days after treatment.	
Chlorine	*Thalassiosira nordensholdii*	SB, SW, LS	0.195(Iod)	24 hr IL_{50}; 20 C	(30) Gentile (1976)*
Chlorine	*Thalassiosira pseudonana*	SB, SW, LS	1.0(Iod)	no growth after ≥ 10 sec exposure.	(30) Gentile et al. (1976)
			0.5	50% reduction in growth after 15 sec exposure. No growth after 30 min exposure.	
			0.15	48% reduction in photosysthesis after 20 min	
Chlorine	*Thalassiosira rotula*	SB, SW, LS	0.330(Iod)	24 hr IL_{50}; 10 C	(30) Gentile et al. (1976)

Table 4. Toxicity of Chlorine to Aquatic Organisms: Plants

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	Reference
	BACILLARIOPHYTA				
Chlorine	*Cyclotella nana*	SB, SW, LS	0.075(Iod)	24 hr IL_{50}; 20 C	(30) Gentile et al. (1976)
	CHLOROPHYTA				
Chlorine	*Chlamydomonas* sp.	SB, SW, LS	1.15(Iod)	Time-lag in growth, 5-10 min exposure.	(31) Hirayama & Hirano (1970)
			20.0	Growth affected, recovered in 9 days.	
Chlorine	*Chlorella pyrenoidosa*	SB, SW, LS	0.4(Amp)	50% decrease in growth after 5 hr exposure.	(32) Kott & Edlis (1969)
			0.6	43% mortality after 20 hr exposure.	
Chlorine	*Chlorella sorokiniana*	SB, FW, LS	0.2-1.0 (Amp)	Algicidal, no additional kills from 2nd dose.	(32) Kott & Edlis (1969)
Chlorine	*Cladophora* sp.	SB, SW, LS	1.0(Iod)	No physical cell damage, 24 hr; 30 C	(33) Betzer & Kott (1969)
			10.0	Complete kill in 2 hr	

Table 4. Toxicity of Chlorine to Aquatic Organisms: Plants

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	Reference
Chlorine	*Dunaliella tertiolecta*	SB, SW, LS	0.110(Iod)	24 hr IL$_{50}$; 20 C	(30) Gentile et al. (1976)
Chlorine	*Nanochloris occulatus*	SB, SW, LS	0.21(Amp)	4 hr EC$_{50}$; 20 C; salinity 30^{o}/oo	(34) Bender et al. (1977)
Chlorine	*Pseudoisochrysis paradoxa*	SB, SW, LS	0.17(Amp)	4 hr EC$_{50}$; 20 C; salinity 30^{o}/oo	(34) Bender et al. (1977)
Chlorine	*Pyramimonas virginica*	SB, SW, LS	0.130(Amp)	4 hr EC$_{50}$; 20 C; salinity 30^{o}/oo	(34) Bender et al. (1977)
Chlorine	*Tetraselmia suecica*	SB, SW, LS	0.06(Amp)	4 hr EC$_{50}$; 20 C; salinity 30^{o}/oo	(34) Bender et al. (1977)

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[1] SB = static bioassay, CB = constant-flow bioassay, SW = saltwater, FW = freshwater study, LS = lab study, FS = field study.

[2] Amp = amperometric titration, OT = acid orthotolodine, DPD = diethylparaphenylene diamine. Iod = Iodometric.

Table 5. Toxicity of Chlorine to Aquatic Organisms: Invertebrates

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	References
	CNIDARIA				
Chlorine	*Bimeria franciscana*	CB, SW, LS	2.5-4.5(Amp)	Slightly decreased growth after 3 hr exposure; 19-23 C; salinity 10.2-11.9°/oo	(35) McLean (1972)
	ROTIFERA				
Monochloramine	*Keratella chochlearis*	CB, FW, LS	0.019(Amp)	4 hr TL_{50}	(36) Beeton et al. (1976)
	MOLLUSCA				
Chlorine	*Crassostrea gigas*	CB, SW, LS	0.44(Amp)	48 hr EC_{50}; 10 day larvae	(37) Thatcher et al. (1976)
Chlorine	*Crassostrea virginica*	CB, SW, LS	0.029(Amp)	48 hr EC_{50} for larvae; salinity 20°/oo	(38) Roberts and Gleeson (1978)
Chlorine	*Crassostrea virginica*	CB, SW, LS	0.18(Amp)	50% decrease in time open after 72 hr exposure	(39) Patrick & McLean (1970)
Chlorine	*Goniobasis virginica*	CB, FW, LS	0.44(Amp)	96 hr LC_{50}; 25 C	(40) Gregg (1974)

Table 5. Toxicity of Chlorine to Aquatic Organisms: Invertebrates

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	References
Chlorine	*Nitocris carinata*	CB, FW, LS	0.086(Amp)	96 hr LC_{50}; 25 C	(40) Gregg (1974)
Chlorine	*Pagurus longicarpus*	CB, SW, LS	0.405(Amp)	24 hr LC_{50}; larvae	(38) Roberts (1978)
			0.310	48 hr LC_{50}; larvae	
			0.098	94 hr LC_{50}; larvae	
			0.21	96 hr LC_{50}; adult	
Chlorine	*Panopeus herbstii*	CB, SW, LS	1.0(Amp)	24 hr LC_{50}; larvae	(38) Roberts (1978)
			0.13	48 hr LC_{50}; larvae	
			0.038	96 hr LC_{50}; larvae	
			0.50	96 hr LC_{50}; adult	
	ARTHROPODA - CRUSTACEA				
Chlorine	*Acartia tonsa*	SB, SW, LS	1.0(Iod)	2 hr LC_{50}; 15 C salinity 30°/oo	(30) Gentile et al. (1976)

Table 5. Toxicity of Chlorine to Aquatic Organisms: Invertebrates

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	References
			2.5	5 min LC_{50}	
			5.0	2 min LC_{50}	
			10.0	0.7 min LC_{50}	
Chlorine	*Acartia tonsa*	SB, SW, LS	0.029(Amp)	48 hr EC_{50}; salinity 20^{o}/oo; 20 C	(38) Roberts and Gleeson (1978)
Chlorine	*Acartia tonsa*	SB, SW, LS	0.062(Amp)	24 hr LC_{50}; salinity 10.4-11.8^{o}/oo; 15 C	(41) Heinle & Beaven (1977)
			0.028	48 hr LC_{50}	
Chlorine	*Acartia tonsa*	CB, SW, LS	0.075(DPD)	30% killed; 20 C; 70% killed, 25 C	(42) Dressel (1971)
			1.15	100% killed; 20 C; pH 7.8	
Chlorine	*Acartia tonsa*	CB, SW, LS	2.5(Amp)	>90% mortality in 96 hr after 5 min exposure; 15 C	(43) McLean (1973)
Chlorine	*Anonyx* sp.	CB, SW, LS	0.145(Amp)	96 hr LC_{50}; 10 C acclimation temp.; 14.8 C exposure temp.; salinity 18^{o}/oo; pH 8	(44) Thatcher (1978)

Table 5. Toxicity of Chlorine to Aquatic Organisms: Invertebrates

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	References
Chlorine	Asellus racovitsai	CB, FW, LS	0.613(Amp)	24 hr LC_{50}; 15 C	(40) Gregg (1974)
Chlorine	Balanus improvisus	CB, SW, LS	2.5(Amp)	80% mortality in 96 hr after 5 min exposure; 15 C	(43) McLean (1973)
Chlorine	Callinectes sapidus	CB, SW, LS	10.0(Amp)	19 hr LC_{50}	(39) Patrick & McLean (1970)
			0.1	96 hr LC_{50}	
Chlorine	Crangon nigricauda	CB, SW, LS	0.134(Amp)	96 hr LC_{50}; 10 C acclimation temp.; 14.8 C exposure temp.; salinity 28^{o}/oo; pH 8	(44) Thatcher (1978)
Chlorine	Crangon septemspinosus	CB, SW, LS	10.0(Iod)	60% mortality after 5 min exposure; 10 C salinity 30^{o}/oo	(30) Gentile et al. (1976)
			5.0	42% mortality after 10 min exposure	
Chlorine	Crangon septemspinosus	CB, SW, LS	0.15	15 hr LC_{50}	(39) Patrick & McLean (1970)

Table 5. Toxicity of Chlorine to Aquatic Organisms: Invertebrates

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	References
Monochloramine	*Cyclops biscupidatus thomasi*	CB, FW, LS	0.084(Amp)	96 hr TL_{50}	(36) Beeton et al. (1976)
			0.069	96 hr TL_{50} free monochloramine	
Chlorine	*Durytemora affinis*	SB, SW, LS	1.0(Iod)	6 hr LC_{50}; 15 C salinity 30^{o}/oo	(30) Gentile et al. (1976)
			2.5	9 min LC_{50}	
			5.0	4 min LC_{50}	
			10.0	2 min LC_{50}	
Chlorine	*Gammarus daiberi*	SB, SW, LS	<0.02(Amp)	avoidance	(46) Ginn & O'Connor (1978)
			1.85	96 hr TL_{50}; salinity 1.6^{o}/oo; pH 7.3; 23.5-23.7 C	
Chloramine	*Gammarus pseudo-limnaeus*	CB, FW, LS	0.0034(Amp)	reduced offspring 17 C	(47) Arthur & Eaton (1971)

Table 5. Toxicity of Chlorine to Aquatic Organisms: Invertebrates

Chemical Compound	Test Organism	Test Conditions^1	Concentration (ppm)^2	Remarks	References
			$\geq$.035	reduced survival in 15 weeks	
			0.22	96 hr-TLm	
Chlorine	*Gammarus tigrinus*	CB, SW, LS	2.5(Amp)	24.8% mortality in 96 hr after 3 hr exposure	(43) McLean (1973)
Chlorine	*Hemigrapus nudus* *H. oregonensis*	CB, SW, LS	1.418(Amp)	96 hr LC_{50}; 10 C acclimation temp.; 14.8 C exposure temp.; salinity 28^{o}/oo; pH 8	(44) Thatcher (1978)
Chlorine	*Eurytemora affinis*	SB, SW, LS	0.10(DPD)	no reproductive effect in 3 mos.; pH 7.8	(45) Bradley (1976)
			0.477	24 hr LC_{50}	
			0.250	reproductive failure in 3 mos.	
Chlorine (free)	*Homarus americanus*	CB, SW, LS	2.90(Amp)	48 hr LC_{50}; 30,60 min exposure; salinity 30-31^{o}/oo; pH 7.9-8.1; 25 C; larvae	(48) Capuzzo et al. (1977)

Table 5. Toxicity of Chlorine to Aquatic Organisms: Invertebrates

Chemical Compound	Test Organism	Test Conditions^1	Concentration (ppm)^2	Remarks	References
Chloramine	*Homarus americanus*	CB, SW, LS	0.30(Amp)	(see above)	
Chlorine	*Melita nitida*	CB, SW, LS	2.5(Amp)	97.2% mortality in 96 hr after 3 hr exposure; 15 C	(43) McLean (1973)
Chlorine	*Mercenaria mercenaria*	SB, SW, LS	0.006(Amp)	48 hr EC$_{50}$ salinity 18.2-20.4^{o}/oo	(49) Roberts et al. (1975)
Chlorine	*Neomysis* sp.	CB, SW, LS	0.162(Amp)	96 hr LC$_{50}$; 10 C acclimation temp.; 14.8 C exposure temp.; salinity 28^{o}/oo; pH 8	(44) Thatcher (1978)
Chlorine	*Palaemonetes pugio*	CB, SW, LS	0.22(Amp)	96 hr LC$_{50}$ salinity; 18.2-20.4^{o}/oo	(49) Roberts et al. (1975)
Chlorine	*Palaemonetes pugio*	CB, SW, LS	2.5(Amp)	98% mortality in 96 hr after 3 hr exposure; 15 C	(43) McLean (1973)
Chlorine	*Pandalus danae*	CB, SW, LS	0.18(Amp)	Total kill 1 mo exposure; 16 C	(50) Gibson et al. (1976)
			0.01, 0.08	reduced growth	
			0.05	increased growth	

Table 5. Toxicity of Chlorine to Aquatic Organisms: Invertebrates

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	References
Chlorine	*Pandalus danae*	CB, SW, LS	0.210(Amp)	96 hr LC_{50}; 15 C	(37) Thatcher et al. (1976)
			0.20	Hatching of eggs inhibited in 6 day test	
Chlorine	*Pandalus goniurus*	CB, SW, LS	0.090(Amp)	96 hr LC_{50}; 10 C acclimation temp.; 14.8 C exposure temp.; salinity 28^{o}/oo; pH 8	(44) Thatcher (1978)
Chlorine	*Pontogenia* sp.	CB, SW, LS	0.687(Amp)	96 hr LC_{50}; 10 C acclimation temp.; 14.8 C exposure temp.; salinity 28^{o}/oo; pH 8	(44) Thatcher (1978)
Chlorine	*Pseudodiaptomus coronatus*	SB, SW, LS	2.5(Iod)	45 min LC_{50}; 15 C salinity 30^{o}/oo	(30) Gentile et al. (1976)
			5.0	9.8 min LC_{50}	
			10.0	5.0 min LC_{50}	
	ARTHROPODA - INSECTA				

Table 5. Toxicity of Chlorine to Aquatic Organisms: Invertebrates

Chemical Compound	Test Organism	Test Conditions[1]	Concentration (ppm)[2]	Remarks	References
Chlorine	*Centroptilium* sp.	CB, FW, LS	0.071(Amp)	24 hr LC_{50}; 6 C	(40) Gregg (1974)
Chlorine	*Ephemerella lata*	CB, FW, LS	0.27(Amp)	48 hr LC_{50}; 15 C	(40) Gregg (1974)
Chlorine	*Hydropsyche bitida*	CB, FW, LS	0.396(Amp)	6 hr LC_{50}; 25 C	(40) Gregg (1974)
Chlorine	*Iron humeralis*	CB, FW, LS	0.0093(Amp)	6 hr LC_{50}; 6 C	(40) Gregg (1974)
Chlorine	*Peltoperla maria*	CB, FW, LS	0.020(Amp)	48 hr LC_{50}; 6 C	(40) Gregg (1974)
Chlorine	*Stenonema ithaca*	CB, FW, LS	0.502(Amp)	6 hr LC_{50}; 25 C	(40) Gregg (1974)

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[1] SB = static bioassay, CB = constant-flow bioassay, SW = saltwater, FW = freshwater, LS = lab study, FS = field study

[2] Amp = amperometric titration, OT = acid orthotolodine, DPD = diethylparapehnylenediamine, Iod = Iodometric

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
	CLUPEIDAE - herrings				
Chlorine	*Alosa aestivalis*	SB, SW, LS	1.1-1.2	30 min LC_{50}	(51) Engstrom & Kirkwood (1974)
			0.67	60 min LC_{50}	
Chlorine	*Alosa aestivalis*	CB, SW, LS	0.33(Amp),	80 hr LC_{50} for eggs	(52) Morgan and Prince (1977)
			0.28-0.32	24 hr LC_{50} for larvae	
Chlorine	*Alosa pseudo harengus*	SB, FW, LS	0.30(Amp)	30 min LC_{50}; 30 C	(53) Seegert & Brooks (1978)
			2.27	30 min LC_{50}; 15 C	
Chlorine	*Brevoortia tyrannus*	SB, SW, LS	1.1-1.2	30 min LC_{50}	(51) Engstrom & Kirkwood (1974)
			0.67	60 min LC_{50}	
Chlorine	*Brevoortia tyrannus*	SB, SW, LS	0.3(Amp)	no mortality in 3 min exposure to larvae	(54) Hoss et al.(1974)
			0.5	reduced survival above 3 min exposure time	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
Chlorine	*Brevoortia tyrannus*	SB, SW, LS	0.1(OT)	No effect in 10 min	(55) Fairbanks et al. (1971)
			0.27	30 min LC$_{50}$	
			0.7	10 min LC$_{50}$	
Chlorine	*Clupea harengus pallasi*	CB, SW, LS	0.057(Amp)	96 hr LC$_{50}$; 10 C acclimation temp.; 14.3 C exposure temp.; salinity 28^{o}/oo; pH 8	(44) Thatcher (1978)
Chlorine	*Dorosoma cepedianum*	CF, FW, LS	0.62(Amp)	some mortality in 10 min exposure	(56) Basch & Truchan (1971)
	SALMONIDAE - trout and salmon				
Chlorine	*Oncorhynchus gorbuscha*	CB, SW, LS	>0.023, <0.052(Amp)	96 hr LC$_{50}$; 10 C acclimation temp., 14.8 C exposure temp., salinity 28^{o}/oo, pH 8	(44) Thatcher (1978)
Chlorine	*Oncorhynchus gorbuscha*	CB, SW, LS	0.5(OT)	7.5 min LC$_{50}$, salinity $20-26^{o}$/oo, pH 7.23-7.80	(57) Stober and Hanson (1974)

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
			0.25	60 min LC_{50}	
			0.10	60 min LC_{50}, +9.9 C temp. shock	
Chlorine	*Oncorhynchus kisutch*	SB, FW, LS	0.29(Amp)	30 min LC_{50}; 20 C	(53) Seegert & Brooks (1978)
			0.56	30 min LC_{50}; 10 C	
Chlorine	*Oncorhynchus kisutch*	CB, SW, LS	0.208(Amp)	60 min LC_{50}, 12.7 C, pH 7.8, salinity 29.6	(58) Stober et al.(1978)
			0.130	60 min LC_{50}, 12.7 C +7.3 C temp. shock, pH 8.0, salinity 28.8	
			0.142	24 hr LC_{50}, 12.1 C temp., pH 7.9, salinity 29.4^{o}/oo	
			0.002-0.5	avoidance, 12 C	
Chlorine	*Oncorhynchus kisutch*	CB, SW, LS	0.032(Amp)	96 hr LC_{50}; 10 C acclimation temp., 14.8 C exposure temp., salinity 28^{o}/oo; pH 8	(44) Thatcher (1978)

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition^1	Concentration (ppm)^2	Remarks	Reference
Monochloramine	*Oncorhynchus kisutch*	CB, FW, LS	0.64(Amp)	96 hr LC_{50}; 12 C, 40 min pulses at 8 hr intervals, pH 6.5-7.7	(59) Heath (1978)
Chlorine	*Oncorhynchus kisutch*	CB, FW, LS	0.05(Amp)	No death in 140 hr	(60) Heath (1977)
Chlorine	*Oncorhynchus kisutch*	CB, FW, LS	0.057(Amp)	96 hr LC_{50} for juveniles, 15 C, pH 7.5	(61) Larson et al. (1977)
Chloramine	*Oncorhynchus kisutch*	CB, FW, LS	0.057(Amp)	96 hr TL_{50} for fry, 10 C, pH 7.5	(61) Larson et al. (1977)
			0.022-0.023	reduced growth in juveniles	
Chlorine	*Oncorhynchus kisutch*	CB, FW, LS	0.016(Amp)	24 hr TLm	(62) Rosenberger (1972)
			0.004	96 hr TLm	
Chlorine	*Oncorhynchus tshawytscha*	CB, SW, LS	>0.038, <0.065(Amp)	96 hr LC_{50}; 10 C acclimation temp., 14.8 C exposure temp., salinity $28^{o}/oo$, pH 8	(44) Thatcher (1978)

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition^1	Concentration (ppm)^2	Remarks	Reference
Chlorine	*Oncorhynchus tshawytscha*	CB, SW, LS	0.5(OT)	15 min LC_{50}, salinity 20-28°/oo, pH 7.66-7.83	(57) Stober and Hanson (1974)
			0.25	60 min LC_{50}	
			0.05	60 min LC_{50}, +10 C temp. shock	
Chlorine	*Oncorhynchus tshawytscha*	CB, FW, LS	1.0(Iod)	100% mortality; 12 min for fry	(63) Collins & Deaner (1973)
Chloramines	*Salvelinus fontinalis*	CB, FW, LS	0.106(Amp)	96 hr TL_{50} for alevins, 10.6 C, pH 7.8	(64) Larson et al. (1977)
			0.082	96 hr TL_{50} for fry, 10.6 C, pH 7.8	
			0.088	96 hr TL_{50} for juvenile, 11.1 C, pH 7.7	
Chlorine	*Salvelinus fontinalis*	CB, FW, LS	0.35(Iod)	Survival time, 9 hr (mean), pH = 6.8; 10 C	(65) Dandy (1972)
			0.08	Survival time, 18 hr (mean).	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition^1	Concentration (ppm)^2	Remarks	Reference
			0.04	Survival time, 48 hr (mean).	
			0.005	Survival for 7 days, but depressant response.	
Chlorine	*Salmo gairdneri*	CB, FW, LS	0.05(Amp)	96 hr LC$_{50}$; 12 C, 40 min pulses at 8 hr intervals; pH 6.5-7.7; free chlorine	(59) Heath (1978)
Chlorine	*Salmo gairdneri*	CB, FW, LS	0.04(Amp)	10% mortality in 7 days	(60) Heath (1977)
		CB, FW, LS	0.15	100% mortality	
Monochloramine	*Salmo gairdneri*	CB, FW, LS	0.75	120 hr LC$_{50}$	(60) Heath (1977)
Chlorine	*Salmo gairdneri*	CB, FW, LS	0.4(Amp)	gill lamellae deformed	(66) Bass et al. (1977)
Chlorine	*Salmo gairdneri*	CB, FW, LS	0.04(Amp)	96 hr LC$_{50}$	(67) Wolf et al. (1975)
Chlorine	*Salmo gairdneri*	SB, FW, LS	0.99(Amp, DPD)	30 min LC$_{50}$; 10 C	(68) Brooks & Seegert (1977)

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
			0.43	30 min LC_{50}; 20 C	
Chlorine	*Salmo gairdneri*	FW, FS	0.014(Amp)	96 hr-TLm	(69) Basch et al. (1971)
			0.029	96 hr-TLm.	
Chlorine	*Salmo gairdneri*	CB, FW, LS	0.001(OT)	Detected avoidance response; soft water, 17 C.	(70) Sprague & Drury (1969)
			0.01	Lethal in 12 days.	
			0.1	Lethal in about 4 days, attracted.	
			1.0	Lethal in <4 hr, strong avoidance.	
Chlorine	*Salmo trutta*	CB, FW, FS	0.14-0.19 (Amp)	48 hr LC_{50}	(71) Basch & Truchan (1976)
			0.02-0.18	96 hr LC_{50}	
			1.19	96 hr ILC_{50};17 C	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
				30 min exposures	
			0.56	96 hr ILC_{50};21 C 30 min exposures	
Chlorine	*Salmo trutta*	CB, FW, LS	0.04(OT)	Lethal, 1 hr	(72) Pike (1971)
			0.01	Lethal, TLm = 43.5 hr	
	OSMERIDAE - smelts				
Chlorine	*Osmerus mordax*	SB, FW, LS	1.27(Amp)	48 hr LC_{50}; 10 C	(53) Seegert & Brooks (1978)
	CYPRINIDAE - minnows and carps				
Chlorine	*Carassius auratus*	SB, FW, LS	15.85(Amp)	0.25 hr TLm	(73) Dickson et al. (1977)
			1.50	2 hr TLm	
			0.40	12 hr TLm	
			0.27	24 hr TLm	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
Monochloramine	*Cyprinus carpio*	SB, FW, LS	2.37(Amp, DPD)	LC_{50}; 10 C; 4-40 min exposures	(74) Brooks & Seegert (1978)
			1.50	LC_{50}; 30 C	
Chlorine	*Cyprinus carpio*	CB, FW, LS	0.245(Amp)	166 hr LC_{50}	(60) Heath (1977)
Chlorine	*Cyprinus carpio*	CB, FW, LS	0.72(Amp)	some mortality in 10 min exposure	(75) Truchan & Basch (1971)
Monochloramine	*Cyprinus carpio*	CB, FW, LS	1.19 (Amp)	166 hr LC_{50}	(60) Heath (1977)
Chlorine	*Notemigonus crysoleucas*	CB, FW, LS	0.19(Amp)	96 hr LC_{50}; 24 C, 40 min pulses at 8 hr intervals, pH 6.5-7.7, free chlorine	(59) Heath (1978)
Monochloramine			0.93	(see above)	
Chlorine	*Notemigonus crysoleucas*	CB, FW, LS	0.06(Amp)	No death	(60) Heath (1977)
Monochloramine	*Notropis atherinoides*	SB, FW, LS	0.63(Amp, DPD)	LC_{50}; 10 C; 4-40 min exposures	(74) Brooks & Seegert (1978)
			0.35	LC_{50}; 30 C	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
Monochloramine	*Notropis cornutus*	SB, FW, LS	0.78(Amp, DPD)	LC$_{50}$; 10 C; 4-40 min exposures	(74) Brooks & Seegert (1978)
			0.45	LC$_{50}$; 30 C	
Chlorine	*Notropis hudsonius*	SB, FW, LS	0.53(Amp)	30 min LC$_{50}$; 20 C	(53) Seegert & Brooks (1978)
			2.41	30 min LC$_{50}$; 10 C	
Chlorine	*Notropis rubellus*	CB, FW, LS	0.102(Amp)	avoidance to total residual chlorine	(76) Cherry et al. (1977)
			0.055	avoidance to combined residual chlorine	
			0.044	avoidance to free residual chlorine	
			0.014	HOC avoidance	
Monochloramine	*Notropis spilopterus*	SB, FW, LS	0.65(Amp)	LC$_{50}$; 10 C 4-40 min exposures	(75) Brooks & Seegert (1978)
			0.41	LC$_{50}$; 30 C	
Chlorine	*Pimephales promelas*	CB, FW, LS	0.082-0.095 (Amp)	LC$_{50}$; 96 hr TLm	(77) DeGraeve & Ward (1977)

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
			0.110-0.138	no mortality in 7 days after previous exposure to sublethal levels	
Chlorine	*Pimephales promelas*	CB, FW, LS, FS	0.16-0.21 (Iod)	Total kill, 96 hr	(78) Zillich (1972)
			0.07-0.19	96 hr LC_{50}	
			0.04-0.09	Sublethal stress	
			0.04-0.05	Threshold concentration	
Chloramine	*Pimephales promelas*	CB, FW, LS	0.0165(Amp)	Lowest level with no significant effect (reproduction); 23 C	(47) Arthur & Eaton (1971)
			0.085	no deaths in 96 hr	
			0.108	60% mortality in 30 days for larvae	
			0.154	All killed in 3 days	
Monochloramine	*Catostomas commersoni*	SB, FW, LS	1.09(Amp, DPD)	LC_{50}, 10 C; 4-40 min exposures	(75) Brooks & Seegert (1978)

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
			0.36	LC_{50}, 30 C	
	ICTALURIDAE - catfishes				
Chlorine	*Ictalurus lacustris*	CB, FW, LS	0.12	No death	(60) Heath (1977)
Monochloramine	*Ictalurus lacustris*	CB, FW, LS	0.25	120 hr LC_{50}	(60) Heath (1977)
Chlorine	*Ictalurus melas*	CF, FW, LS	1.36(Amp)	some mortality in 25 min exposure	(75) Truchan & Basch (1971)
Monochloramine	*Ictalurus punctatus*	SB, FW, LS	0.78(Amp, DPD)	LC_{50}; 10 C; 4-40 min exposures	(74) Brooks & Seegert (1978)
			0.67	LC_{50}; 30 C	
Chlorine (free)	*Ictalurus punctatus*	CB, FW, LS	0.06(Amp)	96 hr LC_{50}; 24 C, 40 min pulses at 8 hr intervals, pH 6.5-7.7	(59) Heath (1978)
Monochloramine		CB, FW, LS	0.33 (Amp)	(see above)	
	CYPRINODONTIDAE - killifishes				

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
Chlorine	*Fundulus heteroclitus*	CB, SW, LS	<0.4(Amp)	complete survival, free chlorine	(79) Capuzzo et al. (1977)
Chloramine	*Fundulus heteroclitus*	CB, SW, LS	<0.8 (Amp)	complete survival	
	ATHERINIDAE - silversides				
Chlorine	*Menidia beryllina*	CB, SW, LS	0.21-0.32 (Amp)	48 hr LC_{50} for eggs	(52) Morgan & Prince (1977)
Chlorine	*Menidia menidia*	CB, SW, LS	0.13(Amp)	46% kill 3 min exposure	(80) Hoss et al. (1977)
Chlorine	*Menidia menidia*	SW, LS	0.03-0.64 (Amp)	avoidance; salinity 0-7%	(81) Meldrin & Fava (1977)
Chlorine	*Menidia menidia*	CB, SW, LS	0.30(Amp)	48 hr LC_{50} for eggs	(52) Morgan & Prince (1977)
Chlorine	*Menidia menidia*	CB, SW, LS	0.037(Amp)	96 hr LC_{50}; salinity 18.2-20.4°/oo	(49) Roberts et al. (1975)
Chlorine	*Menidia menidia*	SB, SW, LS	1.1-1.2	30 min LC_{50}	(51) Engstrom & Kirkwood (1974)
			0.58	90 min LC_{50}	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition^1	Concentration (ppm)^2	Remarks	Reference
	GASTEROSTEIDAE - sticklebacks				
Chlorine	Gasterosteus aculeatus	CB, SW, LS	0.167	96 hr LC$_{50}$; 10 C acclimation temp., 14.8 C exposure temp., salinity 28^{o}/oo, pH 8	(44) Thatcher (1978)
	SYNGNATHIDAE - pipefishes and seahorses				
Chlorine	Syngnathus fuscus	CB, SW, LS	0.27(Amp)	96 hr LC$_{50}$; salinity 18.2-20.4^{o}/oo	(49) Roberts et al. (1975)
	PERCICHTHYIDAE - temperate basses				
Chlorine	Morone americana	SW, LS	1.3		(81) Meldrin & Fava (1977)
Chlorine	Morone americana		0.04-0.35 (Amp)	avoidance	
Chlorine	Morone americana	CB, SW, LS	0.27(Amp)	76 hr LC$_{50}$ for eggs, 15 C,	(52) Morgan & Prince (1977)

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition^1	Concentration (ppm)^2	Remarks	Reference
			0.31	salinity 8-12^o/oo 24 hr LC$_{50}$ for larvae	
Monochloramine	*Morone chrysops*	SB, FW, LS	2.87(Amp, DPD)	LC$_{50}$; 10 C; 4-40 min exposures	(74) Brooks & Seegert (1978)
			1.15	LC$_{50}$; 30 C	
Chlorine	*Morone saxatilis*	CB, SW, LS	0.021(Amp)	no emergence from embryos, 1-3^o/oo salinity, 18 C	(82) Middaugh et al. (1977)
			0.07	3.5% emergence	
			0.01	23% emergence	
			0.04	incipient LC$_{50}$ for 2 day larvae	
			0.07	incipient LC$_{50}$ for 12 day larvae	
			0.04	incipient LC$_{50}$ for 30 day larvae	
			0.29-0.32	avoidance response in 24 day old larvae	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
Chlorine	*Morone saxatilis*	CB, SW, LS	0.20-0.36 (Amp)	48 hr LC_{50} for eggs	(52) Morgan & Prince (1977)
			0.19-0.20	24 hr LC_{50} for eggs	
Chlorine	*Morone saxatilis*	SB, SW, LS	0.05	24, 48, 72, 96 hr LC_{50}; 70 F	(83) Hughes (1970)
	CENTRARCHIDAE - sunfishes				
Monochloramine	*Lepomis macrochirus*	SB, FW, LS	3.00(Amp, DPD)	LC_{50}, 10 C; 4-40 min exposures	(74) Brooks & Seegert (1978)
			1.23	LC_{50}; 20 C	
Chlorine	*Lepomis macrochirus*	CB, FW, LS	0.2-0.3(Amp)	hyperplasia & swelling of gill epithelium	(66) Bass et al. (1977)
Chlorine	*Lepomis macrochirus*	CB, FW, LS	0.52(Amp)	74 hr LT_{50} at 6 C; 45 min exposures, 3/day; pH 7.25-7.55; 20 hr LT_{50} at 32 C same	(84) Bass & Heath (1977)
Chlorine	*Micropterus punctulatus*	CB, FW, LS	0.198(Amp)	avoidance to total residual chlorine; 18 C	(76) Cherry et al. (1977)

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition^1	Concentration (ppm)^2	Remarks	Reference
			0.097	avoidance to combined residual chlorine	
			0.101	avoidance to free residual chlorine	
			0.048	HOCl, avoidance	
Chlorine	*Pomoxis nigromaculatus*	CB, FW, LS	1.36(Amp)	some mortality in 25 min exposure	(75) Truchan & Bascz (1971)
	PERCIDAE - perches				
Chlorine	*Perca flavescens*	SB, FW, LS	8.0 (Amp, DPD)	30 min LC_{50}; 10 C	(68) Brooks & Seegert (1977)
			0.70	30 LC_{50}; 20 C	
	Perca flavescans	CB, FW, LS	0.72(Amp)	some mortality in 60 min exposure	(75) Truchan & Basch (1971)
Chlorine	*Stizostedion canadense*	SB, FW, LS	1.14(Amp, DPD)	LC_{50}; 10 C; 4-40 min exposures	(74) Brooks & Seegert (1978)
			0.71	LC_{50}; 30 C	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition^1	Concentration (ppm)^2	Remarks	Reference
	GERREIDAE - mojarras				
Chlorine	*Eucinostomus argenteus*	CB, SW, LS	0.28(Amp)	75% kill; 10 min exposure 25-26 C; salinity 33-35^o/oo; pH 7.5-7.8	(80) Hoss et al. (1977)
	SCIAENIDAE - drums				
Monochloramine	*Aplodinotus grunniens*	SB, FW, LS	2.45(Amp, DPD)	LC$_{50}$; 10 C; 4-40 min exposures	(74) Brooks & Seegert (1978)
			1.75	LC$_{50}$; 20 C	
Chlorine	*Cynoscion nebulosus*	SB, SW, LS	0.21(DPD)	48 hr TLm for 2 hr eggs; 30^o/oo salinity; 25 C; pH 7.8	(85) Johnson et al. (1977)
			0.21	48 hr TLm 10 hr eggs	
			0.17	48 hr TLm 1 hr post-hatching larvae	
Chloramine	*Cynoscion nebulosus*	SB, SW, LS	14.14	48 hr TLm 2 hr eggs	(85) Johnson et al. (1977)

Table 6. Toxicity of Chlorine to Aquatic Organisms. Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
			0.57	48 hr TLm 10 hr eggs	
			5.75	48 hr TLm 1 hr post-hatch	
Chlorine	*Leiostomas xantherus*	CB, SW, LS	0.14(Amp)	24 hr TLm; pH 7.5, 14.2-16 C	(86) Bellanca & Bailey (1977)
			0.09	96 hr TLm	
			0.04	complete survival	
			0.16	Total kill	
Chlorine	*Leiostomas xanthurus*	CB, SW, LS	0.12(Amp)	8 day ILC$_{50}$ 10 C; salinity 20-24^{o}/oo; pH 7.7-7.9	(82) Middaugh et al. (1977)
			0.06	ILC$_{50}$; 15 C	
	CICHLIDAE - cichlids				
Chlorine	*Tilapia aurea*	SB, FW, LS	>0.5 (OT)	3-5 g fish sensitive	(87) Eren & Langer (1973)
			≤1.0	tolerated by larger fish.	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
	EMBIOTOCIDAE - surfperches				
Chlorine	*Cymatogaster aggregata*	CB, SW, LS	<0.2(Amp)	No mortality. 60 min exposure	(58) Stober et al.(1978)
			0.301	60 min LC_{50}; 13 C; pH 8.1	
			>1.0	100% mortality	
			0.175-0.5	avoidance; 12 C	
Chlorine	*Cymatogaster aggregata*	CB, SW, LS	0.071(Amp)	96 hr LC_{50}; 10 C acclimation temp.; 14.8 C exposure temp.; salinity 28^{o}/oo; pH 8	(44) Thatcher (1978)
	MUGILIDAE - mullets				
Chlorine	*Mugil cephalus*	CB, SW, LS	0.50(Amp)	90% survived 10 min exposure; 25-26 C; salinity 33-35^{o}/oo; pH 7.5-7.8	(80) Hoss et al. (1977)

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition^{1}	Concentration (ppm)^{2}	Remarks	Reference
	AMMODYTIDAE - sand lances				
Chlorine	*Ammodytes hexapterus*	CB, SW, LS	0.082(Amp)	96 hr LC$_{50}$; 10 C acclimation temp.; 14.8 C exposure temp.; salinity 28^{o}/oo; pH 8	(37) Thatcher et al. (1976)
	GOBIIDAE - gobies				
Chlorine	*Gobiosoma bosci*	CB, SW, LS	0.08(Amp)	96 hr LC$_{50}$; salinity 18.2-20.4^{o}/oo	(49) Roberts et al. (1975)
	BOTHIDAE - lefteye flounders				
Chlorine	*Paralichthys* sp.	SB, SW, LS	0.3(Amp)	20% killed in 7 min	(80) Hoss et al. (1977)
			0.3	100% killed in 10 min.	
	PLEURONECTIDAE - righteye flounders				

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
Chlorine	*Limanda ferruginea*	SB, SW, LS	0.10-0.20	24 hr LC_{50}; 10 C salinity 30^{o}/oo	(30) Gentile et al. (1976)
Chlorine	*Parophrys vetulus*	CB, SW, LS	0.073(Amp)	96 hr LC_{50}; 10 C acclimation temp., 14.8 C exposure temp., salinity 28^{o}/oo, pH 8	(44) Thatcher (1978)
Chlorine	*Pleuronectes platessa*	CB, SW, LS	0.105 (DPD, OT)	192 hr LC_{50}; eggs	(89) Alderson (1974)
			0.64	72 hr LC_{50}; eggs	
			0.025-0.071	96 hr LC_{50}; larvae	
			0.094-0.095	96 hr LC_{50}; fish	
Chlorine	*Pseudopleuronectes americanus*	SB, SW, LS	2.5(Iod)	15 min LC_{50}; 10 C; salinity 30^{o}/oo	(30) Gentile et al. (1976)
			5.0	2.5 LC_{50}	
			10.0	0.3 LC_{50}	

Table 6. Toxicity of Chlorine to Aquatic Organisms: Fish

Chemical Compound	Test Organism	Test Condition[1]	Concentration (ppm)[2]	Remarks	Reference
Chlorine	*Solea solea*	CB, SW, LS	0.028-0.059 (DPD, OT)	48 hr LC_{50}; larvae	(88) Alderson (1974)
			0.070-0.089	96 hr LC_{50}; fish	

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[1] SB = Static bioassay, CB = constant-flow bioassay, SW = saltwater, FW = freshwater, LS = lab study, FS = field study.

[2] Amp = amperometric titration, OT = acid orthotolodine, DPD = diethylparaphenylenediamine, Iod = iodometric.

A detailed discussion of factors influencing toxicity is presented in the sections that follow.

3.2 Concentration and Exposure Time

Much of the recent work on the toxicity of chlorine to aquatic organisms has involved the simulation of intermittent chlorination practices employed in power generation facilities and industrial processes for biofouling control. Larson and Schlesinger (89) have pointed out that one of the major problems associated with the analysis and interpretation of results of these types of experiments is the highly variable concentrations, durations, and frequency of exposure. The time concentration relationships found in the field at points of discharge of intermittently chlorinated waters range widely from square to spike patterns (G. Nelson as cited by Larson & Schlesinger (89)). Thus the usual method of representing toxicity in terms of toxicant concentration precludes the application of results obtained from particular patterns of concentration and times of exposure to any other patterns to which organisms might be exposed. Furthermore there has been a tendency to report the results of spike patterns of exposure in terms of peak concentrations (60) and square patterns of exposure in mean plateau concentrations (74). To overcome some of the problems in representing the results of intermittent exposure experiments, Larson and Schlesinger (89) have proposed that the toxicity data be expressed on the basis of the area under the concentration time curves. They have provided evidence that when different groups of largemouth bass (Micropterus salmoides) are subjected to high spike patterns, low spike patterns, and square patterns of exposure the relationship between mortality and the area under the time-concentration exposure curve were not significantly different for the three different exposure patterns. However, the authors suggested that further investigations be conducted with a greater variety of patterns, concentrations and frequencies of exposure to determine the general applicability of this form of representation.

3.3 Species-Dependent Effects

In determining the impact of chlorine on an aquatic community consideration must be given to the species composition of that community. Factors such as life stage (egg, larvae, juvenile, adult), size, and species specific sensitivity appear to influence toxicity.

Thatcher (44) conducted a series of 96-hr LC50 continuous flow bioassays to determine the impact of chlorination on 15 estuarine and marine fish and invertebrates. A thermal stress was included to simulate power plant cooling effluents. Total residual oxidant (TRO) concentrations were measured by an amperometric titration system. In

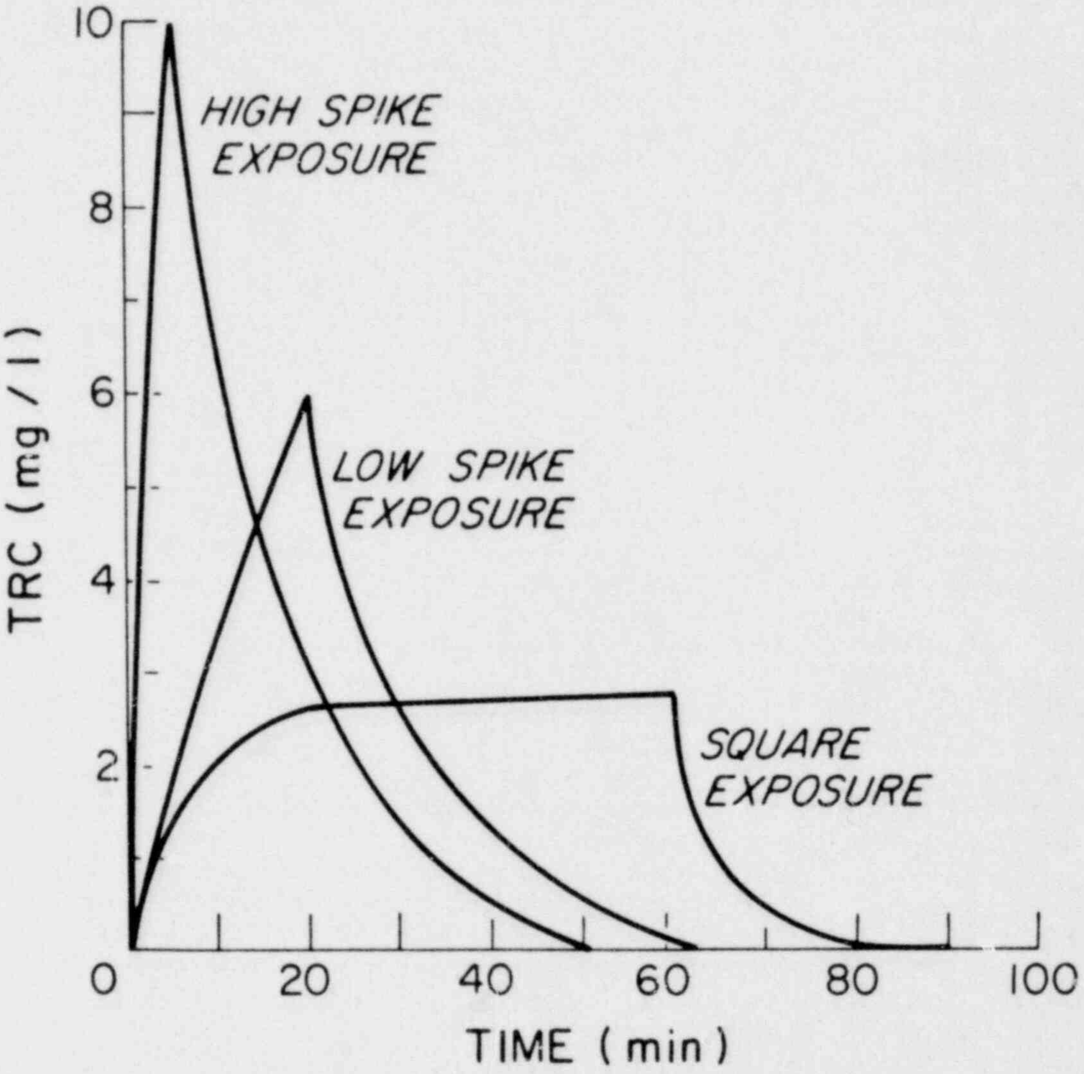

Figure 6. Examples of the time-concentration relationships of the square exposure and the high and low spike exposures with total residual chlorine (TRC). (Taken from Larson and Schlesinger (89)).

general, the fishes were more sensitive than the invertebrates. Based on the LC50 values the 15 species fell into three distinct groups with differing sensitivity to chlorinated seawater. The most sensitive group included coho salmon, (Oncorhynchus kisutch), pink salmon (O. gorbuscha), chinook salmon (O. tshawytscha), Pacific herring, (Clupea harengus), shiner perch (Cymatogaster aggregata), English sole, (Parophrys vetulus) Pacific sand lance (Ammodytes hexapterus) and a shrimp (Pandalus goniurus). The 96-hr LC50 values for this group were 0.026 to 0.119 mg/L TRO. The group of intermediate sensitivity included the shrimp (Crangon nigricauda), the amphipod (Ammyx sp.), the mysid (Neomysis sp.), the threespine stickleback (Gasterosteus aculeatus) and the coon stripe shrimp (Pandalus danae). Their 96-hr LC50 values ranged from 0.118 to 0.199 mg/L. The most resistant group consisted of the amphipod, Pontogeneia sp. and the shore crabs Hemigrapsus nudus and H. oregonesis. Their 96-hr LC50 values ranged from 0.583 to 1.530 mg/L TRO.

Larson et al. (61) studied the acute and sublethal toxicity of inorganic chloramines to early life stages of coho salmon (Oncorhynchus kisutch). The 96-hr TL50 was shown to vary according to life stage with the most sensitive time just after fry stage. Temperature and alkalinity did not affect acute toxicity; however, toxicity at pH 8.1 was greater than pH 7.0 or 7.5. Continuous exposure to chloramine concentration up to 47 μg/L did not affect the survival, development or hatching ability of embryos although alevins died within 9 weeks after hatching. Growth of alevins and juveniles was reduced at 23 μg/L chloramines.

In a complementary study, Larson et al. (64) again showed that the acute toxicity of inorganic chloramines to brook trout (Salvelinus fontinalis) alevin, fry, and juvenile life stages was not constant. Two groups of alevins (TL50 of 105.5 and 90.6 μg/L) were more tolerant than fry (TL50 82 μg/L) which in turn were less tolerant than 2 groups of juveniles (TL50 90.6 and 88 μg/L).

Morgan and Prince (52) provide data on the sensitivity of the eggs and larvae of white perch (Morone americana), striped bass (Morone saxatilis), blueback herring (Alosa aestivalis) and eggs of Atlantic silversides (Menidia menidia), and tidewater silversides (M. beryllina) to various levels of total residual oxidant (TRO). All bioassays were performed in a continuous flow system and TRO was measured by amperometric titration. LC50 values for eggs ranged from 0.20 to 0.40 ppm TRO. Larval LC50 values ranged from 0.20 to 0.032 ppm. In addition, 15% of blueback eggs exposed to 0.31 to 0.38 TRO developed abnormal vertebral columns as larvae. The general response for an egg was decreasing sensitivity to chlorine with egg age. For larvae the trend was increasing sensitivity to chlorine with larval age.

Brooks and Seegert (74) tested the resistance of ten species of warm water fish to quadruple 40 minute exposures of monochloramine administered at five-hour intervals over a 24-hour period at 10, 20, and

30°C. The authors separated the fish into "sensitive" and "resistant" species based on the tolerance to monochloramine. The sensitive group which included the emerald shiner (Notropis atherinoides), common shiner (N. cornutus), spotfin shiner (N. spilopterus), channel catfish (Ictalurus punctatus), white sucker (Catostomus commersoni) and sauger (Stizostedion canadense) had 30°C LC50 values ranging from 0.35 to 0.71 mg/L. Resistant species, freshwater drum (Aplodinotus grunniens) white bass (Morone chrysops), bluegill (Lepomis macrochirus) and carp (Cyprinus carpio) had LC50 values of 1.15-1.50 mg/L at 30°C. Based on concentrations which produced no mortality for the sensitive species, it was suggested that mean monochloramine exposures of 0.2 mg/L should not exceed 11.0 minutes/day. Seegert and Brooks (53) have also shown the thirty minute LC50 values to chlorine for coho salmon (Oncorhynchus kisutch), alewife (Alosa pseudoharengus), spottail shiner (Notropis hudsonius) and rainbow smelt (Osmerus mordax) were species dependent.

In testing the resistance of warm water and cold water fish to an intermittent chlorination regime of three 45-minute daily exposures for up to seven days, Heath (60) found that rainbow trout (Salmo gairdneri), coho salmon (Oncorhynchus kisutch) and channel catfish (Ictalurus punctatus) were the most sensitive species to free chlorine and monochloramine. Carp (Cyprinus carpio) were most resistant and golden shiner (Notemigonus crysoleucas) were intermediate. The comparable sensitivities of channel catfish and the two salmonid species indicated that the distinction of warm water fish generally being more resistant to chlorine than cold water fish cannot always be drawn.

Roberts (90) exposed eggs and larvae of two crab species Panopeus herbstii and Pagurus longicarpus to chlorinated seawater in a continuous flow system. Panopeus herbstii eggs were more tolerant to TRO than larvae. The 96-hr LC50 for adult Panopeus herbstii was approximately 0.50 mg/L, five times the maximum 96-hr LC50 observed for larvae. For Pagurus longicarpus adults the 96-hr LC50 was 21 mg/L, at least twice that for larvae. The greater tolerance of eggs compared to zoeae was explained by the thick egg membranes which serve as protection from the external medium and the relatively poorly calcified exoskeleton of the zoeal stages which may be more permeable. Furthermore, during the molting process the larvae would be particularly sensitive to chlorine toxicity.

Comparing two species in saltwater, Stober et al. (58) found that coho salmon (Oncorhynchus kisutch)proved to be more sensitive to TRO than shiner perch (Cymatogaster aggregata) with a 24 hr LC50 of 0.123 mg/L for shiner perch approximating a 12 hr LC50 for coho salmon of 0.114 mg/L. Coho salmon were also significantly more sensitive to chlorinated seawater for short exposure times of 7.5, 15, 30 and 60 min than shiner perch.

Seasonal influences should also be considered in evaluating chlorine toxicity. Scott and Middaugh (91) chronically exposed adult American oysters (Crassostrea virginica) to chlorine during the fall (45-day exposure), winter (75-day exposure) and spring (60-day exposure) to determine long-term seasonal effects. At high concentrations the TRO produced considerable mortality and at low concentrations severe sublethal effects. Toxicity varied seasonally and was related to seasonal changes in measured TRO concentrations, temperature and physiological condition of the oyster. Reductions in feeding and avoidance of TRO resulted in decreased tissue production, particularly gonadal tissue, as well as increased dependence on glycogen reserves during exposures.

The toxicity of intermittent chlorination to an attached filamentous green algae (Stigeoclonium subsecundum) was found to be related to the biomass of the algae (92). Above a specific level of algal biomass intermittent chlorination had no effect. However, below that level the attached algal cells were completely killed within the week-long bioassay. The importance of this finding lies in the fact that natural periphyton populations are known to vary seasonally and once seasonal changes reduce the periphyton biomass below a critical level survival in an altered environment becomes impossible.

3.4 Temperature-Chlorine Interactions

In addition to simulating intermittent chlorination regimes, several studies have included a thermal stress in view of the fact that heated waters are usually a component of power plant discharges. These studies fall into two categories. One involves testing an organism over a range of acclimation temperatures; the other, which more closely approximates the natural situation, involves exposing the organism to a variety of temperature shocks over a range of chlorine concentrations. While the results of many of these experiments have indicated a general trend of increasing sensitivity of aquatic life to chlorine, particularly fish, at higher temperatures others have demonstrated little or no effect. For example, Brooks and Seegert (74) exposed ten species of warm water fish to quadruple 40 minute exposures of monochloramine administered at five-hour intervals over a 24-hour period at 10, 20 and 30°C and found an inverse relationship between temperature and LC50. The LC50 values generally decreased by a factor of two as the exposure temperature increased from 10 to 30°C. The LC50 values ranged from 0.35 mg/L at 30°C for the emerald shiner (Notropis atherinoides) to 3.0 mg/L at 10°C for the bluegill (Lepomis macrochirus). Stober et al. (58) conducted a series of continuous flow-through bioassays to determine the toxicity of chlorinated seawater to shiner perch (Cymatogaster aggregata) and coho salmon (Oncorhynchus kisutch). A three way matrix test design with chlorine concentration, exposure time, and temperature was used as the test variables. Exposures of 60 min or less to shiner perch produced no mortalities in concentrations less than 0.2 mg/L TRO although increased respiration rates indicated a response to chlorinated seawater at levels of 0.10 mg/L. TRO concentrations of more than 1.0 mg/L produced 100 percent mortality at exposures of 7.5 minutes or more. The shiner perch were significantly more sensitive to chlorine with increasing exposure time (up to 60 minutes) and a temperature shock of +7°C (but not at +3°C). Coho salmon experienced no mortality below 0.10 mg/L TRO for the range of exposures and temperatures tested while 100% mortality was observed at concentrations greater than 0.50 mg/L TRO. A temperature shock of +7°C resulted in a significant decline in LC50 values from exposure times of 7.5, 15 and 30 minutes. A+3°C temperature shock above ambient had no significant effect.

In an earlier experiment using a similar type matrix design, Stober and Hanson (57) found that pink salmon (Oncorhynchus gorbuscha) and chinook salmon (O. tshawytscha) were also more sensitive to residual chlorine at elevated temperatures. Temperature increases of 9.9 - 10°C above acclimation and 0.5 mg/L TRO exerted the greatest toxic effect.

Similarly tests with juvenile spot (Leiostomas xanthurus) acclimated to 15°C seawater and exposed to combinations of two TRO ranges (0.05 - 0.07 and 0.34-0.52 mg/L) and three shock temperatures (20, 25 and 28°C) and four exposure times (7.5, 15, 30 and 60 min) indicated a consistent increase in the sensitivity to TRO as temperature and exposure time increased (93).

Seegert and Brooks (53) exposed coho salmon (Oncorhynchus kisutch), alewife (Alosa pseudoharengus), spottail shiner (Notropis hudsonius) and rainbow smelt (Osmerus mordax) for 30 minutes to residual chlorine at wide ranges of acclimation temperatures. Thirty minute LC50 values were found to be both species and temperature dependent and all species showed an inverse relationship between test temperatures and LC50 values. This was most evident in the alewife tests where almost a 10-fold decrease in LC50 values was observed as test temperatures increased from 10 to 30°C. Brooks and Seegert (68) observed a similar response to temperature in rainbow trout (Salmo gairdneri) and yellow perch (Perca flavescens) subjected to short exposures of chlorine.

Dickson et al. (73) exposed goldfish (Carassius auratus) intermittently for 24 hours to free and combined chlorine at various frequencies and durations of exposure, and various temperatures and also found chlorine to be more toxic at higher temperatures.

The interaction of chlorine and temperature is not restricted to fish alone, Capuzzo et al. (48) demonstrated the importance of temperature when exposing stage I larvae of the American lobster (Homarus americanus) to free chlorine and chloramine. A thirty or sixty minute exposure to free chlorine which produced no significant mortality at 20°C resulted in an estimated LC50 value of 2.5 mg/L at 30°C.

Chloramine toxicity was also more severe as the temperature increased. The estimated LC50's of chloramine were 4.08 mg/L at 20°C and 0.56 mg/L at 30°C.

Goldman et al. (95) recently summarized the results of a series of previously published experiments which were designed to assess the effects of chlorine, ammonia, and temperature on entrained marine plankton and juvenile fish. State I and IV lobster larvae (Homarus americanus), seven-day oyster larvae (Crassostrea virginica), adult copepods (Acartia tonsa), adult rotifers (Brachionus plicatilis) and killifish (Fundulus heteroclitus) were exposed to varying combinations of chlorine, temperature and ammonia in 48-hr bioassays (79, 95). The results indicated a marked increase in both chlorine and chloramine toxicity with elevated temperatures in the tested range 20-30°C (10-30°C for the copepods). The effect was most apparent when the thermal tolerance of the organisms was approached. At the lower temperatures the relative toxicity of the two halogen forms was least pronounced, but it increased with increasing temperature for the four zooplankton species and for the killifish.

In contrast, Heath (60) exposed five species of warm and cold water fish: rainbow trout (Salmo gairdneri), coho salmon (Oncorhynchus kisutch), channel catfish (Ictalurus punctatus), carp (Cyprinus carpio), and golden shiner (Notemigonus crysoleucas), to an intermittent chlorination regime of three 45-minute daily exposures for up to seven days and concluded that temperature had little effect on the lethal concentrations (LC50) for either free chlorine or monochloramine. However, inspection of the data revealed that temperature was an important component of toxicity during the initial 1-5 days of the experiments depending on the species. This suggests that chlorine-temperature interactions are more pronounced for shorter periods of time and the importance of temperature decreases as exposure times increase. These results may not apply to situations in which a continuous chlorination procedure is employed.

3.5 Metal-Chlorine Interactions

Chlorine may interact synergistically or additively with a variety of chemical species present in wastewater and power plant effluents. However, there is a dearth of published information on these interactions. The following studies reflect the importance of this neglected area.

Liden et al (96) examined the effect of a 24-h exposure of 0.08 mg/L TRC on goldfish (Carassius auratus) previously exposed to long-term sublethal metal stress and found significantly less survival compared to fish exposed to TRC alone. The results of this study indicate that fish residing in aquatic systems receiving low levels of metal pollution may be more susceptible to TRC levels commonly occurring in sewage and wastewater treatment effluents than fish inhabiting a "pollution free" environment.

Total residual chlorine and nickel, typical of cooling water discharges, have been shown to exert a synergistic toxic effect on rainbow trout (Salmo gairdneri) (77). Concentrations of each tested separately produced less than 5% mortality, however, in combination accounted for 95% mortality in 96 hr bioassays. This finding represents a significant potentiation of the response of fish to nickel caused by chlorine residuals.

Dickson et al. (98) conducted a field bioassay with caged bluegill (Lepomis macrochirus) and a snail (Amulosa sp.) to evaluate the effects of chlorinated cooling tower blowdown. No fish deaths occurred which could be attributed to the blowdown discharge; however, the snail suffered 50% mortality in 72 hrs with total residual chlorine measured at less than 0.04 ppm and copper at 80 μg/L. The authors felt that the toxicity observed may not be a function of chlorine toxicity alone, but may involve copper acting synergistically with chlorine.

The need for additional study in this area is readily apparent if elucidation of some of the more important interactions is to be achieved.

3.6 Interactions with Other Environmental Factors

Several water quality parameters influence the toxicity of chlorine by determining the specific chlorine species formed. Evidence indicating a direct effect of pH on chlorine toxicity is lacking and it is most likely that the few observed interactions of pH and chlorine are attributable to its effect on the relative proportions of various chlorine species.

There are conflicting reports in the literature on the difference between the toxicity of free chlorine and that of chloramines to fish. Tomkins and Tsai (100) exposed the blacknose dase (Rhinichthys atratulus) by continuous flow bioassay to solutions of free chlorine and chloramines. Using either the median lethal exposure time or median survival time as the toxicity index, chloramines were found to be more toxic than free chlorine at high concentrations. The difference was less pronounced at low concentrations.

When testing the resistance of warm and cold water fish to an intermittent chlorination regime Heath (60) found a three to fourteen-fold greater toxicity of free chlorine compared to monochloramine, depending on fish species and exposure time. Juvenile marine fish: winter flounder (Pseudopleuronectes americanus), common scup (Stenotomus chrysops) and killifish (Fundulus heteroclitus) exposed to varying combinations of chlorine, temperature and ammonia in continuous flow-through bioassays were apparently more susceptible to free chlorine than chloramine and responded in a threshold fashion (79). Several invertebrates: Stage I and IV lobster larvae (*Homarus americanus*), seven-day oyster larvae (*Crassostrea virginica*), adult copepods (*Acartia tonsa*), and adult rotifers (*Brachionus plicatilis*) tested in a like manner were more sensitive to chloramines and responded in a more gradual fashion, i.e., increased mortality with increased toxicant concentrations (94). The above findings suggest that the different forms of chlorine affect various species by different modes of action depending on the concentration.

3.7 Behavioral Responses of Motile Organisms

Behavioral responses are some of the more important sublethal effects which can be used to demonstrate ecological impacts of chlorinated effluents. If organisms are attracted to plumes of chlorinated water, the toxic effects in the field could approximate those seen in an acute bioassay. However, if organisms avoid a chlorinated plume, toxicity may be minimal, but the mixing zone must then be considered an uninhabitable area for those organisms showing avoidance and the ecological impacts assessed in a different manner. The behavioral responses (avoidance or attraction) become important when considering the ecological impact of power plant effluents on individual species.

Stober et al. (58) determined the behavioral response of coho salmon (*Oncorhynchus kisutch*) and shiner perch (*Cymatogaster aggregata*) to chlorinated and heated seawater. A significant avoidance threshold for coho salmon occurred at 2 µg/L TRO and was reinforced with increasing temperature. Shiner perch avoided TRO at 175 µg/L, while a significant preference response at 16°C and 20°C occurred at 10, 25, 50, and 100 µg/L TRO. Thatcher (44) determined that the 96-hr LC50 for shiner perch in chlorinated seawater was 71 µg/L TRO and consequently, continuous discharges of heated seawater having a chlorine TRO of 71-100 µg/L TRO could attract shiner perch and eventually result in adverse sublethal effects.

Behavioral tests by Meldrim, et al. (100) indicated a temperature-dependent avoidance response with white perch, *Morone americana*. At 1.5 ppt salinity and 4°C, avoidance was observed for concentrations of free chlorine ranging from 0.07 to 0.25 mg/L. Free chlorine concentrations of 0.02 to 0.08 mg/L were avoided at 17° and 22°C. Further testing and analysis of multiple variables with white perch indicated the avoidance concentration of total residual oxidant was inversely related to salinity, test temperature, and fish size (81). Middaugh, et al. (82) found that 24-day-old striped bass larvae avoided measured total residual chlorine (TRC) concentrations as low as 0.29-0.32 mg/L at 1.0 to 3.0 ppt salinity and 19° ±1°C. In research with juvenile spot, *Leiostomus xanthurus*, Middaugh, et al. (93) found temperature-dependent avoidance responses to TRC. They reported a TRC concentration of 0.18 mg/L at 19 and 22 ppt salinity and 10°C caused avoidance, while tests at 15° and 20°C, TRC concentrations as low as 0.05 mg/L were avoided.

Avoidance thresholds for TRC and its components in freshwater were reported for spotted bass (Micropterus punctulatus) and rosyface shiner (Notropis rubellus) (76), and for golden shiner, (Notemigonus crysoleucas) (10). The former study indicated an increase in threshold concentration with temperature, however, the relationship was less clear in the latter study. Bogardus, et al. (102) found that coho salmon (Oncorhynchus kisutch) in freshwater exhibited a generally variable avoidance at all temperatures for all concentrations of monochloramine below 0.20 mg/L TRC. Avoidance threshold of TRO could be expected to differ from freshwater to seawater, but additional standardized behavioral testing will be required along with detailed analysis of the chemistry of chlorine in both environments.

3.8 Mode of Action

Several authors have concluded that gills are the primary site of chlorine toxicity (62, 65, 103, 104). In contrast, Fobes (105) exposed gill tissue from white suckers (Catostomus commersoni) to a lethal concentration of chlorine and found no change in the respiration rate. Based on this finding it was concluded that the gills were not the primary site of chlorine toxicity and that chlorine may enter through the gills and affect the nervous system of the fish.

More recent studies have concentrated on examining various histopathologic, hematologic and pathophysiological responses of fish to chlorine toxicity. Bass et al. (66) examined bluegill (Lepomis machrochirus) and rainbow trout (Salmo gairdneri) for histopathological effects following multiple exposures to total residual chlorine (primarily free chlorine). Fish were subjected to 45-minute exposures 3 times a day at 8 hr intervals for 7 days at concentrations of 0.21, 0.31, 0.41 and 0.52 mg/L. Test temperatures were 15, 25, 32°C for bluegill and 15C for rainbow trout. Lesions noted were moderate gill hyperplasia and swelling of the lamellar epithelium at sublethal concentrations. Lethal concentrations caused extensive hyperplasia of gill epithelium and hepatic necrosis and these lesions were more severe at higher temperatures. It was concluded that death resulted from damage to the gill causing blockage of respiratory gas transport.

Zeitoun (106) attempted to assess the mode of chlorine action on fish by exposing rainbow trout to various concentrations of chlorine for different lengths of time and determining changes in certain hematological parameters. Blood of fish exposed to chlorine was darker and thicker than that of controls. Chlorine appeared to diffuse readily through the gills, oxidizing the hemoglobin to methemoglobin and disrupting erythrocyte membranes resulting in hemolysis. The resultant polycythemia was also due to the substantial increase in hematocrit value and hemoglobin concentration. The hemoconcentration seemed to interfere with blood circulation and hinder the delivery of oxygen to tissues. In another study (106) plasma concentrations of phosphorus, magnesium, iron, copper, zinc and potassium increased substantially in rainbow trout (Salmo gairdneri) subjected to shock exposures of chlorine (1.67 -3.86 mg/L). Sodium was the only electrolyte to decrease substantially in the test fish compared with controls. Chlorine toxicity, therefore, appeared to disturb mineral homeostasis.

Physiological responses involving respiratory and osmoregulatory mechanisms, which reflect gill function, were examined in white perch (Morone americana) continuously exposed to an acutely lethal concentration of TRO (0.8 mg/L) at 15°C, salinity 13.6 ppt and pH 7.87 (109). Blood pH decreased from 7.5 to 6.8 and hematocrit and erythrocyte carbonic anhydrase were elevated. It was suggested that these changes resulted from destruction of the gill epithelium by the oxidative properties of TRO. The percentage of methemoglobin did not change which has also been observed with juvenile spot (Leiostomus xanthurus) exposed to TRO levels up to 0.37 mg/L for 95 min. However, freshwater fish exposed to chlorine have shown significant increases in methemoglobin (111, 106). Osmoregulatory breakdown resulted in increased osmolarity primarily due to an influx of Cl^-, Mg^{2+} and Ca^{2+} ions. It was concluded that two of the most sensitive indicators of oxidant stress were blood pH and plasma osmolarity.

It appears from the above studies that the mode of action of chlorine is not well defined and as yet not clearly understood. Chlorine may be acting on more than one site or in more than one fashion and this may be dependent on the chlorine species and environmental conditions.

3.9 Application of Toxicity Data

The exposure of an organism to chlorine under natural conditions is dependent on where and how it interacts with a discharge plume. Consequently, extrapolation of laboratory toxicity data to actual situations in an attempt to establish reasonable limits on chlorine discharge concentrations is dependent on the ability to predict the concentration and time to which an organism would be exposed. Several methods have been proposed. As mentioned previously (Section 3.1) one approach is examining the relationship between the time-concentration curve of toxicity studies and mortality and thereby obtain a more accurate assessment of highly variable concentrations and durations during exposure periods. Lietzke (19) has proposed a kinetic model for predicting the composition of chlorinated water discharge from power plant cooling systems. This model is based upon reaction rate constants that exist for conditions at any given time and concentrations of the various potential reactants. Another method, described by Grieve et al. (110), applies previously derived thermal plume shape, orientation and decay data to chlorine chemical reactions to account for the decline of chlorine concentrations in the plume. Yet another procedure for regulating power plant discharges of chlorine to the aquatic environment has been proposed by Mattice and Zittel (3). This procedure is based on a comparison of estimated exposure of organisms in the discharge area with previously derived toxicity data. Both time and concentration are considered in the comparison so acute and chronic levels could be established. "Safe" levels for marine and freshwater organisms were predicted separately.

Hergott et al. (111) conducted a study to determine the nature, levels and persistence of chlorinated compounds in the discharges of five power plants in Northern California and employed the procedure of Mattice to determine if toxicity would be predicted. Decay studies conducted at the outfalls together with measurement of TRO in the receiving water provided a base for estimating the persistence and zone of influence of the chlorinated compounds. It was determined that all effluents produced receiving water TRO levels that would be predicted to demonstrate chronic toxicity to marine organisms and two plants showed levels that would be predicted to demonstrate chronic toxicity to freshwater organisms. Acutely toxic levels to freshwater and marine organisms existed in the receiving waters at one of the sites.

The majority of toxicity studies to date have emphasized the acute toxic effects of chlorine, however; it may be that long-term exposures will be of major consequence to aquatic organisms in the future. The rapid dilution and degradation of acutely toxic forms of chlorine combined with recent state and federal regulations and required dosage levels point to a decreased importance of acutely toxic forms and to an increased concern of long-term exposures of aquatic life to concentrations of TRC, TRO or chlorination by-products.

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16. ABSTRACT *(200 words or less)*

This report provides a state-of-the-art review of 111 of the most recent available references on the interactive effects of chlorine and various environmental factors on saltwater and freshwater aquatic organisms. The chemistry of chlorine in freshwater and seawater is discussed as it relates to the evaluation of toxicity studies. Those factors found to affect the toxicity of chlorine are concentration, exposure time, temperature, chemical species of chlorine and biotic factors such as species, life stage, and size of organism. Other factors such as pH and metal pollutants modify the toxicity of chlorine; however, the two factors of primary importance in the determination of toxicity are concentration and exposure time. Further research is needed on the reaction products of chlorine in both aquatic environments which will require development of new analytical methodology. Toxicity testing which simulates the transient low level exposures of organisms in cooling water mixing zones is needed to provide more accurate estimates of conditions in the receiving water. References defining the additive, synergistic or antagonistic effects of chlorine with one or more other chemical pollutants were very limited.

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