



Research and Development

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**DRINKING WATER CRITERIA DOCUMENT FOR
CHLORINE, HYPOCHLOROUS ACID AND HYPOCHLORITE ION**

Prepared for
OFFICE OF DRINKING WATER

Prepared by

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This document provides the health effects basis to be considered in establishing the Maximum Contaminant Level Goal. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature providing dose-response information.

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1989; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, $\leq 10\%$ of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
bw	Body weight
DNA	Deoxyribonucleic acid
DWEL	Drinking water equivalent level
FEL	Frank-effect level
GC/MS	Gas chromatography/mass spectrometry
GI	Gastrointestinal
HA	Health Advisory
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
LDL	Low density lipoprotein
LOAEL	Lowest-observed-adverse-effect level
MDR	Minimum daily requirement
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
PEM	Peritoneal exudate macrophage
RfD	Reference dose
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulfate
TSH	Thyroid stimulating hormone

I. SUMMARY

Chlorine is a highly reactive element that is widely distributed in the environment. Elemental chlorine exists as a greenish-yellow gas at 25°C. In water, chlorine reacts to form hypochlorous acid and hypochlorite ion. At pH 7.4 these two species are in equimolar concentration; however, as the pH increases, hypochlorite ion will be the predominant species. Chlorine, hypochlorous acid and hypochlorite ion are the definitive constituents of "free chlorine" in water. When ammonia or nitrogenous compounds are present in chlorinated water, chloramines are formed. Chlorine present in water as chloramines is termed "combined chlorine." "Total chlorine" is, therefore, the sum of free and combined chlorine. Chlorine is known to react with other organic material such as humic and fulvic acids to form a wide variety of chlorinated organic compounds. Each of these chlorine species is important in the disinfection and treatment of water as a result of their respective oxidation potentials and biocidal activities.

Chlorine is a common drinking water additive. The occurrence for chlorine, hypochlorous acid and hypochlorites centers upon their use in drinking water and wastewater disinfection as well as an intermediate in the manufacture and preparation of a number of organic products such as antifreeze, rubber, cleaning agents and pharmaceuticals.

The measurement and classification of the chlorine species in water can be performed by several analytical procedures. Appropriate testing procedures for the detection of chlorine, its oxidants and combined-form species include potentiometry, amperometry, spectrophotometry, colorimetry and titration. These methods differ in their sensitivity depending on the form of chlorine (free or combined) being measured; therefore, selection of the appropriate testing procedure is essential for accurate estimates of chlorine concentrations.

Chlorine hydrolyzes very rapidly in water with a half-life of 0.005 seconds in natural waters to yield hypochlorous acid and hypochlorite ion. Because free chlorine is a strong oxidant, its stability in water is very low and in the presence of light rapidly undergoes oxidation. Vaporization of molecular chlorine at low concentrations is insignificant; however, at acidic pH values and high concentrations, vaporization may become significant.

The toxicokinetics of chlorine, hypochlorous acid and hypochlorite ion in drinking water are largely unknown due to the fact that these compounds readily react with biological molecules forming other Cl⁻ species. Pharmacokinetic studies have also been conducted on these chlorinated species; however, these compounds may not be useful in providing insight in Cl₂ toxicokinetics due to significant differences in the oxidative reactivity of the various compounds.

Studies in rats have shown that following oral administration of radioactive Cl_2 , ^{36}Cl is rapidly absorbed into the blood. Distribution is the highest in plasma followed by bone marrow, kidney, testes, lungs, skin and liver. Chlorine is eliminated from the body primarily through the urine and feces.

Limited information is available regarding the acute effects of chlorine following oral exposure. Short-term exposure of rats to HOCl or Cl in aqueous solution has resulted in transient decreases in blood glutathione and hypothalamic norepinephrine levels and liver alterations.

Exposures of ≥ 90 days in drinking water of rats, mice, rabbits, guinea pigs, pigeons and chicks have shown conflicting results. Exposures of ≥ 150 mg/L chlorine in rats, mice, guinea pigs and chicks have shown no adverse effects; however, rabbits and pigeons exposed to 15 mg/L chlorine experienced increased plasma cholesterol levels and changes in plasma thyroxine and hydroxyproline levels.

When administered in the diet, chlorine concentrations of 60 mg/kg bw had no adverse effects on weanling rats; however, exposures of ≥ 100 mg/kg bw resulted in dose-related increases in kidney and liver weights. No significant histopathologic effects were observed at any dose tested.

A 7-generation study of chlorine in which rats were administered highly chlorinated drinking water (100 mg/L), resulted in no treatment-related effects on

lifespan, fertility, growth, hematologic or histopathologic parameters. Long-term exposure to sodium hypochlorite in drinking water of rats resulted in dose-related decreases in body and organ weights at doses ≥ 13.5 mg/kg bw/day. Chronic exposure to chlorinated drinking water resulted in no adverse effects in rats or mice at doses up to 275 mg/L.

Cardiovascular effects have been reported in both pigeons and monkeys maintained on calcium-deficient or atherogenic diets and exposed to chlorine in their drinking water. Mice exposed to chlorinated drinking water showed no evidence of humoral or cell-mediated immune responses.

No evidence of reproductive or developmental effects have been reported. There is no clear evidence of carcinogenic effects in rats or mice exposed to chlorinated drinking water. Chlorine, hypochlorous acid or hypochlorite ion have not been shown to act as either direct carcinogens or initiators of tumorigenesis. Marginal increases in mononuclear leukemias were reported in female rats; however, these findings were not dose-related nor supported by similar findings in male rats or male and female mice.

Assessment of the mutagenic potential of chlorine is confounded by the reactive nature of the chlorine molecule and by the presence of reaction products, which have been found to be mutagenic. Because of bacteriotoxicity, chlorine or sodium hypochlorite have given variable results in standard plate incorporation assays. In mammalian cell systems chlorine has been shown to produce chromosomal aberrations.

In humans research on the effects of chlorine, hypochlorous acid and hypochlorites has been overshadowed by the investigation of chlorination by-products such as chloramines and trihalomethanes. The results of pertinent studies have indicated that the ingestion of chlorinated drinking water, under most normal circumstances, does not directly produce toxic effects. Consumption of heavily chlorinated drinking water (≤ 90 ppm) produces constriction of the throat and irritation of the membranes of the throat and mouth. Epidemiologic studies that address the association between chlorinated drinking water supplies and cancer have been limited by weaknesses in study objectives, design and confounding factors. The results of these studies do not support or refute a carcinogenic association with exposure to chlorinated drinking water.

The emphasis of this document is on the derivation of drinking water criteria for the protection of human health from potential toxicity due to exposure to chlorine, hypochlorous acid and hypochlorite ion. To that end there is included a discussion of the physico-chemical and biochemical characteristics of these compounds as relates to their biological effects. It should be noted, however, that the organic by-products formed during drinking water disinfection, especially the trihalomethanes, are important contributors to the overall health risks resulting from water chlorination. Because of the importance of these compounds, they are discussed in separate U.S EPA documents on chloramines, trihalomethanes and chlorine dioxide.

Lack of appropriate data precluded derivation of a 1-day HA for chlorine. It is recommended that the 10-day HA be adopted as sufficiently protective. A 10-day HA of 2.5 mg/L for a 10 kg child was derived from a no-adverse-effect level in mice drinking 25 mg/kg free available chlorine in water/day for 33 days.

A 90-day drinking water study was selected for the development of a longer-term HA. Longer-term HA values of 2.0 mg/L (2000 µg/L) for a 10 kg child and 6.0 mg/L (rounded to 6000 µg/L) for a 70 kg adult were derived based on a NOAEL of 16.7 mg/kg bw/day for absence of adverse effects in male rats exposed for 90 days to chlorinated drinking water. A DWEL of 4.0 mg/L (rounded to 4000 µg/L) for a 70 kg adult has been derived based on the NOAEL of 14.4 mg/kg bw/day in female rats exposed for 2 years to chlorinated drinking water. Caution should be applied in the interpretation of this health risk assessment in that it does not address the effects of the chlorinated by-products, especially trihalomethanes, formed during drinking water disinfection.

II. PHYSICAL AND CHEMICAL PROPERTIES

The halogen chlorine (Cl_2) is found as a greenish-yellow gas under standard conditions, or when compressed as a high density amber liquid. The diatomic gas is characterized by a penetrating and irritating odor. Table II-1 lists chemical and physical properties specific to chlorine, hypochlorous acid and sodium hypochlorite.

Chlorine is very reactive and will combine directly with nearly all of the elements except the rare gases (excluding xenon). The high level of chlorine reactivity is related to the structure of the chlorine atomic shell in which there are seven electrons; thus, chlorine will readily accept or share electrons in order to form a more stable electron configuration. This is reflected in chlorine's functional capacity as an oxidant, as a replacement for hydrogen and hydroxyl groups in organic compounds, as an intermediate in organic synthesis, and in its role in the saturation of double bonds. Although chlorine usually forms univalent compounds, it may be found with valences ranging from one to seven.

In pure water, chlorine forms elemental chlorine (Cl_2), chloride ion (Cl^-) and hypochlorous acid (HOCl). As pH increases, hypochlorous acid dissociates to hypochlorite ion (OCl^-). The term "free chlorine" (free available chlorine, free residual chlorine) refers to the concentrations of elemental chlorine, hypochlorous acid and hypochlorite ion that collectively occur in water. Several factors, including chlorine concentration, pH,

TABLE II-1

Physical and Chemical Properties of Chlorine, Hypochlorous Acid and Sodium Hypochlorite*

Property	Chlorine	Hypochlorous Acid	Sodium Hypochlorite
Chemical formula	Cl ₂	HOCl	NaOCl
CAS Registry No.	7782-50-5	7790-92-3	7681-52-9
Molecular weight	70.91	52.47	74.45
Boiling point (25 mm Hg)	-34.05°C		
Melting point	-101°C		18°C
Density Dry gas (0°C/1 atm) Liquid (0°C/3.65 atm) (1.4085 mg/L)	3.209-3.214 mg/L 1.468 mg/L 1.4085 mg/L	--	--
Specific gravity	2.482 (0°C)	--	--
Water solubility	7.3 g/L (20°C) 14.6 g/L (0°C)	-- --	283 g/L (0°C)
Physical form (25°C)	gas	liquid	crystal
Color	greenish-yellow	greenish-yellow	white
Taste threshold (water)	--	--	--
Odor threshold (water) (air)	0.002 mg/L 0.31 ppm	-- --	-- --
Conversion factors	1 ppm=2.9 mg/m ³ 1 mg/m ³ =0.344 ppm		
Residue level (water)	0.2-1.5 mg/L		

*Source: Windholz, 1983; NIOSH, 1976, 1984, 1989; Sconce, 1962

temperature, exposure to light and the presence of catalysts or organic material affect the stability of free chlorine in aqueous solution. As pH increases above 3.0 and the total concentration of chlorine decreases below ~ 1000 mg/L, molecular chlorine quantities diminish, while the hypochlorous acid and hypochlorite ion predominate (NRC, 1980). The dissociation of hypochlorous acid to hypochlorite ion is largely dependent upon pH and temperature. The acid is the prevalent form at $\text{pH} < 7.8$ and 7.5 for 0° and 20°C , respectively (Morris, 1978).

Hypochlorous acid is a greenish-yellow liquid whose stability is determined by reactant concentration, pH, temperature and exposure to light (see Table II-1). Hypochlorous acid is the active form of chlorine in treated water and wastewater. When chlorine reacts with organic compounds in solution, hypochlorous acid acts as an electrophile in the addition, substitution and oxidation reactions that occur (Morris, 1978). In water containing significant amounts of ammonia or other nitrogenous compounds, hypochlorous acid will react to form chloramines. The term "combined chlorine" (combined available chlorine, combined residual chlorine) refers to the amount of chlorine present as chloramines. The chloramine hydrolysis constant may favor the reverse reaction such that hypochlorous acid is produced (at a slower rate) from chloramines in aqueous solution. This reaction serves as the key mechanism in the chlorination of municipal water supplies, wastewater and swimming pools.

Hypochlorite ion is most commonly supplied as a salt. Table II-1 contains chemical and physical properties associated with sodium hypochlorite. Calcium hypochlorite and sodium hypochlorite are used as chlorine sources for the disinfection of drinking water since hypochlorite solutions are more stable than hypochlorous acid. Their decomposition may be enhanced by factors such as reactant concentrations, pH, temperature, exposure to light and ionic strength. Hypochlorous acid is readily formed when hypochlorites undergo hydrolysis. The utilization of hypochlorites as bleaching agents and disinfectants is explained by their high oxidation potential and ability to form hypochlorous acid under favorable conditions.

Environmental Fate, Transport and Distribution

The fate, transport and distribution of chlorine in natural waters is varied and complex. The major chemical reactions known to occur in aqueous solutions serve as the primary source of information (Table II-2). Several compilations of thermodynamic, equilibrium and kinetic data have been made for these reactions. Significant efforts have been made to integrate water quality data with kinetic and equilibrium data in order to develop models for predicting the products of the chlorination of natural waters (freshwater and seawater). Such models will facilitate a more thorough understanding of chlorination, chlorination products and their ultimate fate and distribution in aqueous solutions (Jolley and arpenter, 1983a).

TABLE II-2

Chlorine Reactions Known to Occur in Aqueous Solution*

Reaction Type	Examples
Water	
Hydrolysis	$\text{Cl}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HOCl} + \text{H}^+ + \text{Cl}^-$ $\text{Ca}(\text{OCl})_2 + 2 \text{H}_2\text{O} \rightleftharpoons \text{Ca}(\text{OH})_2 + 2 \text{HOCl}$
Ionization	$\text{HOCl} \rightleftharpoons \text{H}^+ + \text{OCl}^-$ $\text{NaOCl} \rightleftharpoons \text{Na}^+ + \text{OCl}^-$ $\text{Ca}(\text{OCl})_2 \rightleftharpoons \text{Ca}^{2+} + \text{OCl}^-$
Ammonia	
Substitution	$\text{NH}_3 + \text{HOCl} \rightarrow \text{NH}_2\text{Cl} + \text{H}_2\text{O}$
Oxidation	$2\text{NHCl}_2 + \text{H}_2\text{O} \rightarrow \text{N}_2 + \text{HOCl} + 3\text{H}^+ + 3\text{Cl}^-$
Inorganic oxidation	$\text{Mn}^{+2} + \text{HOCl} + 2\text{H}_2\text{O} \rightarrow \text{MnO}(\text{OH})_2 + 3\text{H}^+ + \text{Cl}^-$
Disproportionation	$3\text{OCl}^- \rightarrow 2\text{Cl}^- + \text{ClO}_3^-$
Decomposition	$2\text{HOCl} \rightarrow 2\text{H}^+ + 2\text{Cl}^- + \text{O}_2$
Organic reactants	
Oxidation	$\text{RCHO} + \text{HOCl} \rightarrow \text{RCOOH} + \text{H}^+ + \text{Cl}^-$
Addition	$\text{RC}=\text{CR}^1 + \text{HOCl} \rightarrow \text{RC}(\text{OH})\text{C}(\text{Cl})\text{R}^1$
Substitution	
N-Cl bond	$\text{RNH}_2 + \text{HOCl} \rightarrow \text{RNHCl} + \text{H}_2\text{O}$
C-Cl bond	$\text{RCOCH}_3 + 3\text{HOCl} \rightarrow \text{RCOOH} + \text{HCCl}_3 + 2\text{H}_2\text{O}$

*Source: Jolley and Carpenter, 1983a

Chlorine hydrolyzes very rapidly in water with a hydrolysis constant ranging from 1.5×10^{-4} at 0°C to 4.0×10^{-4} at 25°C . The half-life of chlorine in natural waters is 0.005 s^2 . Complete hydrolysis occurs in fresh and wastewaters at $\text{pH} > 6$ with the formation of hypochlorous acid (HOCl) and chloride ion (Cl^-) (Morris, 1978). Hypochlorous acid ionizes rapidly with a dissociation constant ranging from 1.6×10^{-8} at 0°C to 3.2×10^{-8} at 25°C yielding hydrogen ion (H^+) and hypochlorite ion (OCl^-). At $\text{pH} > 5$ and 25°C , the concentration of HOCl and OCl^- are essentially equimolar (Morris, 1966). At higher pH values OCl^- becomes the major form of chlorine, while at lower pH values HOCl will dominate.

Because free chlorine (Cl_2 , HOCl and OCl^-) is a strong oxidant in natural water, its stability is very low. In the presence of ultraviolet light, free chlorine oxidation of water to O_2 proceeds at a significant rate. Half-lives of 8-28 minutes have been measured in secondary effluents with sunlight. For similar chlorinated effluents without sunlight, a 10-fold greater persistence was measured with a half-life of 1.3-5 hours (Johnson, 1978).

Vaporization of molecular chlorine (Cl_2) is insignificant at low concentrations of chlorine and neutral pH values. At acidic pH values and high concentrations (pH 2 and 3500 mg/L chlorine) significant amounts of Cl_2 may be vaporized into the atmosphere (White, 1972).

Free chlorine reacts rapidly with inorganics such as bromide and more slowly with organic material present in natural and wastewaters. The primary chemical reactions of chlorine, hypochlorous acid and hypochlorite ion in freshwater and seawater are depicted in Figure II-1 (Sugam and Helz, 1980). Free oxidant forms (Cl_2 , HOCl , OCl^- , HOBr , OBr^-), combined oxidant (chloramines, organic chloramines, bromamines, organic bromamines, mixed halamines), and reaction products (chloro- and bromoorganics, oxidized organics, chlorate and bromate ion, oxidized metal ions, nitrogen and oxygen) participate in a complex set of interactions. Factors such as reactant concentrations, pH, temperature, salinity and exposure to light control the extent of reactions. In freshwaters and wastewaters (reactions on the right side of Figure II-1) the concentrations of ammonia, bromine and chlorine, and the pH serve as important regulators of reaction products of which monochloramine appears to be the most persistent compound formed. Free chlorine in natural waters is transformed to chloride, oxidized organics, chlororganics, oxygen, nitrogen, chlorate, bromate and bromoorganics. The term "chlorine demand" describes the quantity of chlorine required in these reactions. The actual concentrations, distribution and fate of the products associated with chlorine demand reactions have not been determined (Jolley and Carpenter, 1983a).

Analytical Methods

The measurement of chlorine, hypochlorous acid and hypochlorite ion in drinking water is complicated by such factors as confusion associated with water chlorination terminology, selection of appropriate testing procedures, and the sensitivity and accuracy

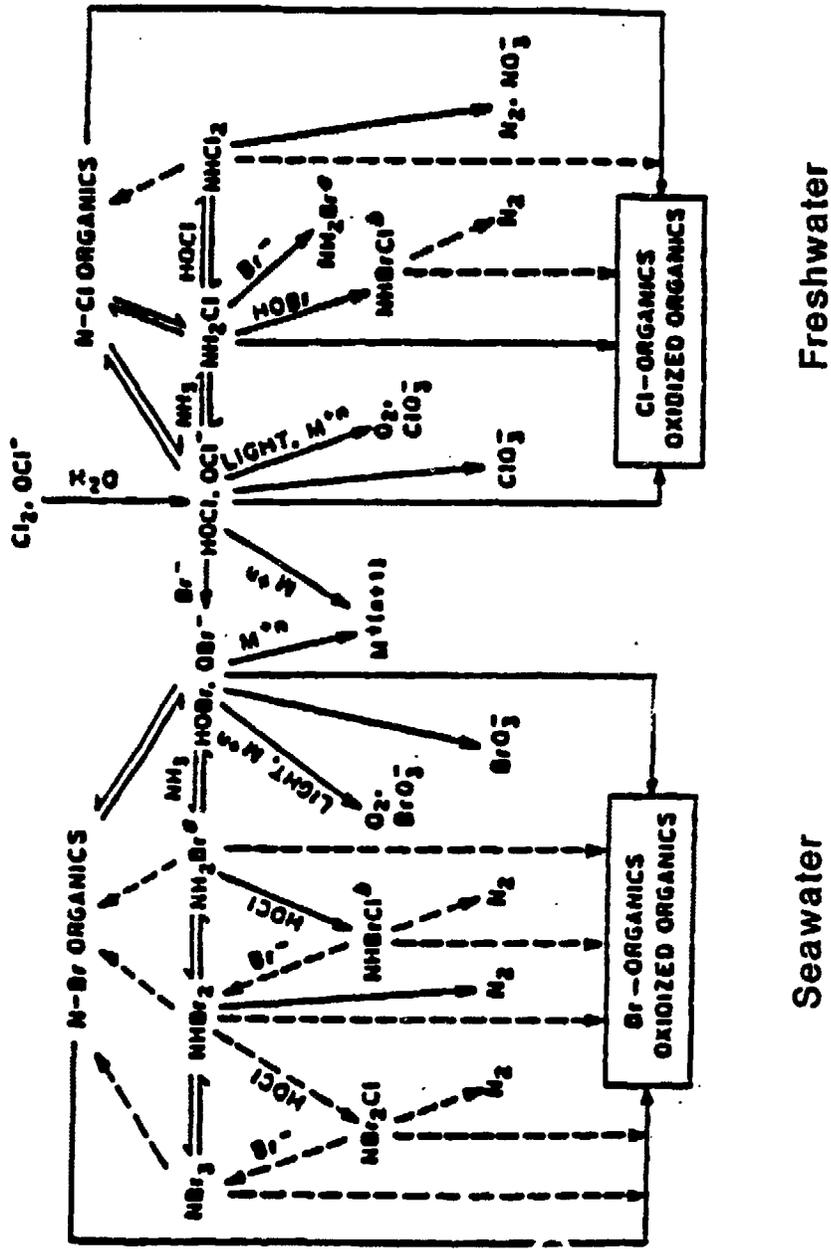


FIGURE II-1

Principal chemical pathways for reaction, degradation and environmental fate of free available chlorine in the aquatic environment. Presumed pathways (not yet proven) are delineated by dashed arrows. Compounds formed at different places in the reaction scheme are designated by superscripts a and b to assist in understanding interrelationships. Halides (Cl^- and Br^-) are not depicted but are products of many of the chemical pathways.

of the available instrumentation. Combined chlorine, which is associated with the presence of ammonia or nitrogenous compound, is often analyzed as free chlorine. Iodine, hypobromous acid and bromamines may also be detected as free chlorine. The terms "free oxidant" and "combined oxidant" are used when referring to these compounds. When considering both free and combined chlorine, the term "total chlorine" (total available chlorine, total residual chlorine) is applied (Jolley and Carpenter, 1983b).

Appropriate testing procedures for the detection of chlorine, its oxidants, and combined-form species include potentiometry, amperometry, spectrophotometry, chemiluminescence, colorimetry and titration (Jolley and Carpenter, 1983b). These methods differ in their sensitivity for detection of the various chlorine compounds found in water. Potentiometric methods utilize measurement of the spontaneous electrical potential generated between two dissimilar ion selective electrodes and can be used to detect total oxidant formation. Limitations associated with the technique include the logarithmic (Nernstian) response of the electrode and a poor level of precision as compared with other techniques (U.S. EPA, 1981). The amperometric method of chlorine detection involves the monitoring of the current generated from the application of a constant external potential across indicator and reference electrodes. Amperometric methods can be used to determine both free and total chlorine concentrations depending upon the composition of the buffer solution (Johnson, 1978). The cell used in the amperometric method can be modified for the measurement of available chlorine, hypochlorous acid and hypochlorite

ion. Continuous monitoring amperometric devices are considered to be fairly accurate, typically $\pm 1\%$ (NRC, 1976), but are subject to interference from MnO_2 (Johnson, 1978).

Ultraviolet spectrophotometry, while not commonly employed, evaluates the chemical speciation and degradation reactions of chlorine and bromine compounds. Total and free available chlorine are measured by this process (Opresko, 1980).

Chemiluminescence involves the oxidation of luminol to azaquinone by hypochlorous acid or molecular chlorine in aqueous solution and is useful as an indicator method for very low concentrations of free available chlorine (U.S. EPA, 1981). Hypochlorite ion levels can be detected at concentrations from 10^{-4} to $10^{-6.5}$ M.

The two most commonly applied methods for the detection of chlorine or its related compounds in water are colorimetry and titration. Colorimetry has proven to be more popular based upon its simplicity. In colorimetric techniques indicator chemicals are reacted with chlorine compounds to produce colors that can be analyzed by comparison with standard color scales. Generally, colorimetric methods are subject to interference from sample color, turbidity, MnO_2 and organic contaminants. However, the measurement of chlorine or oxidant residuals at concentrations ≤ 0.1 mg/L as chlorine can be readily accomplished with colorimetric indicators (Jolley and Carpenter, 1983b).

Titrimetry consists of four techniques (iodometry, amperometry, ferrous-orthotolidine and ferrous-DPD) in which chlorine compounds are reacted with a reducing reagent. The iodometric method involves the titration of iodine with a standard sodium-thiosulfate solution and is used in measuring total chlorine concentrations ≤ 1 mg/L (APHA, 1985). The amperometric method is a more sensitive test used for the determination of free or combined chlorine by titrating at pH 7 in the absence of iodide by a standard solution of phenylarsine oxide or sodium arsenite. The third and fourth methods of titrimetry include the ferrous-orthotolidine and ferrous-DPD techniques. The orthotolidine procedures have been deleted from Standard Methods due to the low accuracy of this technique (APHA, 1985). The ferrous-DPD technique serves as an operationally simple procedure for determining free available chlorine, total available chlorine, combined available chlorine and chloramine levels in water. Titrimetric methods are subject to a variety of interferences.

The reliability or accuracy of any information involving chlorine or chlorine-containing compounds in drinking water is dependent upon the proper selection, execution and analysis of results of a particular analytical method. Overall, electrochemical amperometric methods are recommended for the detection of low concentrations of free or combined chlorine, while iodometric and DPD-titrimetry and DPD-colorimetry are considered appropriate for the determination of total chlorine levels in water. It is suggested that measurement techniques and test conditions be described in reports involving the analysis of chlorine levels in water. Pertinent information would include the following: water quality, analyst expertise, methodology and statistical significance (Jolley and Carpenter, 1983b).

Environmental Sources, Production and Use

Chlorine exists largely as chloride in combination with sodium, potassium, calcium or magnesium in compounds such as salt (NaCl), carnalite (KMgCl₃·H₂O) and sylvanite (KCl) (Sconce, 1962).

As of January 1, 1986, 25 manufacturers with an estimated total chlorine production capacity of 26.1 billion pounds were reported for the United States (SRI, 1986). Total chlorine production for the United States was 20.8 and 21.9 billion pounds for 1986 and 1987, respectively (Reisch, 1988). In addition to domestic production of chlorine, the U.S. Department of Commerce (1986) estimated chlorine imports to be 576.9 million pounds.

Over 95% of the chlorine manufactured in the United States is formed by the electrolysis of chloride salts, most commonly sodium chloride (Windholz, 1983). In addition, chlorine is produced through the oxidation of HCl in the presence of catalytic metal oxides or oxidation with SO₂ or HNO₃, also as a by-product of potassium nitrate production and as a co-product in the production of potassium hydroxide (U.S. EPA, 1981). Hypochlorous acid is generated *in situ* by chlorine hydrolysis. Hypochlorite solutions are prepared by chlorination of caustic or lime slurry (Wojtowicz, 1978).

Chlorine, hypochlorous acid and hypochlorites are used mainly as intermediates in the production of organic chemicals. They are required to produce antifreeze, rubber, pharmaceuticals, home permanents and cleaning agents, construction materials,

automobiles, food and paper. In addition, they are used as intermediates in the production of inorganic chemicals, which in turn are used for a multitude of processes and products. Chlorine has also been extensively used in the paper industry as a bleaching agent for pulp and paper products.

Elemental chlorine and hypochlorites are also utilized in various water treatment processes. As a result of their oxidizing characteristics and toxicity to microorganisms, they are used to disinfect drinking water, sewage and wastewater, swimming pool water, seawater, reservoirs, drainage ditches, in-plant water supplies (food industry) and industrial cooling waters (Wojtowicz, 1978). The chlorine compounds function to sanitize, control odors, reduce biochemical oxygen demand, implement chemical precipitation, reduce color and treat cyanide (Dychdala, 1977).

In the food industry, chlorine and hypochlorites are employed for general sanitation, and bacteria and odor control. Dairies, wineries, breweries, canneries, beverage bottling plants and food processing plants use chlorine compounds to disinfect the equipment and utensils, as well as the ingredients associated with the production of food and beverages (Dychdala, 1977).

Summary

Chlorine, hypochlorous acid and hypochlorite ion are compounds that are noted for their high oxidation potentials. Hypochlorous acid and hypochlorite ion are formed when

chlorine is added to water. The concentration of each agent in water is a function of reactant concentrations, pH, temperature, exposure to light and the presence or absence of catalysts or organic material. As a result of their highly reactive nature, chlorine and its aqueous dissociation products are involved in the synthesis of many other chemical species. The measurement of chlorine, hypochlorous acid and hypochlorite concentrations in water involves the use of potentiometry, amperometry, spectrophotometry, chemiluminescence, colorimetry and titrimetry. Electrochemical amperometric methods are recommended for evaluation of low levels of free or combined chlorine; total chlorine levels in water are best determined through iodometric and DPD-titrimetry and DPD-chlorimetry. The ultimate fate of hypochlorous acid and hypochlorite in water is dependent upon environmental conditions. Decomposition products may include chloride, oxidized organics, oxygen, nitrogen, chlorate and nitrate.

III. TOXICOKINETICS

The toxicokinetics of chlorine, hypochlorous acid and hypochlorite ion in drinking water are largely unknown due to the fact that chlorine readily reacts with biological molecules and is short-lived in organisms. Pharmacokinetic studies of Cl_2 , HOCl and OCl^- have been performed using radio labeled chlorine compounds. However, because these molecules are so highly reactive in biological systems, these studies may reflect more the fate of the chlorine ion (Cl^-) or other reaction products generated than that of the parent molecules. Chlorine is known to react with purine and pyrimidine bases and amino acids, but the toxicokinetics of these compounds are not known. Pharmacokinetic studies have also been conducted on chlorination by-products such as trihalomethanes as well as alternate disinfectants such as chlorine dioxide (ClO_2) and its metabolites. Studies using these compounds may not be useful in providing insight into Cl_2 toxicokinetics due to significant differences in oxidative reactivity of the various compounds.

Absorption

Data pertaining to the absorption of chlorine, hypochlorous acid and hypochlorite ion in humans and animals are limited. The rates of absorption, the factors influencing absorption, and the forms in which these compounds are absorbed are among topics that have not been fully addressed. A few recent studies, however, have examined the absorption of chlorine after oral exposure.

Abdel-Rahman et al. (1983) investigated the pharmacodynamics of hypochlorous acid. Radioactive hypochlorous acid (HO^{36}Cl) was administered by gavage to four fasted and four nonfasted male Sprague-Dawley rats. Blood samples were taken and analyzed for ^{36}Cl levels at intervals ≤ 72 hours in nonfasted rats and ≤ 96 hours in fasted animals. A peak ^{36}Cl plasma level was reached in 2 hours in fasted rats versus 4 hours in nonfasted rats following the oral administration of 0.6 mg (3 mL of 200 mg/L HO^{36}Cl) and 0.75 mg (3 mL of 250 mg/L HO^{36}Cl) HO^{36}Cl per animal, respectively. Body weights for fasted and nonfasted rats ranged between 220 and 240 g. Doses of HO^{36}Cl were calculated (using mean body weight of 230 g) to be 2.61 and 3.26 mg/kg, respectively. The absorption half-life was 2.2 hours for both fasted and nonfasted rats. Plasma elimination half-lives were 44.1 hours in the fasted rats and 88.5 hours in the nonfasted rats. In an earlier study, Abdel-Rahman et al. (1982b) found an absorption half-life of ^{36}Cl in plasma of 4.42 ± 1.31 hours in rats receiving an oral dose of HO^{36}Cl in 3 mL of 250 mg/L aqueous solution of ^{36}Cl . Approximately 77% of the initial dose was absorbed within 72 hours. These differences in the absorption and elimination of ^{36}Cl (derived from HO^{36}Cl) may reflect the greater tendency for chlorine, hypochlorous acid and hypochlorites to produce chlorinated organic by-products in the blood or GI tract of nonfasted animals (Vogt et al., 1979; Mink et al., 1983).

Suh and Abdel-Rahman (1983) studied the kinetics of Cl^- absorption in four fasted male Sprague-Dawley rats administered an oral dose of 3 mL of 200 mg/L Na^{36}Cl solution (~ 1.6 mg/kg bw). The blood samples were collected by heart puncture and ^{36}Cl determined

by liquid scintillation spectrometry. Levels of ^{36}Cl in the plasma reached a maximum after 8 hours. The absorption and elimination half-lives from plasma were 19.2 and 51.9 hours, respectively. The absorption half-life for the chloride was ≤ 4 times that observed for any oxygenated forms (Table III-1).

Distribution

Two studies by Abdel-Rahman et al. (1982b, 1983) examined the tissue distribution of orally-administered hypochlorous acid in male Sprague-Dawley rats. In the first study (Abdel-Rahman et al., 1982b), four animals were gavaged with 3 mL of 250 mg/L HO^{36}Cl (3.26 mg/kg bw). After 72 hours the distribution of HO^{36}Cl was found to be highest in the plasma (0.77% of the initial dose), followed by bone marrow, kidney, testes, lung, skin, duodenum, spleen, liver and carcass, and was lowest in the ileum (0.14% of the initial dose).

In the Abdel-Rahman et al. (1983) study four fasted animals received an average dose of 0.6 mg HO^{36}Cl (2.61 mg/kg bw). Ninety-six hours after administration, the percentage of ^{36}Cl was highest in the plasma (1.92 $\mu\text{g/g}$), followed by whole blood, bone marrow, testes, skin, kidney, lung, packed blood cells, duodenum, stomach, spleen, thyroid, thymus, liver, carcass and ileum, and was lowest in fat (0.09 $\mu\text{g/g}$).

TABLE III-1

Summary of Selected ³⁶Cl Absorption Studies in Male Sprague-Dawley Rats

Test Compound	Dose*	Rats Fasted/ Nonfasted <i>t</i> _{1/2} (hours)	Plasma Absorption <i>t</i> _{1/2} (hours)	Plasma Elimination Plasma ³⁶ Cl (hours)	Hours to Peak	Reference
HO ³⁶ Cl	3 mL of 200 mg/L (-2.61 mg/kg bw)	Fasted	2.2	44.1	2	Abdel-Rahman et al., 1983
HO ³⁶ Cl	3 mL of 250 mg/L (-3.26 mg/kg bw)	Nonfasted	2.2	88.5	4	
HO ³⁶ Cl	3 mL of 250 mg/L (3.26 mg/kg bw)	NR	4.4	77.0	NR	Abdel-Rahman et al., 1982b
Na ³⁶ Cl	3 mL of 200 mg/L (-1.60 mg/kg bw)	Fasted	19.2	51.9	8	Suh and Abdel-Rahman, 1983

*All materials administered orally.

NR = Not reported

The subcellular distribution of ^{36}Cl in rat liver 24 hours following HO^{36}Cl administration was also analyzed by Abdel-Rahman et al. (1983). They found that for hypochlorous acid, 75% of the total ^{36}Cl activity of the liver homogenate was recovered in the cytosol, 2.5% in the microsomal fraction, 1.5% in the nuclear and <0.1% in the mitochondrial fraction. Only 4% of the total ^{36}Cl activity in the whole homogenate was recovered in the trichloroacetic acid precipitate of the homogenate following HO^{36}Cl treatment.

Metabolism

Free residual chlorine, as Cl_2 , OCl^- and HOCl , is a strong oxidizing agent that readily reacts with organic molecules to produce a wide variety of chlorinated compounds; therefore, it is short-lived in biological systems (Seegert and Bogardus, 1980). This reactivity in biological systems makes it difficult to study the *in vivo* metabolism of free residual chlorine and to separate the effects of parent compounds from those of free residual metabolites. The majority of data concerning the metabolism of free residual chlorine is either indirect or derived from *in vitro* preparations.

Mink et al. (1983) studied the *in vivo* biotransformation of sodium hypochlorite in fasted and nonfasted male Sprague-Dawley rats. In these experiments, rats weighing ~400 g were divided into control and treatment groups of six each (three fasted and three nonfasted animals). The six treated rats were dosed with 7 mL of an 8 mg/mL solution of sodium hypochlorite by gavage (140 mg/kg bw). One hour post-treatment all rats were

sacrificed and the blood and stomach were removed for analysis by GC/MS. Trichloroacetic acid and dichloroacetic acid were detected in the stomach contents of both fasted and nonfasted rats. This indicated that *in vivo* formation of these chlorinated acetic acids was not dependent on NaOCl interactions with organic material in the gut. Trichloroacetic acid was detected in 2/6 plasma samples from dosed rats, and dichloroacetic acid was detected in 5/6 plasma samples. The minimum detection limits of dichloroacetic acid (0.3 $\mu\text{g/mL}$) are lower than trichloroacetic acid (1.3 $\mu\text{g/mL}$) by broad spectrum capillary GC/MS scanning. This could account for the higher incidence of dichloroacetic acid observations. While chloroform was found in the gut contents of all of the treated rats, it was found in the plasma of only one dosed (nonfasted) rat. Dichloroacetonitrile was detected in the gut contents of 2 of the 3 nonfasted rats. None was present, however, in the gut of fasted rats, indicating that the presence of food can have an influence on the development of chlorination by-products. Dichloroacetonitrile was not detected in any plasma samples. These findings emphasize the high reactivity of chlorine and the difficulty in ascertaining the *in vivo* metabolic and toxicokinetic profile of chlorine.

The data of Abdel-Rahman et al. (1983) provide some insight into the terminal product of chlorine metabolism. They found that 96 hours after rats were orally dosed with HO^{36}Cl , 51% of the total ^{36}Cl dose was excreted through urinary and intestinal routes. The major metabolite of HO^{36}Cl in plasma was chloride ion (81%).

Baker (1947) and Pereira et al. (1973) studied the *in vitro* reactions of hypochlorite and hypochlorous acid, respectively, with protein. Baker (1947) summarized the earlier work of several authors with the following observations: 1) amino acids and their residues are attacked by sodium hypochlorite at rates that differ greatly from one acid or residue to another; 2) groups other than the peptide linkage are attacked; 3) both chlorination and oxidation occur; 4) active chlorine groups of varying stability are formed; and 5) in acidic conditions, chlorination predominates over oxidation. Baker (1947) showed that in the course of protein degradation by NaOCl, -2.9 molecules of hypochlorite were reduced per amino acid residue attached to protein. Pereira et al. (1973) found that hypochlorous acid converts several α -amino acids into a mixture of the corresponding nitriles (major product) and aldehydes (minor product) by oxidative decarboxylation. Chlorination of the ring of tyrosine was also observed. Cysteine when reacted with HOCl yielded cystine and cysteic acid as the only identified products. They also found that the amide nitrogen bond of several dipeptides was resistant to HOCl at room temperature and chlorination of these compounds yielded the corresponding N, N-dichlorodipeptide.

The reaction of hypochlorous acid with nucleotide bases has been examined fairly extensively in recent years. Patton et al. (1972) found that one equivalent of hypochlorous acid reacted with cytosine, 5-chlorocytosine or 5-methylcytosine to yield the corresponding 4-N-chloro derivatives. Reaction of three equivalents of HOCl with cytosine produced two unstable compounds identified as di- and trichlorocytosine in addition to 4-N-chloro derivatives. Reaction of cytosine with five equivalents of HOCl produced unstable tri- and

tetrachloro- derivatives. Hoyano et al. (1973) found both stable and chlorinated products and labile intermediates formed by the reaction of aqueous HOCl with thymine, uracil and 5-bromouracil. The purine ring system of guanine and adenine was more resistant to HOCl attack than thymine and uracil. but, with sufficient reaction time, parabanic acid was produced. Dennis et al. (1978) found reaction of uracil with a 10-fold excess of HOCl resulted in rapid degradation to trichloroacetic acid, carbon dioxide and nitrogen trichloride.

An estimation of the eventual metabolic fate of such chlorinated pyrimidines in mammals can be extrapolated from experiments in mice where 5-chlorouracil was added to the drinking water. The chlorinated base was found to be incorporated into the DNA of tissue samples in the liver and testes (Cumming, 1978). Thus, it is presumed that the bases are phosphorylated and eventually incorporated into nucleic acids.

Excretion

The elimination of ^{36}Cl -hypochlorous acid and its metabolites from the body has been studied by Abdel-Rahman et al. (1983). In this study, the urine, feces and expired air were collected over 4- and 5-day periods after the administration of 3 mL of a 200 mg/L solution of HO^{36}Cl (2.61 mg/kg bw) to four fasted male Sprague-Dawley rats. During the first 24-hour period, 7.05% of the initial HO^{36}Cl dose was excreted in the urine and 7.45% in the feces. After 96 hours, 36.43% of the administered dose was excreted in the urine and 14.80% in the feces. In an earlier study (Abdel-Rahman et al., 1982b), a gavage dose of 3.26 mg/kg bw HO^{36}Cl was administered to each of four male Sprague-Dawley rats. It

was observed that after 72 hours, 76 and 24% of the excreted ^{36}Cl residues of HO^{36}Cl were found in the urine and feces, respectively. ^{36}Cl compounds were not found in the expired air from any treated rats throughout the experiment.

Summary

At present, the study of the toxicokinetics of chlorine, hypochlorous acid and hypochlorite ion has been limited to analyses of the gross movement of ^{36}Cl following oral administrations of various ^{36}Cl -containing compounds. Studies in rats have shown that following oral administration of radioactive HO^{36}Cl , ^{36}Cl is rapidly absorbed into the blood with peak concentrations reached in 2 hours for fasted animals and 4 hours for nonfasted. Absorption half-life was 2.2 hours for both fasted and nonfasted animals. When administered as Cl^- (Na^{36}Cl) in nonfasted animals, peak plasma concentrations were reached in 8 hours. Seventy-two to 96 hours after oral administration, the distribution of ^{36}Cl was highest in the plasma, followed by bone marrow, kidney, testes, lungs, skin and liver. Lowest concentrations were found in the ileum and adipose tissue.

Chlorine, as Cl_2 , OCl^- or HOCl , is a strong oxidizing agent that reacts readily in biological systems producing a wide variety of chlorinated organic compounds. *In vivo* studies in fasted rats have reported that 81% of the total $^{36}\text{Cl}_2$ excreted after 96 hours was in the form of chloride ion. *In vitro* studies have shown that hypochlorite and hypochlorous acid react with proteins and nucleotide bases to form various chlorinated organic compounds. Chlorine is eliminated from the body primarily through the urine and feces.

IV. HUMAN EXPOSURE

Text to be provided by the Office of Drinking Water.

V. HEALTH EFFECTS IN ANIMALS

Introduction

The importance of chlorine, hypochlorous acid and hypochlorites in the treatment of drinking water is evidenced by the relative decline in the incidence of waterborne infectious diseases in modern times. Although chlorine has proven effective in its inactivation of bacterial, viral and protozoal pathogens, concern over its use has been generated by the discovery that water chlorination may result in the formation of potentially carcinogenic organic by-products, in particular trihalomethanes (Bellar et al., 1974; Rook, 1974, 1976). Thus, attention has focused upon the utilization of alternative water disinfectants such as chlorine dioxide, chloramines and ozone (Akin et al., 1982). Most studies of the effects of chlorine-containing compounds in water have either investigated these alternative disinfectants or the by-products (for example, trihalomethanes, chloramines) formed as a result of water chlorination. The extent of research on the effects of exposure to chlorine and its dissociation products in drinking water on animals and humans is very limited.

Acute Toxicity

Inhalation. Information on the acute toxicity of chlorine has historically been based upon the results of inhalation studies. Table V-1 lists lethal concentration levels of chlorine gas administered by inhalation to various animal species.

TABLE V-1

Lethal Concentrations of Chlorine Gas*

Species	Effect	Exposure Conditions
Rat	LC ₅₀	293 ppm/1 hour
Mouse	LC ₅₀	137 ppm/1 hour
Dog	LC _{LO}	800 ppm/30 minutes
Cat	LC _{LO}	660 ppm/4 hours
Rabbit	LC _{LO}	660 ppm/4 hours
Guinea Pig	LC _{LO}	330 ppm/7 hours
Mammals	LC _{LO}	500 ppm/5 minutes

*Source: NIOSH, 1986

Withers and Lees (1985) reanalyzed the available acute inhalation toxicity data for mice, rats and dogs. Using the Litchfield and Wilcoxon method of probits and adjusting for a common exposure period of 30 minutes, these authors estimated LC₅₀ values of 256, 414 and 650 ppm for mice, rats and dogs, respectively.

Several studies have been conducted to evaluate sensory irritation and pulmonary function in mice, rats and rabbits following acute exposures to chlorine gas (Barrow and Smith, 1975; Barrow et al., 1977; Barrow and Steinhagen, 1982; Chang and Barrow, 1984). RD₅₀ values of 9.3 and 25.4 ppm were reported for mice and rats, respectively. Pretreatment of rats for ≤ 2 weeks at concentrations of 1-10 ppm increased the RD₅₀ values 20-fold (Barrow et al., 1977; Barrow and Steinhagen, 1982; Chang and Barrow, 1984). Rabbits exposed to 50-200 ppm chlorine gas for 30 minutes had decreased pulmonary compliance and edema. Recovery time (0.5 hours to 3 days) was related to the level of exposure and the extent of damage (Barrow and Smith, 1975).

Oral. An oral LD₅₀ of 850 mg/kg bw was reported in rats ingesting calcium hypochlorite (NIOSH, 1986). Toxicity data on acute oral exposures to chlorine, hypochlorous acid and hypochlorites are summarized in Table V-2.

TABLE V-2

Acute Toxicity Studies in Rats

No./Sex of Animals Studied	Study Length	Chlorine Exposure	Mode of Administration	Effects	Reference
4 groups of 6/NR	1.5 hours	0, 20, 50 or 80 mg equivalent chlorine in 5 mL NaOCl solution (0, 100, 250 or 400 mg/kg), pH not reported	intragastric intubation	Amount of CHCl_3 in blood, liver, kidney and fat increased with increasing doses of NaOCl	Vogt et al., 1979
4/males	2 hours	0, 10, 20 or 40 mg/L HOCl solution (0, 0.2, 0.4 or 0.8 mg/kg)	drinking water gavage	Decrease in blood glutathione after 30 minutes in the 10 or 40 mg/L dose groups; recovery after 2 hours	Adbel-Rahmen et al., 1982
4 groups of 4/males	3 hours-7 days	0 or 50 mg equivalent free chlorine in 5 mL NaOCl solution (250 mg/kg), pH not reported	intragastric intubation	Significant decreases in hypothalamic norepinephrine levels and increases in normetanephrine levels after 3 and 24 hours; recovery to normal levels after 7 days	Vogt et al., 1982
4 groups of 4/males	2-10 days	0 or 50 mg equivalent free chlorine in 5 mL NaOCl solution (0 or 142.9 mg/kg), pH not reported	intragastric intubation	Morphological and biochemical liver changes within 2 days of dosing; recovery after 10 days	Chang et al., 1981
4 groups of 5/males	9 days	0, 40, 200 or 1000 mg/L available chlorine (0, 38.1, 180.5 or 902.4 mg/kg/day), pH not reported	in milk ad libitum	No significant effect on body weight gain or organ weights	Cunningham, 1980
4 groups of 10/females	14 days	0, 80, 400 or 2000 mg/L available chlorine, (0, 8, 40 or 200 mg/kg/day), pH not reported	milk fed by gavage	Body weight gain enhanced at 80 mg/L; kidney enlargement in 2000 mg/L group	

NR = Not reported

Vogt et al. (1979) administered aqueous sodium hypochlorite by gavage to fasted Sprague-Dawley rats at 0, 100, 250 or 400 mg/kg bw (0, 20, 50 or 80 mg chlorine). Animals were sacrificed within 1.5 hours after dosing. Chloroform was measured in blood, liver, kidney, fat and brain. Chloroform concentration was found to be a function of increasing doses of sodium hypochlorite in all tissues except the brain, in which chloroform levels remained constant.

A single dose of 10, 20 or 40 mg/L (0.2, 0.4 or 0.8 mg HOCl/kg bw) was administered to groups of four male Sprague-Dawley rats (Abdel-Rahman et al., 1984). Decreases in blood glutathione were observed in the low- and high-dose animals by 30 minutes post administration. Recovery was complete in all groups by 2 hours. All treated animals also showed an increase in red blood cell osmotic fragility at the earliest time point (15 minutes); this effect was not observed 1 hour after treatment.

Administration of chlorinated water was found to influence the levels of hypothalamic norepinephrine in rats (Vogt et al., 1982). Three groups of four male Sprague-Dawley rats were given a single intragastric dose of 5 mL sodium hypochlorite solution (250 mg/kg), containing 50 mg equivalent free chlorine. Animals were killed after 3 hours, 24 hours, or 7 days after dosing. A control group of four rats was given saline solution 12 hours prior to sacrificing. Measurement of neurotransmitter levels in the hypothalamus at the different

time intervals revealed that significant treatment-related decreases had occurred in the levels of norepinephrine ($p < 0.05$). Hypothalamic norepinephrine had decreased by 25% at the 3- and 24-hour time intervals. Recovery to the levels found in control animals was completed in the treated rats after 7 days. Concurrent with the decreased norepinephrine levels, normetanephrine, a metabolic product of the o-methyl transferase system, was found to be increased. Normetanephrine levels returned to normal in a pattern that corresponded (in the opposite direction) to the changes seen in norepinephrine levels. Histamine and dopamine levels were decreased, but the changes were not statistically significant.

Chang et al. (1981) reported that intragastric administration of chlorinated water to rats resulted in the development of a condition resembling "fatty liver" syndrome. Adult male Sprague-Dawley rats (4/group) were given a single 5 mL dose of sodium hypochlorite solution which contained 1% (50 mg) equivalent free chlorine (142.9 mg/kg). The animals were sacrificed at 2, 5 or 10 days after treatment. A control group was given saline solution and killed after 12 hours. After 2 days, morphological changes occurred in the livers of treated rats such that the liver acquired a fatty, pale colored appearance. Total triacylglycerols increased 250% in the dosed rats. The composition of the triacylglycerols was altered in both liver mitochondria and whole liver homogenate. Analysis of the acyl groups revealed the presence of small but significant amounts of long chain polyunsaturated fatty acids (PUFA). It was noted by the author that hepatic triacylglycerols

generally contain only trace levels of PUFA. Recovery from the morphological and biochemical changes had occurred in 50% of the rats examined at 5 days and in all of the rats examined after 10 days.

Cunningham (1980) reported that weight gain in weanling Wistar specific-pathogen-free rats and albino guinea pigs was enhanced by administration of water or milk treated with sodium hypochlorite. Four experiments, two of which entailed short-term exposure, were performed to aid evaluation of practices within the dairy industry. In the first, sodium hypochlorite was mixed with cow's milk on a daily basis such that the nominal concentrations of available chlorine in milk were 0, 40, 200 or 1000 mg/L (0, 36.1, 180.5 or 902.4 mg/kg/day). Five male weanling rats/treatment group were given the milk *ad libitum* for 9 days. Body weights were recorded 5 times in 9 days. The animals were given free access to a commercial rat diet, but no water during the exposure period. Weight gain was found to be enhanced, though not at statistically significant levels, in rats exposed to the lower two chlorine concentrations. Organ weights (liver, kidneys, heart and brain) as a percentage of body weight were unaffected.

Similar results occurred in the second experiment in which female rats (10/group) were given sodium hypochlorite in milk at nominal doses of 0, 8, 40 or 200 mg/kg bw/day as available chlorine. Treatment was by twice daily gavage at a rate of 1 mL/10 g bw/day. Water was available *ad libitum*. Female rats were weighed 10 times over a 2-week study

period. Weight gain was determined to be significantly enhanced by comparison with controls in those animals receiving 8 mg/kg/day ($p < 0.05$). Kidney weights of animals of the highest dose group were also significantly increased ($p < 0.01$).

Other Routes. Sodium hypochlorite was tested in a screening bioassay wherein deionized distilled water solutions were injected into fertile hens' eggs. An LD_{50} was determined to be 1650 mg/kg bw. This was only slightly toxic by comparison with other halogenated hydrocarbons evaluated in the same assay (Hekmati et al., 1983).

Subchronic Toxicity

Limited data are available regarding the subchronic toxicity of chlorine, hypochlorous acid and hypochlorite ion. Available studies are summarized in Table V-3.

Cunningham (1980) tested the effects of hypochlorite added to the drinking water of male weanling rats (10/group) for 6 weeks at exposure levels of 0, 20, 40 or 80 mg/L available chlorine. The average intake of free residual chlorine was calculated to be 0, 4.1, 8.1 and 15.7 mg/kg bw/day for the control, low-, mid- and high-dose groups, respectively. Enhanced weight gain was observed in all treated animals. Weight gain was significantly ($p < 0.05$) different from controls for those rats in the 8.1 mg/kg bw/day group only.

TABLE V-3

Subchronic and Chronic Animal Studies Involving Chlorine Administration in Drinking Water

Species	Number of Animals Studied	Study Length	Chlorine Exposure	Effects	Reference
Shaver broiler chicks	240 females 240 males	28 days	0, 300, 600 and 1200 mg/L available chlorine, pH not reported	In 1200 mg/L: significant increase in mortality, decrease in feed efficiency, heart, kidney, liver and testes weight. In 600 mg/L: reduced heart weights (in both sexes) and testes weight. In 300 mg/L: significantly lower mean body weights, linear decrease in water consumption with increasing chlorine concentration.	Hulan and Proudfoot, 1982
Shaver broiler chicks	240 females 240 males	28 days	0, 37.5, 75 and 150 mg/L available chlorine, pH not reported	No effects on organ weights, water consumption, feed efficiency or mortality.	
Mice	10 females 10 males 10 male controls	33 days	0 or 200 mg/L free available chlorine (0 or 25 mg/kg/day), pH 5.9-6.2	No adverse effects on weight gain, food or water consumption, histological or pathological changes.	Blabau and Nichols, 1956
Guinea pigs	2 groups of 10 males	5 weeks	0 or 50 mg/L available chlorine (0 or 13.4 mg/kg/day), pH not reported	No significant effects on water consumption or body weight gain.	Cunningham, 1980
Rats	4 groups of 10 males	6 weeks	0, 20, 40 or 80 mg/L available chlorine (0, 4.1, 8.1 or 15.7 mg/kg/day), pH not reported	Significant increase in total body weight gain at 40 mg/L, only at 6 weeks.	
Mice	10 males 10 male controls	50 days	0 or 100 ppm free available chlorine (0 or 12.5 mg/kg/day), pH 6.2-6.5	No adverse effects on weight gain, food or water consumption, histological or pathological changes.	Blabau and Nichols, 1956

TABLE V-3 (cont.)

Species	Number of Animals Studied	Study Length	Chlorine Exposure	Effects	Reference
Carnau pigeons	groups of 12	3 months	0, 2 or 15 mg/L (0, 1.0 or 7.5 mg/kg/day), pH 6.5 or 8.5	Increase in plasma cholesterol after 2 and 15 mg/L with normal diet and at 15 mg/L with high cholesterol diet; increase in aortic plaque size in pigeons fed normal diets and exposed to 2 and 15 mg/L chlorine. changes in plasma thyroxine levels after treatment with 2 and 15 mg/L chlorine	Revis et al., 1986
New Zealand rabbits	NR	3 months	0, 0.1 or 15 mg/L (0, 0.01 and 1.6 mg/kg/day)	Increase in hydroxyproline in heart tissue after 15 mg/L treatment	Revis et al., 1985
Sprague-Dawley rats	70 males 70 females	3 months	0, 25, 100, 175 or 250 mg/L (0, 2, 7.5, 12.8 or 16.7 mg/kg/day males) (0, 3.5, 12.6, 19.5 or 24.9 mg/kg/day females)	No consistent treatment-related adverse effects.	Daniel et al., 1990
Carnau pigeons	5 groups of 5	9 months	0, 0.1, 10, 15 or 30 mg/L (0, 0.05, 5.0, 7.5 or 15.0 mg/kg/day)	Contractile properties of the heart decreased after 15 and 30 mg/L; relative heart weight increased at 15-30 mg/L; endocardial and myocardial fibrosis more intense at 30 mg/L; more atherosclerotic plaques at 30 mg/L; increased hydroxyproline in heart tissue after 0.1 mg/L; decrease in serum thyroxine and its metabolites after 10, 15 and 30 mg/L.	Revis et al., 1985

TABLE V-3 (cont.)

Species	Number of Animals Studied	Study Length	Chlorine Exposure	Effects	Reference
Sprague-Dawley rats	groups of 4/males	12 months	0, 1.0, 10.0 or 100 mg/L (0, 0.14, 1.4 or 14 mg/kg bw/day)	Significant decreases in red blood cell count and hematocrit reversed at 6 months; increased osmotic fragility 1-100 mg/L only significant at 1 and 100 mg/L; increased mean corpuscular hemoglobin.	Abdel-Rahman et al., 1984
F344 rats	groups of 50/ males & females	2 years	0, 0.05 and 1% males (5.5 and 21.9 mg/kg bw) 0, 0.1 and 0.2% females (0, 15.5 and 54.7 mg/kg bw)	Significant decreases in body and liver weights all treated animals; decreased brain and heart weights in males and salivary gland and kidney weight in females.	Hasegawa et al., 1986
F344 rats	groups of 70/ males & females	2 years	0, 70, 140, 275 ppm (0, 4.2, 7.2, 13.3 mg/kg bw males) (0, 4.2, 7.8, 14.4 mg/kg bw females)	No treatment-related adverse effects. Decreased water consumption, and body weights.	NTP, 1990
B6C3F1 mice	groups of 70 males & females	2 years	0, 70, 140, 275 ppm (0, 7.4, 14, 24 males) (0, 7.6, 14, 24 females)		
BDII rats	236/sex NR	7 generations	100 mg/L free residual chlorine (10 mg/kg/day), pH not reported	No adverse effects on weight gain, food consumption, water consumption, fertility, lifespan, growth pattern, hematology, histology (liver, spleen, kidney or other organs).	Druckrey, 1968

NR = Not reported

Cunningham (1980) also tested male albino guinea pigs to determine whether the enhancement of weight gain was a specific response in rats. Ten guinea pigs/group were administered either nonchlorinated water or water containing 50 mg/L (13.4 mg/kg bw/day) available chlorine *ad libitum*. At the end of 5 weeks, body weight gain was increased over controls; however, this increase was not statistically significant. No treated animals, rats or guinea pigs, were observed to decrease their water consumption or to exhibit any signs of toxicosis.

Interest in the results of the Cunningham (1980) studies provided the impetus to determine if similar effects might occur in Shaver Broiler chicks (Hulan and Proudfoot, 1982). Two experiments were conducted in which sodium hypochlorite was added to drinking water to obtain available chlorine concentrations of 0, 300, 600 and 1200 ppm (experiment 1), or 0, 37.5, 75.0 and 150 ppm (experiment 2). In each experiment, water was made available *ad libitum* for 28 days starting when the chicks were 1 day old. The 480 chicks were randomly assigned so that each treatment was offered to three replicate units of 20 male and 20 female chicks. Food and water consumption was recorded weekly for the entire unit of 20 rather than for individual birds. On day 28, two birds from each of the eight treatment groups were killed and hearts, livers, kidneys and testes were removed and weighed. Administration of 1200 ppm available chlorine resulted in significantly ($p < 0.01$) increased mortality (as compared with controls), "lowered feed efficiency," reduced water consumption, and decreased heart, liver, kidney and testes weights. Mean

body weights and water consumption were significantly ($p < 0.01$) lowered in chicks receiving 300 ppm or more available chlorine in drinking water. There was some indication of a growth stimulus at the lower concentrations (37.5-150 ppm) but none of the responses were statistically significant. The results of this study are difficult to interpret, since the investigators did not provide information on the causes of death for the chicks at the 1200 ppm HOCl treatment concentration. The decreases in body and organ weight may have been an indirect effect of the unpalatable taste of the highly chlorinated drinking water. The study lacked any discussion of quality control procedures as to whether the available chlorine concentrations were measured or confirmed over the exposure period, or if precautions were taken to prevent volatilization and photodegradation of residual chlorine. Also, water consumption data are not adequate for deriving a mg/kg bw dose of chlorine.

Although the Cunningham (1980) and Hulan and Proudfoot (1982) studies indicated that toxic effects may occur after oral administration of chlorine, studies by Blabaum and Nichols (1956) did not report any evidence of toxicity. Blabaum and Nichols (1956) administered chlorine to weanling white mice (strain not specified) obtained from a stock solution prepared by bubbling chlorine gas through city water. In the first part of the investigation, a group of 10 male mice were given free access for 50 days to 100 ppm (12.5 mg/kg/day) available chlorine in water (pH ranged from 6.2-6.5). In the second part of the experiment two groups of mice (10 females, 10 males) were given 200 ppm (25 mg/kg/day) free available chlorine in drinking water (pH ranged from 5.9-6.2), *ad libitum*,

for 33 days. A single control group of 10 male mice was used for both parts of the experiment. The control group received city water from Madison, Wisconsin (pH=7.3), which contained 10 ppm Cl. Water supplies were changed every 12 hours, at which time both the old and new drinking water supplies were analyzed with the idometric method to determine the total residual chlorine. Chlorine test papers were employed to confirm that the chlorine content of the dispensed water drops were not appreciably different from that in the bottle. At the end of the experiments the mice were sacrificed, autopsied, and their stomachs, intestines, kidneys, livers and spleens were removed for histological examination. By comparison with controls, the mice receiving 100 and 200 ppm free available chlorine experienced no differences in weight gain, growth or water consumption. No gross or physical abnormalities were found upon autopsy or histologic examination. A NOAEL of 25 mg/kg/day can be identified in this study.

Fisher et al. (1983a) fed weanling rats (9/sex/group) diets containing 0, 1257 or 2506 ppm chlorinated flour for 28 days. TWA chlorine concentrations were 118 and 236 mg/kg bw for males and 125 and 249 mg/kg bw for females for the low- and high-dose groups, respectively. Body weights, food consumption and organ weights were measured. Histological examination of the kidneys, livers, hearts and brains was performed. There was no difference in body weight in males. In the females, there was a significant ($p<0.05$) inverse correlation between body weight and chlorine treatment at 28 days. No significant differences were seen in absolute organ weights; however, there were significant ($p<0.05$)

dose-related increases in kidney weights (males) and liver weights (both sexes) when organ weights were adjusted for body weight covariance. No significant histopathological effects were found.

In a study by Kotula et al. (1987), weanling F344 rats (24/sex/group) were fed diets containing 0, 50, 200 or 600 ppm (0, 5, 20 or 60 mg/kg/day) chlorine for 92 days. Rats were weighed and food consumption recorded weekly. After 92 days, rats were sacrificed and histopathologic examination was performed on all major organs (adrenals, brain, liver, kidneys, lungs, ovaries, heart, thyroid, testes, pancreas, colon and stomach). Rats fed the chlorine-treated diets did not exhibit any adverse treatment-related effects. Male rats fed the meat diets with or without chlorine developed moderate fatty livers. This was attributed to an excess intake of fat in the diet (33% fat). A NOAEL of 60 mg/kg/day can be identified from this study.

Revis et al. (1985) exposed male New Zealand rabbits (5/group) that had been maintained on calcium-deficient diets (80% minimum daily requirement) and supplemented with 10% lard to drinking water containing 0, 0.1, 10, 15 or 30 ppm (mg/L) chlorine for 3 months. Chlorine doses were estimated to be 0, 0.01, 1.6 and 3.2 mg/kg bw assuming an average body weight of 3.8 kg and 0.41 L/day water consumption. Analyses of the hydroxyproline content of the rabbits hearts exposed to 0, 0.01 and 1.6 mg/kg bw chlorine showed a dose-related increase in the hydroxyproline concentration. This increase was

statistically significant ($p < 0.05$) at the high dose (1.6 mg/kg) only. No other effects were reported in the rabbits.

Revis et al. (1986) exposed groups of 12 male White Carneau pigeons to 0, 2 or 15 mg/L chlorine in drinking water at pH 6.5 or 8.5 for 3 months. The birds were maintained on either a calcium-deficient (80% minimum daily requirement) or a calcium-deficient diet supplemented with 10% lard and 0.5% cholesterol. At 1-month intervals, blood samples were collected and analyzed for development of atherosclerosis. The authors reported significant increases in plasma cholesterol levels as compared with controls after 3 months of exposure to 1.0 and 7.5 mg/kg/day chlorine at pH 8.5 and a normal cholesterol (but calcium-deficient) diet. Of birds on the high cholesterol diet, plasma cholesterol was increased in pigeons exposed to 1.0 mg/kg/day chlorine (pH 6.5 and 8.5), but significant increases ($p < 0.05$) were only observed in those pigeons given 7.5 mg/kg/day at pH 8.5. Significant increases in the mean aortic plaque size in pigeons fed the normal diet were observed only in the group exposed to chlorine at pH 8.5 (2 and 15 mg/L), suggesting a relationship between mean aortic plaque size and plasma cholesterol levels. In an attempt to determine if the increase in plasma cholesterol level is associated with thyroid function, the levels of plasma thyroxine (T_4) and its metabolite triiodothyroxine (T_3) were quantified. Significant changes in the levels of these hormones were observed in both the 1.0 and 7.5 mg/kg/day treatments at pH 8.5. Although the implications of these results may be complicated by the fact that the experiment utilized calcium-deficient diets, the data have

identified a potential sensitive subpopulation and suggest possible adverse effects on the cardiovascular system as a consequence of chlorine exposure.

Daniel et al. (1990) administered chlorine in the drinking water of adult male and female Sprague-Dawley rats (10/sex/dose) for 90 days. Dose levels were 0, 25, 100, 175 and 250 mg/L which correspond to 0, 2, 7.5, 12.8 and 16.7 mg/kg/day for males and 0, 3.5, 12.6, 19.5 and 24.9 mg/kg/day for females, respectively. Food and water consumption, and body weight were monitored and detailed hematologic, clinical chemistry and histopathologic examinations were conducted. There were no treatment-related effects on survival, body weight or histopathologic lesions for either sex. At the highest dose level (200 mg/L), there was a 62-64% decrease in water consumption compared with controls. The authors concluded that the highest dose level (200 mg/L) was a NOAEL.

In a more recent report (Daniel et al., 1991) chlorine was administered in the drinking water of adult male and female B6C3F1 mice (10/sex/dose) for 90 days. Dose levels were 0, 12.5, 25, 50, 100 and 200 mg/L which corresponded to 0, 2.7, 5.1, 10.3, 19.8 and 34.4 for males and 0, 2.8, 5.8, 11.7, 21.2 and 39.2 mg/kg/day for females. Mortality, body weight, food and water consumption, hematology, clinical chemistry, organ weights and histopathology were monitored. There was no reduction in survival or food consumption or clinical signs of toxicity or nontreatment-related histopathologic lesions. A concentration-related decrease in water consumption was observed in both males and

females. Decreases were statistically significant ($p < 0.05$) in females at the highest dose levels (100 and 200 mg/L). A concurrent decrease in body weight gain was also observed in both sexes with a significant reduction in males at the two highest dose levels. Reductions in organ weights and serum enzymes were also observed; however, these decreases were not consistent in males and females and were not reported at all dose levels. The authors concluded that these effects were consistent with the decreased water consumption rather than any specific treatment-related toxicity, especially in the absence of any histopathologic lesions or signs of clinical toxicity. The 50 mg/L dose was considered to be a NOAEL by the authors, while the two highest doses of 100 and 200 mg/L were considered mild LOAELs.

Bercz et al. (1990) studied the influence of chlorinated water on the development of hyperlipidemia in monkeys consuming an atherogenic diet. Rhesus (4 females) and African Green (5 males and 4 females) monkeys were exposed for 89 weeks to chlorinated drinking water (0, 5 or 30 ppm) while being maintained on either normal or high cholesterol diets. The study design consisted of eight treatment periods. In period I (6 weeks), animals were maintained on a normal diet and given nonchlorinated drinking water, which served to establish baseline lipid parameters. During periods II-V, animals were fed an atherogenic diet (15% lard and 1% cholesterol) and given drinking water containing 0, 5 or 30 mg/L chlorine. Fasting blood samples were drawn weekly and analyzed for total cholesterol, LDL, HDL and triglycerides. Statistical evaluation of measured responses was

performed by comparing each animal's median response with that of the previous treatment period. The results indicated that exposure to 5 mg/L chlorine had no effect on lipid metabolism. Exposure to 30 mg/L chlorine (14 weeks) resulted in increased LDL and decreased HDL levels when compared with previous exposure period. It should be noted, however, that cholesterol levels were initially increased with the introduction of the atherogenic diet (previous treatment period) and may have reached a plateau during the following period in which animals were exposed to high chlorine levels (30 mg/L). No effect was seen on triglyceride levels. After 61 weeks, the animals were returned to a normal diet and allowed to stabilize for 9 weeks with distilled water. Monkeys exposed to 30 mg/L chlorine and normal diets showed no alterations in lipid metabolism.

Abdel-Rahman et al. (1984) administered HOCl in drinking water to groups of four male Sprague-Dawley rats for ≤ 12 months at chlorine concentrations of 0, 1, 10 or 100 mg/L (0, 0.14, 1.4 or 14 mg/kg bw/day). Blood samples were collected after 2, 3, 4, 6, 7, 10 and 12 months of treatment and blood osmotic fragility and glutathione (GSH) levels were determined. Data from this study indicated increased GSH levels at 2 months in 10 and 100 mg/L groups followed by decreased levels at 7 months. After 3 months of treatment, there were significant decreases in red blood cell count and hematocrit; however, these effects were reversed after 6 months of treatment. Increased osmotic fragility was observed in all treatment groups; however, this effect was statistically significant ($p < 0.05$) in only the 1 and 100 mg/L dose groups. The authors concluded that,

based on these results, there was an increased mean corpuscular hemoglobin concentration indicative of erythrocyte damage. The results of this study should, however, be viewed with caution in light of the relatively small sample size involved as well as the inconsistencies in the dose response and duration of effects.

Revis et al. (1985) also exposed groups of five white male Carneau pigeons maintained on a calcium-deficient diet (80% MDR) to drinking water containing 0, 0.1, 10, 15 or 30 ppm (mg/L) chlorine (as sodium hypochlorite) for 9 months. Based on an average body weight of 500 g and water consumption of 250 mL/day, this corresponds to -0, 0.05, 5.0, 7.5 and 15.0 mg/kg/day. In the pigeons, blood pressure, ventricular pressure (left side) and pressure-time measurements were made as well as body and heart weights. Morphological studies were also conducted on the heart tissue. Although the diastolic and systolic blood pressure increased in the pigeons exposed to 5-15 mg/kg/day chlorine, this change was not statistically significant. Contractile properties of the pigeon hearts (described as dp/dt , the rate of rise of ventricular pressure) decreased significantly in pigeons exposed to 7.5 and 15 mg/kg/day chlorine. Heart weight was also significantly increased ($p < 0.01$) at these dose groups. Endocardial and myocardial fibrosis as well as atherosclerotic plaques in coronary arteries appeared to increase in incidence and severity with increasing doses. An analysis of the hydroxyproline content of the pigeon hearts showed an increased concentration with increased dosage, significant at 0.05 mg/kg in the pigeons. Serum thyroxine (T_4) and thyroxine metabolite (T_3) were also measured in

pigeons exposed to chlorinated water. The authors reported a significant decrease in the levels of these hormones in pigeons given 5, 7.5 and 15.0 mg/kg/day chlorine.

Chronic Toxicity

Druckrey (1968) studied the effects of highly chlorinated drinking water (100 mg/L) given daily to 7 consecutive generations of BD II rats. Solutions were prepared weekly by bubbling chlorine gas through tap water (Freiberg, Germany). Chlorine content was monitored by titration with $\text{Na}_2\text{S}_2\text{O}_3$. In order to insure a stable total dietary chlorine concentration, dry rat chow was cooked with the chlorinated water prior to distribution to the parental generation. Subsequent generations received chlorine only in the water and ate a standard diet, resulting in an average daily dose of -10 mg/kg bw chlorine.

Parental animals began treatment at 100 days of age. Repeated matings were done and rats remained on treatment during pregnancy and lactation. Selected progeny were separated from their dams at 30-40 days and were designated the subsequent generation at this time. Animals of the F_3 and F_4 generations consumed chlorinated water only until the birth of progeny. Subsequent generations remained on test for the entire lifespan. Two groups of animals served as controls at the beginning and ending of the experimental period.

After a period during which the rats became acclimated to the taste of the chlorinated water, the test solution was well tolerated. Weight gain among neonates was somewhat depressed during the first few days of life. By maturity the average body weight for all generations of test animals was -5-10% greater than that of the untreated rats. Of 236 rats observed, no treatment-related effects were noted on the lifespan, fertility, growth, hematological measurements, or histology of liver, spleen, kidney and other organs. The incidence of malignant tumors in the treated rats was not found to differ from that of control group rats (see the Carcinogenicity Section). A NOAEL of 10 mg chlorine/kg bw could be identified from this study.

Hasegawa et al. (1986) studied the potential adverse effects resulting from long-term exposure to sodium hypochlorite in drinking water. Male and female F344 rats (50/sex/dose) were given sodium hypochlorite in their drinking water at concentrations of 0.05 and 0.1% for males and 0.1 and 0.2% for females for 104 weeks. Similar numbers of male and female rats received distilled water. All animals were observed daily and body weights, mortality and water consumption were measured at regular intervals. All animals surviving until the termination of the experiment were sacrificed, fasting blood samples were taken and then autopsied. Complete gross and microscopic examination was also performed on all moribund rats or animals dying spontaneously during the experiment. Based on information provided by authors, chlorite (OCl⁻) concentrations of 13.5 and 27.7 mg/kg bw for males and 34.3 and 63.2 mg/kg bw for females were estimated. These

values, however, appear extremely high and inconsistent with those reported by other investigators who have seen marked reductions in water consumption at similar or lower chlorine concentrations.

As requested by the U.S. EPA, NTP conducted a 2-year bioassay to evaluate the chronic toxicity and carcinogenicity of chlorinated drinking water. In this study F344 rats (70/sex/dose) and B6C3F1 mice (70/sex/dose) were administered chlorinated drinking water at 0, 70, 140 or 275 ppm available chlorine (as sodium hypochlorite) for 104 weeks. Based on body weights and water consumption values reported in the study, these doses correspond to doses of 4.2, 7.3 and 13.6 mg/kg/day for male rats; 4.2, 7.8 and 14.4 mg/kg/day for female rats; 7.4, 14.0 and 24 mg/kg/day for male mice, and 7.6, 14.2 and 24.2 mg/kg/day for female mice. Interim sacrifices were performed at 14 and 66 weeks on 10 animals/sex/dose. Body weights, organs weights, histopathology and hematology food and water consumption were evaluated throughout the study.

In rats, there was a dose-related decrease in water consumption. During the second year of the study, water consumption was decreased 21% in males and 23% in females. Mean body weights were slightly lower in high-dose females and in all treated males. However, these decreases were within 10% of the control animals. There were no biologically significant differences in organ weights or organ-to-body weight ratios. In

addition, there were no changes in hematology, and no treatment-related gross or microscopic lesions observed.

In mice, body weights were 5-8% lower for high-dose males and 5-7% lower in females. There were no treatment-related significant differences in organ weights or organ-to-body weight ratios. No alterations were reported in hematologic or gross or microscopic histopathologic parameters. Although there was a high rate of mortality, the survival rate was similar for all groups of rats, controls and treated animals. Absolute liver weights were decreased significantly in male and female treated rats when compared with controls. Significant decreases in weight, salivary gland and kidney weight were observed in females. In the males, there was a significant decrease in brain and heart weights. There was no significant increase in the incidence of gross or histopathologic nonneoplastic lesion of any type in treated animals. No changes in serum chemistry, food consumption or water consumption were observed. The biological significance of the reduced organ and body weights is unclear although the authors concluded that these effects may be suggestive of chronic toxicity.

Several studies have been done to ascertain possible health effects of chlorine as an additive in flour (Fisher et al., 1983a,b; Ginocchio et al., 1983). Wistar rats (60/sex/group) were fed diets consisting of cake prepared from flour containing 0, 1250 or 2500 ppm Cl for 104 weeks (Fisher et al., 1983b). All animals were inspected daily, body

weights and food intake were recorded weekly. Blood and urine analyses were performed at 1, 3, 12, 18 and 24 months. Organ weight and histological evaluations were also performed. Chlorine intake was estimated by the investigators to be 12.8 and 25.3 mg/kg bw/day for males and 17.0 and 35.0 mg/kg bw/day for females for the low- and high-dose groups, respectively. No differences in mortality were observed in treated animals when compared with similarly fed control animals. Dose-related increases in hematological parameters were seen in both males and females; however, these effects were statistically significant in the males only ($p < 0.05$). A dose-related statistically significant ($p < 0.05$) reduction in spleen weight was seen in the females. In addition to these effects, histological lesions characteristic of the aging process were observed in the liver, kidney and spleen in both control and treated rats; however, these effects appeared earlier in treated animals. A LOAEL of 12.8 mg/kg can be identified in this study.

In a similar study, Ginacchio et al. (1983) fed Theiller original strain mice (60/sex) cakes prepared from chlorinated flour containing 0, 1257 or 2506 ppm chlorine for 70-73 weeks. Body and organ weights, food consumption and mortality rates were measured. Blood and urine analysis as well as histological evaluations were also conducted. Estimated chlorine intakes were 143 and 286 mg/kg bw/day for males and 175 and 350 mg/kg bw for females for the low- and high-dose groups, respectively. The results showed an increase in obesity in both male and female treated mice. In females, there was a statistically significant ($p < 0.01$) increase in heart and kidney weights and a significant

($p < 0.05$) decrease in ovary weight. There was also increased mortality in females ($p < 0.001$) in both dose groups. A LOAEL of 175 mg/kg bw can be derived from this study.

Target Organ Toxicity

Cardiovascular Effects. A reduction in the weight of the heart occurred in both sexes of Shaver broiler chicks studied by Hulan and Proudfoot (1982) at available chlorine concentrations of both 600 and 1200 ppm in drinking water.

As pigeons may provide a useful model for cardiovascular disease, Revis et al. (1985) used these birds in an investigation of the effect of chlorinated drinking water on the cardiovascular system. Groups of five male white Carneau pigeons were fed calcium-deficient diets (80% MDR) and drinking water containing either 0 (deionized water), 0.1, 10, 15 or 30 mg/L (0, 0.05, 5.0, 7.5 or 15.0 mg/kg/day) chlorine *ad libitum* for 9 months. After 9 months of exposure, pigeons were cannulated and blood pressure, left ventricular pressure and pressure-time (dp/dt) were measured. Systolic and diastolic pressure increased in the range of 8-13 mm Hg and 9-17 mm Hg in pigeons exposed to 5.0-15.0 mg/kg/day chlorine, respectively. These changes in blood pressure were not statistically significant. Significant increases in heart weight of 22-49%, however, were observed in all treatment groups. Body weight of treated pigeons did not change by comparison with controls. Pigeons given 7.5 or 15 mg/kg/day chlorine showed a significant decrease in dp/dt, which is frequently observed in chlorine heart failure secondary to

cardiac hypertrophy. Morphological changes of the heart such as endocardial and myocardial fibrosis appeared to increase with higher concentrations. Decreases in T_4 levels ranging from 25-45% were measured in pigeons given 10, 15 and 30 mg/L chlorine. The authors suggest that hypothyroidism may be a disease condition associated with enlargement of the heart.

In a more recent study, Revis et al. (1986) examined the relationship of chlorine to plasma cholesterol and thyroid hormone levels in pigeons. Groups of 12 male white Carneau pigeons (age 3-4 months) were fed altered diets and drinking water containing either 0 (deionized water), 2 or 15 ppm chlorine *ad libitum* for 3 months (0, 1.0 or 7.5 mg/kg/day). Diets were either (A) reduced to 0.35% calcium (80% MDR for a pigeon), or (B) reduced to 0.35% calcium with the addition of 10% lard and 0.5% cholesterol. Treated drinking water was prepared and changed daily. At 1-month intervals blood samples were collected and plasma levels of cholesterol and T_4 were determined. Following a 3-month exposure to diet (B) and either deionized water or water containing 15 ppm chlorine (pH 8.5), pigeons were observed to have significant increases in plasma cholesterol. Plasma T_4 levels were significantly decreased in pigeons fed a normal diet (A) or high cholesterol diet (B) and drinking water containing 2 and 15 ppm chlorine (pH 8.5). Plasma T_3 levels appeared to be increased at the 2 ppm level and decreased at 15 ppm in both diets A and B. There was no clear dose response effect for plasma cholesterol observed in any of the treatment groups. Thus, factors associated with the effects of chlorine on plasma

cholesterol were not clearly elucidated by these data. The authors suggest that the changes in plasma cholesterol may be mediated by products formed when chlorine reacts with organic matter in the upper GI tract.

Studies by Bercz et al. (1990) indicated that in monkeys maintained on high atherogenic diets, the consumption of chlorinated water (0, 5 or 30 ppm chlorine) may influence the development of hyperlipidemia. Exposure of both male and female African Green and female rhesus monkeys to 30 mg/L chlorine resulted in increased LDL and decreased HDL levels. There was also an increase in total serum cholesterol levels in these animals. It should be noted, however, that when these animals were returned to normal diets and exposed to similar levels of chlorine (30 mg/L) no change in lipid metabolism was observed.

Hasegawa et al. (1986) found that long-term exposure of F344 rats (50/sex/dose) to sodium hypochlorite in their drinking water resulted in statistically significant ($p < 0.05$) decreases in heart weights of males exposed to chlorine concentrations of ≤ 13.5 mg/kg bw/day. This effect was not seen in females.

In a study in which mice (Theiller original strain) were fed diets of cakes prepared from flour containing 0, 1257 or 2506 ppm chlorine for 70-73 weeks, there was a

statistically significant ($p < 0.01$) increase in heart weight in female mice (Ginacchio et al., 1983).

Immunological Effects. As part of a study on water disinfectants (Hermann et al., 1982) 30 outbred CRI-1:CD-1 mice received chlorinated drinking water for 120 days. Control groups drank deionized distilled water (0.1 ppm Cl, pH 6.1-6.4) or tap water (0.9 ppm Cl, pH 7.0 ± 0.2); experimental groups received hyperchlorinated water (15 or 30 ppm Cl, pH 6.4 or 6.8, respectively), chlorinated/acidified water (15 ppm Cl, pH 2.5) or hyperacidified water (0.1 ppm Cl, pH 2.0). Fifteen mice from each group were tested for delayed hypersensitivity to sheep red blood cell, and 15 were assayed for serum antibody responses to the same antigen. Five mice/group were not immunized but were measured for delayed hypersensitivity. Nonimmunized mice were used for assessment of reticuloendothelial phagocytosis of colloidal carbon. None of the mice drinking chlorinated waters showed evidence of a statistically significant change in humoral or cell-mediated immune responses.

Fidler (1977) examined the relationship between drinking water hyperchlorination and peritoneal exudate macrophage (PEM) functions in female C57Bl/6N mice. A control group received tap water (0.5-1.0 ppm chlorine) and the treatment group received hyperchlorinated tap water (numbers of animals not specified). The latter treatment was prepared by adding sodium hypochlorite to tap water twice a week so that total residual

chlorine (assayed by the o-toluidine method) was maintained at levels between 25 and 30 ppm. At weekly intervals, five control mice and five treated mice were injected i.p. with 2 mL thioglycolate broth in order to collect PEMs. Beginning with the first week of treatment, there were significant decreases in the number of PEMs collected from mice given hyperchlorinated water. Furthermore, during the first 2 weeks of treatment, PEMs from mice receiving hyperchlorinated water showed significant decreases in *in vitro* cytotoxicity against mouse melanoma (B16) and fibrosarcoma (UV-112) target cells, and a complete absence of tumoricidal activity in the remaining 2 weeks. Hyperchlorination of drinking water was, therefore, determined to have an adverse effect upon the macrophage defense mechanism of laboratory mice.

Carcinogenicity

Chlorine, hypochlorous acid and hypochlorite ion have not been shown to act as direct carcinogens or initiators of tumorigenesis. In the 7-generation toxicity study conducted by Druckrey (1968), the incidence of malignant tumors in rats consuming drinking water with a free available chlorine level of 100 mg/L was not different from the incidence in control rats. Average daily dose was determined to be 10 mg/kg bw/day. Of a total of 236 individuals from all generations of treated rats, 24 malignant tumors were found. Among the total of 56 control animals, five tumors considered malignant were found. The authors considered these incidences to be equivalent. There was, however, an increase in the number of ileocecal sarcomas in the F₂ generation of treated rats.

These were undifferentiated large-cellular sarcomas originating in the retroperitoneal or mesenteric lymph glands: there was evidence of some metastasis. F₅ and F₆ rats consumed test water throughout their lifespan. Of 49 F₅ rats only two ileocecal sarcomas were observed, and among 63 F₅ rats only one was noted. The author concluded that the increased incidence of ileocecal sarcoma in the F₂ rats was not attributed to the chlorine ingestion, as an increase was not noted in the subsequent generations.

In a more recent study, Hasegawa et al. (1986) studied the carcinogenic potential of sodium hypochlorite on F344 rats. Groups of 50 male and female rats were given NaOCl in their drinking water at concentrations of 0.05 and 0.1% for males and 0.1 and 0.2% for females for 104 weeks. Although a variety of tumors developed in all groups (controls and treated), no dose related increase was seen in either incidence, type or latency period of tumors for any organ or tissue in either sex. Survival was also similar for all groups. The most frequent tumors and proliferative lesions found were leukemias, adenomas of the pituitary, C-cell adenomas of the thyroid gland and adrenal cortical hyperplasia. These tumors are the most commonly found spontaneous tumors in F344 rats. NTP (1990) evaluated the potential carcinogenicity of chlorinated drinking water in F334/N rats and B6C3F1 mice (70/sex/dose). Animals were administered drinking water containing 0, 70, 140 or 275 ppm chlorine (as available atomic chlorine) for 2 years. Interim sacrifices of 10 animals/sex/dose were performed at 15 and 66 weeks. Food and water consumption, body weights, organ weights, survival, clinical chemistry, hematology,

and gross and microscopic histopathology were evaluated. Survival among treated animals (rats and mice) was similar to controls. In rats there was an increased incidence of mononuclear cell leukemia in mid- and high-dose females. This increase was statistically significant ($p=0.014$) for the mid-dose females only. The incidence of leukemias in females was 8/50, 7/50, 19/51 and 16/50 for the control, low-, mid- and high-dose groups, respectively. The proportion of animals with leukemia that died before the termination of the study (104 weeks) and the mean time to death were comparable for all dose groups and controls. No leukemia were observed in male rats. Other neoplasms, renal tubular cell adenomas and squamous cell carcinomas in males and islet cell adenomas and lymphoid hyperplasia in females were also observed. However, the occurrence of these neoplasms was sporadic, nondose-related or lacked supportive evidence in either males or females. These neoplasms, therefore, were not considered treatment related.

In mice there was no increase in neoplasms that were statistically significant when compared with controls and that could clearly relate to chlorinated drinking water exposure. Renal tubular cell neoplasms were observed in two high-dose male mice (one carcinoma and one adenoma). Additional examination of renal tissue from all male mice (treated and controls) resulted in the observation of one additional tubular cell carcinoma in a low-dose male and focal hyperplasia in controls and treated animals from all dose groups. Since there was no dose-related increase in the occurrence of these renal neoplasms and the

incidence of hyperplasia was comparable in controls and treated mice, it was concluded that these neoplasms were not treatment related.

Dermal application studies performed with sodium hypochlorite in conjunction with other chemicals have produced conflicting results. In a study by Hayatsu et al. (1971), three groups of 40 female ddN mice underwent dermal applications of 4-nitroquinoline-1-oxide alone, commercial sodium hypochlorite solution alone ($\leq 10\%$ free residual chlorine), or a combination of both. Application of sodium hypochlorite solution alone for a total of 60 treatments in 300 days did not produce malignant or benign tumors after a total observation time of 450 days. However, when 45 applications of sodium hypochlorite solution were given after 20 treatments of 4-nitroquinoline-1-oxide (0.05 mg of 0.25% w/w benzene solution), 9/32 mice developed skin tumors. This may indicate a possible cocarcinogenic potential of sodium hypochlorite.

A later experiment by Pfeiffer (1978) used a study design similar to that used by Hayatsu et al. (1971). In this case a 0.22% or 0.44% acetone solution of benzo[a]pyrene was applied to the depilated skin of female NMRI mice twice a week for 10 weeks, providing total doses of 750 ug or 1500 ug/animal. Sodium hypochlorite (1%) was applied to groups of 100 mice either before, during, or after treatment with benzo[a]pyrene. Results indicated that skin tumors in animals administered NaOCl prior to benzo[a]pyrene treatment were reduced in size and number. The number of carcinomas among the tumors

observed was decreased by 40% in all the treatment groups that received chlorine. Neither the time to tumor induction nor the mortality rates were affected by chlorine treatment. Thus, these experiments showed chlorine to reduce rather than to increase the tumor incidence.

In a more recent study, Robinson et al. (1986) treated the skin of female SENCAR mice with aqueous solutions of HOCl and NaOCl for 4 days. Animals were sacrificed on days 1, 2, 3, 4, 5, 8, 10 or 12 following the last treatment. The shaved backs of animals were treated with 0, 1, 10, 100, 300 or 1000 ppm HOCl or NaOCl. For animals treated with HOCl there was a dose-related statistically significant ($p < 0.05$) increase in epidermal thickness. Treatment with NaOCl resulted in increased epidermis only at the 1000 mg/L dose level.

Mutagenicity

Assessment of the mutagenicity of chlorine is complicated by the reactive nature of the chlorine molecule. Mutagenicity testing of chlorine is confounded by the presence of its reaction products, which have been found to be mutagenic.

Sodium hypochlorite has been determined to be directly mutagenic at the histidine locus in *Salmonella typhimurium* (Wlodkowski and Rosenkranz, 1975). Sodium hypochlorite gave variable results in standard plate incorporation assays, because of the

bacteriotoxicity of the test compound. This toxicity was reduced by incubating the bacteria and hypochlorite in suspension cultures and adding ascorbic acid at various time intervals to decompose the residual hypochlorite. Under these conditions sodium hypochlorite at a concentration of 0.28 μ moles/mL was mutagenic for the base substitution mutant TA1530 but did not revert the frameshift mutation in TA1538.

Sweeney and Chek (1985) demonstrated that XAD-4 resin concentrates of free residual chlorine were mutagenic in the Ames assay. Dose-related increases in the number of revertants/L were formed when chlorine concentrations of 0, 0.5, 1.0 and 2.0 ppm were tested without S-9 using *Salmonella* strain TA100.

In a study by Ishidate et al. (1984), sodium hypochlorite and calcium hypochlorite were shown to be direct-acting mutagens in the Ames assay using *Salmonella typhimurium* TA100 at nonbacteriotoxic concentrations.

Mickey and Holden (1971) reported that chlorine can directly produce chromosome aberrations in mammalian cells. These authors added chlorine to the culture medium of three different mammalian tissue culture cell systems at 13 different concentrations ranging from 2.5-60 ppm. No significant increase in chromosomal aberrations of human lymphocytes was detected below concentrations of ~20 ppm. Above this concentration there was an exposure dependent increase in chromatid and chromosome breaks,

translocations, dicentric chromosomes and gaps. Chlorine was added to Chinese hamster lung cells or to Indian muntjac cells 24 hours after subculture, and cells were harvested and processed 24 hours later. Extensive exposure-dependent chromosomal breakage occurred at concentrations ≤ 20 ppm. Endomitosis also increased at concentrations of ≤ 20 ppm. Thus, in all three test systems, the higher concentrations of chlorine produced significant increases in chromosomal aberrations and endomitotic figures over control values and completely inhibited mitosis at still higher chlorine levels.

Patton et al. (1972) showed that aqueous hypochlorous acid reacts with cytosine to produce various chlorinated cytosine derivatives. Many investigators have shown that the chlorination of uracil in water produces chlorinated uracil derivatives, the most abundant of which is 5-chlorouracil (Dennis et al., 1978). Furthermore, Walton and Cumming (1976) demonstrated 5-chlorouracil to be mutagenic for bacterial test strains. A 300-fold increase in *Escherichia coli* WP-1 mutants was observed after the incubation of the bacteria with 50 ug/mL. Cumming (1978) found that 1 g/L 5-chlorouracil given to mice in their drinking water was incorporated into DNA. These authors, however, could not demonstrate any mutagenic activity in a mouse dominant lethal assay from 5-chlorouracil and concluded that it does not constitute a significant mutagenic hazard to the human population. The formation of chlorinated nucleic acids *in vivo* as a consequence of drinking water exposure is speculative. 5-Chlorouracil has, however, been identified in chlorinated sewage effluents (Cumming, 1978).

Sodium hypochlorite was assayed for its DNA-damaging potential in the *E. coli polA* test. Preferential growth inhibition of the bacterial strain lacking the DNA repair polymerase was observed in a spot test of sodium hypochlorite (0.01 and 0.006 umoles/spot). This was indicative of the hypochlorite ability to interact with DNA. The author hypothesized that the hypochlorite attacked the pyrimidine 5-6 double bond to yield 6-hydroxy-5-chloro-5,6-dihydropyrimidine (Rosenkranz, 1973).

Calcium hypochlorite, likewise, produced chromosomal aberrations in CHL cells in the absence of exogenous mammalian metabolic enzymes (Ishidate et al., 1984).

Meier et al. (1985) evaluated the cytogenetic effect of oral administration of hypochlorite ion or hypochlorous acid to Swiss CD-1 mice. Test solutions were prepared from a stock of NaOCl by adjusting the pH; at pH 8.5 the predominant chlorine species was OCl⁻, and at pH 6.5 it was HOCl. Five males and five females (or four each for bone marrow aberration studies) were administered -1.6, 4.0 or 8.0 mg/kg/day. Dosing was either for 5 days with sacrifice 6 hours after the last treatment (both assays) or a single dose followed by sacrifice at 6, 24 or 48 hours postexposure (bone marrow aberrations only). None of the treatments resulted in significant increases in erythrocyte micronuclei or chromosome aberrations of bone marrow cells.

Reproductive and Developmental Effects

Animal studies in general have demonstrated no evidence of reproductive or teratogenic effects of chlorine. C3H/HEJ and C57B1/6J mice were administered drinking water which had been treated with sodium hypochlorite and hydrochloric acid (10-13 ppm) to maintain the water at pH 2.5 over a 6-month trial period (Les, 1968). Control animals received tap water, which varied in pH from 9.2-9.8, but was usually 9.6. In the treated animals, the number of mice born and the number weaned/dam were greater than in the control ($p < 0.01$). The authors concluded that the treatment of C3H/HEJ and C57BL/6J mice with chlorine and hydrochloric acid had no adverse effects on their reproductive performance.

McKinney et al. (1976) noted a periodic increase in reproductive failure among CD-1 mice. Mating, number of embryos per fertile female, and embryonic development were affected. The effect was seasonal and was most severe in the winter. In the absence of any other observed variations in the animal husbandry, the authors attributed the reproductive deficiencies to the heavily chlorinated Durham city water consumed by the mice.

Two attempts were made to repeat the observations reported by McKinney et al. (1976). Chernoff et al. (1979) found no significant difference in the reproductive parameters of CD-1 mice consuming Durham, NC drinking water as compared with the

control group maintained on distilled water. The animals were maintained on the test or control water for a 2-week period after which mating was begun. Dams were sacrificed on day 18 of gestation. Exposure was continued throughout the 8-month course of the study (December-September). Dams were analyzed for differences in numbers inseminated, number pregnant, weight gain during gestation, organ weight, and percent resorptions. Fetuses were examined for skeletal and visceral anomalies as well as mortality, and body weight. No statistically significant maternal or fetal effects were noted, except for a 28.1% incidence of supernumerary ribs in the tap water consuming group compared with 21.1% in the control group ($p < 0.05$).

In a second study, Staples et al. (1979) reported no significant overall influence on the incidence of malformed fetuses (skeletal or visceral malformations) that could be attributed to the chlorination of drinking water. The incidence of malformations in the controls was 8.1% compared with 7.8 in treated animals. Differences were in the opposite direction from the finding of McKinney et al. (1976). Two significant effects occurred in the month of January, and one occurred in the month of February. In January, a lower number of mated females CD-1 became pregnant, and the average number of implants/pregnant female was lower in the purified water group. In February, the average fetal weight was lower in the purified water group than in the tap water group. Indeed, the presence of chlorine in the water seemed to confer a beneficial effect. The authors concluded that the results of their study did not support the findings of McKinney et al. (1976), although there

were complicating factors in the Staples et al. (1979) study. There appears to be no evidence that would link the process of chlorination at levels consistent with current practice to any adverse reproductive effects in mammals.

Hulan and Proudfoot (1982) studied the effects of sodium hypochlorite in drinking water on Shaver broiler chickens. Sodium hypochlorite was added to the drinking water of chicks (240/sex) at doses of 0, 300, 600 and 1200 ppm. A significant ($p < 0.01$) reduction was found in the weight of chick testes at dose levels of 600 and 1200 ppm of available chlorine. However at these higher concentrations there was also a decrease in total body weight, food and water consumption and an increase in mortality.

Meier et al. (1985) demonstrated that oral administration of a sodium hypochlorite solution, but not hypochlorous acid, resulted in dose-related increases in the amount of sperm-head abnormalities in male B6C3F1 mice. Ten animals/group were given 1 mL of a free residual chlorine solution daily for 5 days. Test solutions were prepared bubbling Cl_2 into a 1 M solution of NaOH and adjusting the pH to either 8.5 (predominant species OCl^-) or 6.5 (predominant species HOCl). The solutions were diluted with distilled water to 200 mg/L, 100 mg/L and 40 mg/L chlorine equivalents (8.0, 4.0 or 1.6 mg/kg bw/day respectively). The mice were then sacrificed at 1, 3 or 5 weeks after the last dose was administered. In mice given OCl^- , significant increases in sperm-head abnormalities were observed only at the 3-week interval at doses of 1.6 and 4.0 mg/kg bw/day. These results

were reproduced in retrials of the experiment. HOCl administration at any dose was not associated with increases in sperm-head abnormalities.

Druckrey (1968) conducted a 7-generation study wherein rats were given drinking water chlorinated to a concentration of 100 mg free chlorine/L. Animals were mated repeatedly and continued to drink the test water throughout gestation and lactation. There was a total of 108 matings among the test group resulting in 80 litters for a total of 609 viable progeny. The average number of viable progeny/litter was 7.9 for test animals and 8.2 for controls. Mortality among neonates of test animals was -20% higher than that observed for controls. This was attributed to decreased milk consumption as a consequence of the chlorinated odor of the milk. Microphthalmia of one or both eyes was noted in 17 treated progeny. This finding was observed irregularly, and it was stated that this condition has been known to occur spontaneously in BDII rats. Mating of microphthalmic rats produced normal offspring, indicating that the condition did not arise from a stable germ cell mutation. Druckrey (1968) concluded that no reproductive effects could be attributed to chlorine ingestion in this study.

Pilot experiments have been performed to determine the possible teratogenic effects of disinfectants on the developing rat fetus (Abdel-Rahman et al., 1982a). Six virgin Sprague-Dawley rats were administered 0, 1, 10 or 100 mg HOCl/L in drinking water for 2.5 months prior to mating. Animals were maintained on the treated water after pregnancy

was confirmed (day 0) and killed on day 20. Maternal weight at time of death was not reported. Incidence of fetal anomalies associated with exposure to hypochlorous acid solutions were not found to be statistically significant. Mean fetal weight from the 10 and 100 mg/L groups were less than control, but this decrease was not statistically significant. Neither was there a significant difference in numbers of resorptions between control and treated groups. Examination of general trends in the study indicated an increase (not significant) in skeletal anomalies in animals treated with 10 mg HOCl/L. Soft tissue anomalies for the 100 mg HOCl/L treatment group were increased significantly by comparison with control. The findings of these experiments were limited by the small number of study animals. Some of the calculations of anomaly percentages reported in the paper were incorrect. Furthermore, the rate of both skeletal and soft tissue anomalies appeared to be higher in the control group than in the low-dose treatment groups.

Summary

The majority of research dealing with acute effects of chlorine exposure has been in the area of inhalation. Short-term exposure of rats by gavage to HOCl or Cl in aqueous solution (to ~250 mg/kg/day) has resulted in transient decreases in blood glutathione and hypothalamic norepinephrine and reversible morphological and biochemical liver changes. Exposure by gavage in milk to 200 mg/kg available chlorine/day for 14 days resulted in kidney enlargement in rats. No effects were observed in rats when as much as 902 mg Cl/kg/day was consumed *ad libitum* in milk.

Shaver broiler chicks were observed to have decreased body weights and organ weights (including testes) when maintained for 28 days on water with 1200 mg/L available chlorine. As there was also a dose-dependent decrease in water consumption by the birds, the effects noted may have been because of the unpalatability of the water. Longer term studies in rodents have shown mixed results as a consequence of consumption of chlorinated drinking water. A 7-generation study wherein BDII rats consumed 10 mg Cl/day in water produced no evidence of adverse effects. However, exposure of F344 rats for 2 years resulted in decreased body and organ weights. Additional studies in F344 rats and B6C3F1 mice observed no treatment-related adverse effects.

In these same studies, no clear evidence of increased treatment-related tumor incidence was reported for rats or mice. Increases in mononuclear cell leukemias in female rats and renal tubular cell neoplasms in male mice were reported. These increases were not considered dose or treatment related. There are conflicting data regarding cocarcinogenicity of sodium hypochlorite. Commercial sodium hypochlorite applied dermally to female ddN mice enhanced tumor development initiated by 4-nitroquinoline-1-oxide. By contrast sodium hypochlorite applied to the skin of female NMRI mice treated with benzo[a]pyrene reduced the tumor incidence. It should be noted that the data discussed here do not address the possible carcinogenicity associated with organic by-products formed during water chlorination.

Chlorine has been shown to produce chromosomal aberrations in mammalian cells. Sodium hypochlorite both damages DNA and causes base substitution mutations. Neither hypochlorite ion or hypochlorous acid, however, caused chromosomal aberrations.

No reproductive dysfunction was noted in a 7-generation rat study wherein chlorine was administered in drinking water. Hypochlorite ion, but not hypochlorous acid, produced dose-related increases in sperm-head abnormalities in male B6C3F1 mice.

VI. HEALTH EFFECTS IN HUMANS

Introduction

Studies of the relationship between chlorine in drinking water and human health effects are few. The majority of information about chlorine in regard to adverse health effects pertains to the inhalation of chlorine gas and is likely of questionable relevance to assessing exposures from ingestion of chlorine in water. Although these data are considered in an overview of chlorine, the findings presented here will principally address the results of exposure to chlorine, hypochlorous acid and hypochlorite ion in drinking water. Some of the more recent information in this chapter, particularly the clinical studies and analytical epidemiologic studies, was previously summarized in another technical report (unpublished) for the Office of Drinking Water (Craun et al., 1989) and is presented here in its original form.

There is no question that disinfection of drinking water with chlorine has clearly been a beneficial practice. Waterborne infectious disease has been brought under control, and while outbreaks still occur in the United States, they are of a different magnitude and nature than occurred before the widespread practice of disinfection was instituted (Craun, 1986). The current focus on the possible relationship of this practice to cancer and cardiovascular disease should be weighed against the clearly positive effects of disinfection.

Clinical Case Studies

A few early clinical case reports of the toxic effects of chlorine in drinking water have been documented (Muegge, 1956). Muegge reported that a group of 150 persons at a military base consumed water with chlorine levels of 50 ppm (~1.4 mg Cl/kg/day) during a period of water main disinfection. No adverse health effects were noted. Another incident of elevated chlorine levels in drinking water occurred during a flood where residents consumed water containing 50-90 ppm (~1.4 and 2.6 mg Cl/kg/day). Muegge noted that when chlorine levels exceeded 25 ppm, people refused to drink the water. In this same article, the author cited an incident in which a group of Army personnel drank water containing >90 ppm chlorine. This ingestion resulted in constriction of the throat and irritation of the membranes of the throat and mouth of those exposed. Muegge's overall conclusion was that human beings have a high tolerance to highly chlorinated water. It must be noted that this was only an anecdotal case report.

Lubbers et al. (1982) investigated the effects of oral ingestion of chlorine, chloramine, chlorine dioxide and chlorate administered in drinking water under two clinical study protocols. Study subjects were healthy male volunteers between 21 and 35 years of age. Phase I consisted of an increasing dose tolerance analysis in which progressive doses of chlorine were administered in water as chlorate, chlorine dioxide, chlorite, chlorine and chloramine to six groups: 10 subjects/compound, given every 3 days for a total of 18 days, at concentrations of 0.1, 1.0, 5.0, 10.1, 18.0 and 24.0 mg/L (0.001, 0.014, 0.071, 0.143, 0.257 and 0.343 mg/kg, respectively) in a total volume of 1000 mL. Phase II consisted of 60 subjects who ingested the various compounds listed above, in

six groups: 10 subjects/compound at daily concentrations of 5 mg/L in a volume of 500 mL of water for 12 consecutive weeks. In both phases, the sixth group consumed untreated water. Three participants who were deficient in glucose-6-phosphate dehydrogenase (G6PD) and considered to be theoretically more susceptible to oxidative stress were included as a separate group in the second phase. They were given daily 5 mg/L of sodium chlorite only in a volume of 500 mL for 12 consecutive weeks.

Sixty-eight biochemical and physiological parameters were measured in each participant at multiple time points for each phase in the study. The data were analyzed using analysis of variance parameters in the different exposure groups but no data were presented to indicate either the direction or magnitude of change. With so few people per experimental group and so many significance tests computed, it would be inappropriate to consider the changes to be a true effect of the exposures. The changes appeared to be randomly distributed among the participants and unrelated to any particular protocol. No findings were specific to the three subjects who were deficient in G6PD. It is unclear why the investigators would even attempt any formal statistical testing in a group comprised of only three individuals. No clinically significant changes in any variables were noted; all fluctuations were within normal range of measurement. Although the study was limited by the failure to control variables such as diet and other sources of drinking water, the fact that there were no overt adverse health effects suggests that ingestion of chlorine at these levels over a relatively short period of time produces no toxicity in healthy adult males. The most reasonable interpretation of the noted changes

is that it likely represents a combination of interindividual variability and random laboratory error.

In a recent series of controlled clinical studies, Wones et al. (1989, 1991) investigated the effects of chlorinated drinking water on human lipid metabolism. In the study 19 healthy males (24-62 years old) were given 0, 2, 5 or 10 ppm chlorine in their drinking water for a total of 15 weeks. Each subject was allowed to serve as his own control and was required to drink 1.5 L of water/day. All subjects were maintained on a high fat, high cholesterol diet throughout the study period and intake was weighed and measured daily. Total serum cholesterol, lipoproteins, apolipoproteins and thyroxine levels were monitored. Baseline levels were obtained during the first 4 weeks of the study in which subjects were given the protocol diet and chlorine-free water, after which chlorine was added to the drinking water at 2, 5 or 10 ppm for a period of 4 weeks each. A 3% increase in total serum cholesterol occurred during the treatment period and was statistically significant ($p < 0.05$) for the 5 and 10 ppm dose groups. Total thyroxine levels were statistically significantly ($p < 0.05$) increased above baseline at all doses throughout the study. However, these changes were numerically small, not uniform in all subjects and were not clinically significant. Because there was no separate comparison group that did not receive chlorine, it is possible that the small observed increases were due to the protocol diet or other uncontrolled factors and not to the chlorine.

To correct for these methodologic deficiencies, a second study was conducted to determine if drinking water containing 20 ppm chlorine affected lipid or thyroid metabolism

in healthy women unselected for baseline total cholesterol levels or in healthy men selected to have baseline total cholesterol levels above the 50th percentile for their age (Wones et al., 1991). Because the impact of drinking water chlorine on lipid metabolism was unimpressive in the first study (Wones et al., 1989), the second study was restricted to men with baseline cholesterol levels above the 50th percentile in order to determine whether elevated baseline cholesterol might be associated with an increased responsiveness to chlorinated drinking water. The protocol consisted of a 4-week dietary stabilization period during which all subjects drank distilled water followed by a 4-week treatment period during which half of the subjects were assigned randomly to continue consuming distilled water while the other half were assigned to consume 1.5 L/day of drinking water containing 20 ppm chlorine. There were 15 men and 15 women in each group. There were no differences between the chlorine and distilled water groups in any of the lipid or thyroid parameters. No subgroups (age, race, baseline total cholesterol, source of pre-study drinking water) were differentially affected by exposure to chlorine. These findings do not support the concern for chlorine's effect on lipid and/or thyroid metabolism that was raised in response to the animal studies reviewed in Chapter v.

Acute exposure to chlorine has occurred through the ingestion of household bleach. This occurs most commonly in children, and the bleach usually consists of 3-6% solutions of sodium hypochlorite in water with pH values averaging ~11.0. The typical amount of bleach ingested by a child has been estimated to be ~4-5 mL. Intake of this small amount of bleach generally results in irritation of the oropharynx and esophagus, a burning sensation in the mouth and throat, spontaneous emesis, and in rare instances,

permanent injury to the esophagus with perforation or stricture formation dependent upon the pH of the solution (Mack, 1983).

Strange et al. (1951) reported a case in which a 49-year-old male ingested a quart of liquid bleach containing roughly 5% free available chlorine in the form of sodium hypochlorite (~6557 mg/kg). Injury to the stomach eventually necessitated a total gastrectomy. The individual's esophagus appeared to be healthy.

Ingestion of a few teaspoons of bleach proved fatal for an 18-month-old girl (Done, 1961). The bleach solution was apparently aspirated into the trachea where it caused acute tracheobronchitis.

The list of reported adverse health effects associated with chlorine gas exposure ranges from bronchitis, asthma and pulmonary edema to headaches, meningitis and heart disease. Not all of these findings have been confirmed in more than one report (NRC, 1976; WHO, 1982).

Shortly after World War I, it was believed that chlorine gas in small amounts decreased the incidence of respiratory diseases among exposed workers. Small amounts of chlorine gas were also used to treat the common cold, influenza and bronchitis (NRC, 1976).

TABLE VI-1

Threshold Levels for Chlorine Gas Inhalation Effects*

Effect	Cl Threshold Levels	
	mg/m ³	ppm
Odor perception/irritation	0.06-5.8	0.02-2.0
Perceivable sensory irritation	2.9	1.0
Intolerable sensory irritation	11.6	4.0
Chronaxie/visual adaptation changes	1.5	0.52
Pronounced dyspnea, anxiety, vomiting, cyanosis, pulmonary edema	87.0-116.0	30.0-40.0

*Source: WHO, 1982

solution (Chlorox), containing 5.25% sodium hypochlorite and 5% available chlorine, were added to the dialysis bath. It was estimated that ~30 mL (~30 mg/kg) of sodium hypochlorite solution crossed the dialysis membrane and entered the patient's circulating volume. The patient experienced massive hemolysis, hyperkalemia, cyanosis and cardiopulmonary arrest. Serum electrolytes drawn before and after the sodium hypochlorite exposure indicated a sudden rise and fall in serum sodium, potassium and chloride concentrations. Blood count, arterial pH and prothrombin time did not change. The patient recovered in 1 week.

Epidemiologic Studies

General Considerations. Since the early 1970s, a number of epidemiologic studies have attempted to assess the relationship between drinking water quality and cancer. These studies differ markedly in both their objectives and design which in turn limits specific inferences to be drawn from their findings. The studies have evolved from general descriptions of disease rates in various geographic areas with different drinking water sources and presumed contaminants to well-designed interview case-control studies with incident cancer cases (Murphy and Craun, 1989).

These studies were never intended to assess whether chlorine itself is responsible for adverse health effects but rather were designed to test the hypothesis that trihalomethanes or other organic compounds occurring in drinking water as a result of chlorination are associated with an increased risk of gastrointestinal and urinary cancer. To date, chlorine itself has not been found to be carcinogenic in animals.

Exposure measures utilized in these studies have been quite variable and are determined by both study design and availability of information. Ecologic studies have used information that is available for larger geographic areas such as proportion of a population served by different water sources using different treatment practices. Case-control death certificate studies are limited to the information that is recorded on the death certificate. Place of usual residence as recorded has been used as a surrogate variable for presumed exposure to different sources and types of drinking water. Rarely will there be sufficient information available to assess and control for confounding with these study designs. This is particularly important when considering the relationship of low-level, long-term exposure to chronic diseases with long latent periods. Inadequate control of confounding and exposure misclassification may obscure any small elevations in risk that truly exist. Many of these limitations, however, do not exist in the most recent studies of this problem, although there will always be some methodologic problems to consider in assessing the final reported results (Craun, 1985; Murphy and Craun, 1989).

With these limitations in mind, the following is an overview of the major studies that have assessed the relationship between exposure to chlorinated drinking water and several site-specific cancers.

Ecological and Retrospective Studies. The earliest studies of this problem were ecologic and used a variety of cancer endpoints and surrogate exposure variables. The detailed results have been summarized and critiqued by many others (Crump and Guess,

1982; Shy, 1985; Craun, 1985; Craun et al., 1989; Murphy and Craun, 1989) and will not be presented in this document. Although there are clear limitations to the interpretation of ecologic studies (Morgenstern, 1982), the studies were instrumental in raising the issue of a possible hazard associated with a long-accepted public health practice (Craun, 1988) and provided the impetus for further, better designed studies to address this potential problem.

The earliest case-control studies of the association between chlorinated drinking water and cancer involved decedent cancer cases and had only limited information on individual risk factors and exposures. Odds ratios derived from these data range from 1.13 (Kanarek and Young, 1982) to 1.93 (Alavanja et al., 1978) for rectal cancer, 1.05 (Gottlieb et al., 1981) to 1.61 (Alavanja et al., 1978) for colon cancer and 1.04 (Kanarek and Young, 1982) to 1.69 (Alavanja et al., 1978) for bladder cancer. These relative risk estimates, in light of differences in study populations, exposure variables, etc. are not strong enough to discount the effects of possible confounding factors; neither do the studies have sufficient statistical power to rule out a relationship between chlorine and its dissociation products and adverse health effects.

More recent case-control studies have used improved study designs to further examine the relationship between exposure to chlorinated drinking water and colon and bladder cancers (Cragle et al., 1985; Cantor et al., 1985, 1987, 1989; Zierler et al., 1986, 1988; Young et al., 1987, 1989).

Cragle et al. (1985) investigated the relationship between water chlorination and colon cancer using 200 incident cases of colon cancer from seven hospitals in North Carolina and 407 hospital-based comparison subjects without evidence of cancer and no history of familial polyposis, ulcerative colitis, adenomatous polyposis, or any other major chronic intestinal disorder. Both cases and comparison subjects were required to be residents of the state for at least 10 years to be included in the study. Comparison subjects were matched on age, race, gender, vital status and hospital to prevent potential confounding by these characteristics. Additional information on potential confounders, including alcohol consumption, genetic risk (number of first-degree relatives with cancer), diet, geographic region, urbanicity, education and number of pregnancies, was obtained by either mailed questionnaire or telephone interview. These characteristics were assessed and statistically controlled in the analysis. Approximately 71% of the eligible population was included in the study. Water exposures were verified for each address and categorized as chlorinated and unchlorinated for the analysis. Logistic regression analysis showed genetic risk, a product term between alcohol consumption and high fat diet, and an interaction term between age and chlorination to be positively associated with colon cancer. The association between chlorinated water and colon cancer was found to be highly dependent upon age. Rate ratios for persons who drank chlorinated water at their residence for ≥ 16 years were consistently higher than those exposed to chlorinated water < 16 years, but a statistically significant association between water chlorination and colon cancer, controlling for possible confounders, was found only for those above age 60. For example, 70-79 year olds who drank chlorinated water for ≥ 16 years had twice (RR=2.15) the risk of colon cancer compared with 70-79 year olds who

drank unchlorinated water; in the same age group the risk of colon cancer was about 50% (RR=1.47) higher in those who drank chlorinated water for <16 years compared with those who drank unchlorinated water.

Young et al. (1981) in an earlier case-comparison mortality study had reported an association between colon cancer mortality in Wisconsin and exposure to chlorinated drinking water estimated by the average daily chlorine dosage 20 years past. The study included 8029 cancer deaths and 8029 noncancer deaths in white females matched on county of residence, year of death, and age. Death certificates provided information on urbanicity, marital status and occupation, and these were considered as potential confounders. This association was further pursued in an interview study of 347 incident cases of colon cancer, 611 population-based comparison subjects, and 639 comparison subjects with cancer of other sites (Young et al., 1987; Kanarek and Young, 1989). Lifetime residential and water source histories and information on water-drinking habits, diet, sociodemographics, medical and occupational histories, lifestyle and other factors were obtained by questionnaire. Data on past THM levels in drinking water were estimated using a predictive statistical model based on current, quantitative THM levels and routinely recorded operating data for Wisconsin water supplies. Multivariate analyses, controlling for variation in water consumption, population size and other factors, were used to estimate the colon cancer risk for various drinking water related factors. While this case-comparison study associated colon cancer incidence with use of chlorinated drinking water for 0-10 years prior to diagnosis, no increased risks were observed between colon cancer incidence and use of chlorinated drinking water for >10

years prior to diagnosis nor were estimated THM exposure in Wisconsin associated with colon cancer incidence. However, current THM levels in these water supplies were generally low with 98% of samples <100 µg/L. Colon cancer cases were also found to more frequently consume water from municipal groundwater supplies, which are more likely to be chlorinated than not chlorinated. Since groundwaters in the United States have also been shown to be contaminated with synthetic volatile organic compounds, the possibility of other water exposure was considered. A study that considered exposures of this population to organic contaminants in groundwater showed higher estimated relative risks (RR=1.7-2.4) of colon cancer incidence in populations exposed to tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane in municipal groundwaters (Kanarek and Young, 1989).

Cantor et al. (1985) reported results from a collaborative Environmental Protection Agency (EPA)-National Cancer Institute (NCI) study of the association between water chlorination and bladder cancer. Included were a total of 2982 persons (73% of those eligible to participate) between the ages of 21 and 84 diagnosed with cancer of the urinary bladder in 1978 and residing in 10 areas of the United States (Connecticut, Iowa, New Jersey, New Mexico, Utah, and the metropolitan areas of Atlanta, Detroit, New Orleans, San Francisco, and Seattle) and 5782 population-based comparison subjects, randomly selected and frequency matched on gender, age and study area. Subjects were interviewed at home by a trained interviewer, and data were collected for a number of possible confounders including smoking, occupation, artificial sweetener use, coffee and tea consumption, and use of hair dyes. A complete residence history was obtained

to categorize individuals according to water sources and chlorination status on a year-by-year basis, and information was obtained on use of bottled water and fluid consumption. Of the 587,568 person-years lived by all residents since 1940, 76% were at a known water source. Logistic regression analysis was used to control for potential confounders. Among persons in all study areas combined, risk was not elevated in those respondents living in areas with chlorinated water supplies for 20, 20-39, 40-59 and 60 or more years. However, it should be noted that this study was originally designed to determine if saccharin was a human carcinogen rather than to determine cancer risks associated with water chlorination. The study areas were not selected to provide the optimal variability of water sources and treatment, and the statistical power of the study is less than suggested by the large number of individuals studied, as the five metropolitan areas were served primarily by chlorinated water supplies. Among the 10 study areas, participants from the three states with agricultural land use did show evidence of increased risk for bladder cancer with the number of years at a surface source, but the number of participants in these areas was small compared with the other areas. It is curious that another analysis of these same data (Cantor, 1987) reported this trend to be in the other nonagricultural areas. Among nonsmokers who were never employed in a high risk occupation (a group otherwise at low risk for bladder cancer) the risk was elevated among those served by chlorinated surface sources with some evidence of a duration of exposure-response relationship. However, only in nonsmokers who resided 60 or more years at a residence served by chlorinated water was the risk statistically significant; in this instance the risk of bladder cancer was more than double (RR=2.3) the risk among nonsmokers who resided in areas served by unchlorinated water.

Previous studies have reported an increased risk of bladder cancer associated with a high total fluid intake but this has not been thoroughly studied. A high volume of total fluid may affect the bladder by increasing its work load or the types of fluid consumed may contain constituents that are either carcinogenic or protective. Cantor et al. (1987, 1990) has recently reported a further analysis of the EPA-NCI national bladder cancer study according to beverage intake level and type of water source/treatment. Among the white participants, complete information on beverage consumption and cigarette smoking was available for 5793 males and 1983 females. Risk of bladder cancer was reported to be associated primarily with the tap water component and to increase with greater consumption of tap water. After correcting for age, smoking and other potential confounding characteristics, it was observed that people who reported drinking the most chlorinated tap water had a bladder cancer risk about 43% higher (RR=1.43) than people who drank the least. When tap water consumption was analyzed separately for males and females, however, only among males was the association between water ingestion and bladder cancer risk statistically significant.

Evaluation of bladder cancer risk by the combined effects of duration of chlorinated surface water use and tap water intake showed that only among the study participants who drank chlorinated surface water for 40 or more years did the bladder cancer risk increase with the higher tap water consumption. A risk gradient with water consumption was not found among consumers of chlorinated surface water for <40 years or among long-term consumers of unchlorinated groundwater. Duration of exposure to chlorinated surface water was associated with bladder cancer risk among women whose tap water

consumption was above the median. However, the rate ratios were statistically significant in only one category, females who had resided 60 or more years at a residence served by chlorinated surface water and whose tap water consumption was above the median. Evaluation of risk by smoking status revealed that most of the duration effect was seen in nonsmokers. Among nonsmokers who consumed tap water in amounts above the median, a risk gradient was apparent only for males. However, a high risk was also seen for nonsmoking females who consumed less than the median. It is difficult to accept these findings as indicative of an association between tap water consumption and bladder cancer because of the random variation found when the results are analyzed separately by gender; the lack of statistical significance in all but a few categories of water consumption; and inconsistent high risks among nonsmoking males who consumed more water than the median and females who consumed less water than the median. In addition, recently reported results from a population-based incident case-consumption study in Utah (Slattery, 1988) found neither total fluid intake nor tap water consumption to be related to bladder cancer after adjustment for cigarette smoking, age, gender, history of diabetes and bladder infections.

Because chloramination has been shown to produce very low levels of THMs, epidemiologic studies were conducted in Massachusetts where chlorine and ammonia have been used since 1938 to disinfect surface water provided to most communities in the Boston metropolitan area. A recently completed mortality study (Zierler et al., 1986) showed a slight increase in bladder cancer in populations in Massachusetts receiving chlorinated surface water compared with populations receiving chloraminated surface

water. To address problems in interpretation of the results of this study, a case-comparison study of 614 individuals who had died of primary bladder cancer and 1074 individuals who had died of other causes was also conducted (Zierler et al., 1988). Confounding by age, gender, smoking, occupation, and socio-economic status was controlled by multiple logistic regression.

The largest water utility in the state, the Massachusetts Water Resources Authority (MWRA), provides water to Boston and to more than 25 additional towns and cities. The water is of high quality because of restricted access to much of the watershed. Chloramine disinfection began in 1938, and only recently have chemicals been added for fluoridation and pH adjustment. The Authority has provided chloraminated water directly to some 20 cities and towns; an additional 10 cities and towns have purchased untreated water from the MWRA or use the MWRA water sources and provide chlorine for disinfection. Other communities using surface water sources of similar quality with chlorination were also included in the studies. Exposure was defined according to duration of residence in communities using chlorine or chloramine disinfection. Individuals who resided from 1938 until the year of diagnosis exclusively in communities supplying chlorinated drinking water were classified as having lifetime exposure; individuals residing exclusively since 1938 in communities with chloraminated drinking water were classified as having no exposure to chlorinated water and lifetime exposure to chloraminated water. Because all of the subjects died between 1978 and 1984, lifetime use means that only one type of disinfectant was used in the residential water supply 40-46 years before death. All individuals not meeting this definition for lifetime

exposure were included in a separate analysis considering "usual" exposure to either disinfectant. Also analyzed separately using the definition for lifetime exposure were data among the subset of residents in communities using only MWRA water, which is obtained from a common source and is disinfected with either chlorine or chloramine by different communities.

A positive association was detected between both usual and lifetime chlorinated drinking water exposure classification and bladder cancer mortality. The bladder cancer association was highest for lifetime residents of chlorinated drinking water communities relative to lifetime residents of chloraminated drinking water communities. Using lymphatic cancers as the comparison group, the risk of bladder cancer mortality among lifetime consumers of chlorinated water was almost three times ($RR=2.7$) the risk of bladder cancer mortality among lifetime consumers of chloraminated drinking water; among usual consumers the risk was doubled ($RR=2.0$). A slightly higher risk ($RR=3.5$) was observed when the subset of lifetime residents using MWRA water was analyzed. When all deaths were used for comparison, the bladder cancer mortality risks were higher than was observed when only lymphoma deaths were used for comparison. The rationale for the lymphoma comparison resulted from consideration of possible associations between disinfected water and other diseases in the comparison group whereas there are no data to suggest that lymphoma may be associated with disinfected water. Because the observed bladder cancer risks were consistently lower when the analysis included all deaths, it is possible that one or more of the causes of death in the comparison group are related to water chlorination and resulted in the observation of this lower risk. More

careful consideration should be given to the proper selection of a comparison group for future epidemiology studies of water contaminants. For example, use of a comparison group that includes cardiovascular deaths could dilute the observed magnitude of effect if cardiovascular disease is associated with chlorinated water.

Although most of the studies reviewed here have looked at colorectal or bladder cancer risk, one recently published work investigated the risk of pancreatic cancer in relation to presumed exposure to chlorinated drinking water. IJsselmuiden et al. (1992) conducted a population-based case-control study in Washington County, Maryland, using the same population data that was originally ascertained during a private population census for an earlier cohort study (Wilkins and Comstock, 1981). The cohort study did not find any association between pancreatic cancer and chlorinated drinking water (OR=0.80, 95% CI=0.44-1.52).

The case-control study was conducted to reexamine chlorinated drinking water as a possible independent risk factor for pancreatic cancer in this population. Cases were residents reported to the County cancer registry with a first time pancreatic cancer diagnosis during the period July 1975 through December 1989, and who had been enumerated in the 1975 census (101 cases). Controls were randomly selected by computer from the 1975 census population (n=206). Drinking water source, as obtained during the 1975 census, was the exposure variable used. In univariate analyses, municipal water as a source of drinking water, increasing age, and not being employed were significantly associated with increased risk of pancreatic cancer. Multivariable

analyses that controlled for confounding variables indicated that the use of municipal chlorinated water at home was associated with a significant odds ratio of 2.23 (95% CI=1.24-4.10).

A clear interpretation of these findings is hampered by several problems regarding the assessment of exposure, including the fact that information obtained in 1975 on type of water and other variables is really cross-sectional and may not truly reflect actual exposure patterns, and there is no information on the actual amounts of water consumed. Additionally, different residential criteria were used for the cases and controls. The cases had to still be residing in the County at the time of their cancer diagnosis to be included in the study, but the controls may not have been current residents. If controls emigrated out of the county differentially on the basis of exposure, the odds ratio may be an over- or underestimate of the true value. Finally, it can not be ruled out that the exposure variable used for this and other studies — residence served by a particular water source — is simply a surrogate for some other unidentified factor associated with nonrural living. The nonspecific relationship of several different causes of death and water source at home observed in the earlier cohort study (Wilkins and Comstock, 1981) lends some support to this possibility. Better studies with more valid individual exposure information are needed to confirm or refute the findings of this and other studies.

Morris et al. (1992) conducted a meta-analysis of 11 epidemiologic studies of cancer and presumed exposure to chlorinated water and its byproducts. The authors reported a combined relative risk estimate of 1.21 (95% CI, 1.09-1.34) for bladder cancer and 1.38

(95% CI, 1.01-1.87) for rectal cancer. Odds ratios for 10 other site-specific cancers including colon and pancreas were reported to be not significantly elevated. This attempt to quantitatively summarize the available epidemiologic studies is hindered by the previously discussed methodologic problems in the individual studies, particularly inadequate control of important covariates and a lack of individual exposure estimates. The individual studies have been considered to be inconclusive primarily because of these resolved methodologic problems, not because of a lack of statistical significance due to inadequate sample sizes. Additionally, the extreme variability in the composition of drinking water over time and across geographic areas means it is unlikely that the exposures across all these studies are really the same and therefore raises a serious question as to whether a statistical meta-analysis of this body of data is a valid exercise.

The use of meta-analytic techniques in the area of environmental epidemiology requires a great deal of careful consideration particularly with regard to the problems of exposure assessment and variability. A major problem in the application of meta-analysis is deciding when a collection of studies are indeed combinable. The decision to combine the water chlorination studies appears to have been based more on statistical rather than logical grounds. Instead of encouraging additional studies designed to resolve some of these important exposure assessment and other methodologic issues, the study of Morris et al. (1992) may have the unintended opposite effect. Further high quality epidemiologic research of this issue may be stifled because of the authors' erroneous interpretation that a clear and significant association between cancer and consumption of chlorinated drinking water has been demonstrated by the meta-analysis.

Cross-Sectional Studies. Results have recently been reported for a cross-sectional epidemiologic study of 1520 adult residents, aged 40-70 years, in 46 Wisconsin communities supplied with chlorinated and unchlorinated drinking water of varying hardness (Zeighami et al., 1989, 1990). This study was designed to determine whether differences in calcium or magnesium intake from water and food and chlorination of drinking water affect serum lipids.

The study communities from central Wisconsin: 1) were small in population size (300-4000) and not suburbs of larger communities; 2) had not undergone more than a 20% change in population between 1970 and 1980; 3) had been in existence for at least 50 years; 4) obtained water from groundwater sources with no major changes in water supply characteristics since 1980 and did not artificially soften water. The water for the communities contained total hardness of either ≥ 80 mg/L or ≥ 200 mg/L CaCO_3 ; 24 communities used chlorine for disinfection and 22 communities did not disinfect. Eligible residents were identified through state driver's license tapes and contacted by telephone; an age-gender stratified sampling technique was used to choose a single participant from each eligible household. Only persons residing in the community for at least the previous 10 years were included and participants were required to spend at least 9 months of each year in the community. A questionnaire was administered to each participant to obtain data on occupation, health history, medications, dietary history, water use, water supply, and other basic demographic information. Water samples were collected from a selected subset of homes and analyzed for chlorine residual, pH, calcium, magnesium, lead,

cadmium, and sodium. Specimens of fasting blood were collected from each participant and analyzed for total cholesterol, triglycerides, high density and low density lipoproteins.

Among females, serum cholesterol levels were found to be significantly higher in chlorinated communities than in nonchlorinated communities. Serum cholesterol levels were also higher for males in chlorinated communities, on the average, but the differences were smaller and not statistically significant. LDL cholesterol levels followed a similar pattern to that for total serum cholesterol levels, higher in chlorinated communities for females, but not different for males. However, for both sexes, HDL cholesterol levels are nearly identical in chlorinated and nonchlorinated communities and there were no significant differences found in the HDL/LDL ratios. The implications of these findings for cardiovascular disease risk are unclear at this time given the inconsistencies in the data. The possibility exists that the observed association in females may have resulted from some unknown or undetermined variable in the chlorinated communities.

The results from a second study, designed to further explore the findings among female participants in the Wisconsin study (Riley et al., 1992, manuscript submitted for publication). Participants were 2070 white females, aged 65-93 years who were enrolled in the Study of Osteoporotic Fractures (University of Pittsburgh Center) and had completed baseline questionnaires on various demographic and lifestyle factors. Total serum cholesterol was determined for all participants. Full lipid profiles (total cholesterol, triglycerides, LDL, total HDL-2, HDL-3, Apo-A-I, and Apo-B), were available from fasting

blood samples for a subset of 821 women. Interviews conducted in 1990 ascertained residential histories and type of water source used back to 1950 and all reported public water sources were contacted for verification of disinfectant practices. Private water sources were presumed to be nonchlorinated. A total of 1896 women reported current use of public, chlorinated water, 201 reported current use of nonchlorinated springs, cisterns or wells, and 35 reported having mixed sources of water. Most of the women had been living in the same home with the same water service for at least 30 years.

Overall, there were no meaningful differences detected in any of the measured serum lipid levels between women currently exposed to nonchlorinated water and those exposed to chlorinated water (246 mg/dL vs. 247 mg/dL, respectively, for total cholesterol). The data were also stratified by age and person-years of exposure to chlorinated water at home. There was some suggestion that women with no exposure to chlorine had lower total cholesterol levels but this was very inconsistent. This finding may be spurious since there was no trend noted with LDL cholesterol or Apo-B, both of which are known to correlate with total cholesterol. There was also no association between increasing duration of exposure to chlorine and HDL cholesterol, Apo-A-I, or triglycerides.

The only notable differences were that women with chlorinated water reported significantly more cigarette and alcohol consumption than the women with nonchlorinated drinking water (Riley et al., 1992). This was evident in all age groups and across strata of duration of exposure. This finding leads support to the possibility that the previously reported association of chlorinated drinking water and elevated total serum cholesterol

(Zeighami et al., 1990) may have arisen due to incompletely controlled lifestyle factors which were differentially distributed across chlorination exposure groups.

High Risk Subpopulations

At present, there are no definitive studies pointing to specific high risk populations. The information presented in this section consists of a few clinical cases of asthma linked with chlorinated water, a question of a possible risk group of persons deficient in G6PD and a postulated risk to those persons who are already at risk of cardiovascular disease.

Reports of the precipitation of asthma attacks as a result of exposure to chlorinated water have been published (Watson and Kibler, 1933; Sheldon and Lovell, 1949; Cohen, 1933). Severe asthma attacks in a 53-year-old female subsided when iodized salt was removed from her diet, though milder symptoms persisted until chlorinated drinking water was replaced by distilled or spring water. Municipal water supplies in this case contained 0.2-0.4 ppm chlorine. Further investigations revealed that asthmatic symptoms returned within 8-10 hours after the subject had ingested municipal water, distilled water or spring water to which sodium hypochlorite had been added. The subject experienced asthmatic attacks upon a few hours of exposure to air contaminated with chlorinated (4-6 ppm load) swimming pool water. Asthma attacks were also precipitated by the subject's weekly washing with a chlorine rinse (NRC, 1976; Sheldon and Lovell, 1949). It must be noted that this report and others describing a link with asthma are anecdotal case reports published over 35 years ago. The lack of subsequent reports in the literature may be an indication that these cases may have been erroneously attributed to chlorine exposure.

In the study of male college student volunteers by Lubbers et al. (1982), three participants were noted to have G6PD deficiency. These three were treated as a separate group for treatment and analysis because, theoretically, they were believed to be more susceptible to oxidative stress. Although no adverse effects were noted among these three individuals, a larger sample size is needed to properly address this question.

Another possible high risk group are those persons already at risk of cardiovascular disease. As discussed in Chapter V of this document, Revis et al. (1985) showed an increase in cholesterol levels as well as atherosclerotic lesions in pigeons receiving a calcium-deficient diet after exposure to chlorine in water. However, these findings have not been reproduced in either animals or humans. The recent study of Wones et al. (1992), designed to investigate a group at higher risk for cardiovascular disease, does not support the earlier findings of a chlorine-induced elevation of either total serum cholesterol or the specific cholesterol subfractions, so it is very questionable as to whether this is truly a high risk subgroup.

The results from the EPA-NCI bladder cancer study (Cantor, 1985, 1987) indicated that if there is indeed a causal relationship of long-term consumption of chlorinated surface with bladder cancer, it may only be occurring in nonsmokers with no known occupational risks, a group considered to be at otherwise low risk for development of bladder cancer. This finding should be followed-up in a study specifically designed to address this risk in a large sample of nonsmokers, as very little is otherwise known about

bladder cancer risks that are independent of cigarette smoking status (Kabat, 1986; Slattery, 1988).

Summary

At this time, there are no known long-term adverse human health effects from exposure to chlorine, hypochlorous acid or hypochlorite ion in drinking water. The majority of literature on the adverse health effects of chlorine deals with the inhalation of chlorine gas. Acute exposure to chlorine gas produces pulmonary congestion, respiratory failure, pulmonary edema and bronchopneumonia. If a person survives the initial exposure, however, symptomatic treatment leads to rapid and complete recovery.

Consumption of heavily chlorinated drinking water (>90 ppm) produces constriction of the throat and irritation of the membranes of the throat and mouth. Levels of 25 ppm chlorine in drinking water make the water unpalatable. Ingestion of household bleach (pH=11) produces irritation of the oropharynx and esophagus. Cases of dermatitis have been linked with the use of sodium hypochlorite disinfectants.

Epidemiologic studies that address the association between chlorinated drinking water supplies and cancer are of limited use in defining adverse health effects of chlorine, hypochlorous acid and hypochlorite ion primarily because of differences in study objectives and relative nonspecificity regarding exposures from disinfected drinking water. The most sophisticated of these studies have attempted to assess whether there are adverse health effects associated with by-products created during the disinfection process

and were not designed to specifically look at the disinfectant, i.e., chlorine, itself as a potential etiologic agent in human disease.

The study of Cantor et al. (1987) has created a lot of concern regarding the risk of bladder cancer in long-term consumers of chlorinated drinking water. It must not be forgotten that this is the first study to date that has analyzed water consumption data in incident bladder cancer cases and population-based controls. Because it stands alone at this time, substantial confirmation will be required before any interpretation regarding causality can be made (Deresa et al., 1990). The results of Zeighami et al. (1989, 1990) must be viewed in a similar light.

With the paucity of data pointing to the adverse health effects of chlorine, hypochlorous acid and hypochlorite ion in drinking water, high risk subpopulations are very questionably defined based on old case reports, animal studies and theory. They include asthmatics, persons deficient in G6PD and possibly persons already at risk of cardiovascular disease.

VII. MECHANISMS OF TOXICITY

Chlorine is a highly reactive element that readily combines with a variety of organic compounds and radicals. This property makes it difficult to characterize the toxic mechanisms of chlorine, hypochlorous acid and hypochlorite ion in mammalian organ systems. Thus, much of the information on the mechanism of toxicity has been hypothesized from studies with microorganisms and *in vitro* experimentation with macromolecules. Only a limited amount of research has been performed with mammals to delineate further the mechanisms of toxicity of these compounds.

The mechanism of action for the bactericidal properties of chlorine, hypochlorous acid and hypochlorites has not been clearly defined. Several theories have suggested that an interaction occurs between chlorine and proteins in the cell membrane, which interferes with normal cell metabolism and results in the destruction of the cell wall (Dychdala, 1977).

Cell wall damage may be the most immediate effect of free available chlorine upon bacteria. Studies by Friberg (1957) demonstrated that small amounts of chlorine were effective in disrupting bacterial wall permeability, which led to leakage of the marker, P32, from nucleoproteins of the bacterial cell. Similar disruptions of the cellular membrane may explain the acute edematogenic and corrosive effects of chlorine on exposed surfaces in mammalian tissues.

In addition to cell wall lesions, key enzymatic reactions have been shown to be inhibited in bacteria exposed to chlorine. Enzymatic reactions involving carbohydrate metabolism have received the most attention. Green and Stumpf (1946) postulated that chlorine irreversibly inhibits the metabolism of glucose in bacteria at the point of oxidation of triosephosphoric acid to phosphoglyceric acid. Knox et al. (1948) suggested that the inhibition of carbohydrate metabolism was a result of chlorine reaction with sulfhydryl groups of enzymes. This hypothesis has been challenged by more recent experiments with viruses. In these studies it has been shown that compounds that are reactive toward sulfhydryl groups are not always lethal. It appears that the inactivation of RNA may be more critical to the viricidal action of chlorine than is inhibition of sulfhydryl-dependent enzymes (NRC, 1976).

Further evidence that chlorine may act through its toxic effects on enzymes or other cellular proteins was provided by Baker (1947). In experiments with egg albumin, the addition of sodium hypochlorite resulted in the denaturation of protein and the formation of N-chloro-groups. Pereira et al. (1973) demonstrated that by a process of oxidative decarboxylation, hypochlorous acid converts several amino acids into a mixture of corresponding nitriles and aldehydes. Hypochlorous acid also chlorinated the ring of tyrosine and reacted with cysteine yielding cystine and cysteic acid.

Hypochlorous acid is also able to interact with the nucleotide bases of RNA and DNA. Patton et al. (1972) demonstrated that hypochlorous acid reacts with cytosine to

form γ -N-chloro derivatives, di-, tri- and tetrachlorocytosine, depending on the hypochlorous acid:cytosine ratio. Hoyano et al. (1973) found that aqueous hypochlorous acid interacted with purine and pyrimidine bases forming both labile intermediates and stable end products. Dennis et al. (1978) reported that uracil reacted with an excess of hypochlorous acid and formed trichloroacetic acid, carbon dioxide and nitrogen trichloride as the break-down products. This interaction of hypochlorous acid with nucleotide bases led the authors to believe that active chlorine might modify or destroy cellular DNA. This could result in direct death of the cell, or initiation of a mutagenic event.

Other research with rodents has indicated that the components of the plasma membrane may be a site of toxic effects of chlorine. Vogt et al. (1979) discussed *in vitro* experiments where decreases in membrane-bound ($\text{Na}^+ + \text{K}^+$) -ATPase activity occurred in rat cells after application of increasing concentrations of hypochlorite. Alterations in membrane structure and function were also noted by Sun et al. (1980) when *in vitro* preparations of mitochondria from rat liver were exposed to various concentrations of chlorine. Although mild chlorine treatment (0.9-1.8 ppm) did not result in changes of protein profiles on SDS gel electrophoresis, mitochondria were observed to lose respiratory control. At chlorine concentrations of 4-8 ppm, changes were found in the protein profiles and the ADP-coupling mechanism in the oxidative phosphorylation was lost. At the highest levels (40-80 ppm) chlorine caused the loss of membrane proteins and lipids and the inhibition of mitochondrial respiratory activity. At the lower concentrations, chlorine was thought to cause changes in the microenvironment of the mitochondrial enzymes, which were part

of the ADP-coupling mechanism. The higher chlorine levels induced more severe degradation of the membrane structure and function.

Disturbances in the enzymatic processes in liver membranes were hypothesized to have caused liver changes that occurred in rats after acute administration of chlorinated water (Chang et al., 1981). Rats were sacrificed 2, 5 or 10 days after a single intragastric dose (5 mL) of sodium hypochlorite water containing 1% free residual chlorine; that is, 50 mg of residual chlorine was given to each animal. Morphological and biological changes occurred largely at the 2-day interval, and recovery was complete within 10 days. Liver triacylglycerols increased and the acyl group composition of triacylglycerols and phospholipids was altered in liver mitochondria and whole homogenate. Long-chain polyunsaturated fatty acid levels increased. These effects suggested that the chlorinated compounds had altered the lipoprotein secretion system and membrane transport function.

Vogt et al. (1982) determined that the synaptic membrane in the hypothalamus of rats was a site of effects occurring after administration of chlorinated water. Each rat was treated with 50 mg of free residual chlorine in 5 mL of a sodium hypochlorite solution and then sacrificed after 3 hours, 24 hours or 7 days. Hypothalamic norepinephrine was decreased at the 3- and 24-hour intervals, but recovery occurred within 7 days. Administration of the chlorinated water was thought to have affected either the release, reabsorption or metabolism of the neurotransmitter. A corresponding increase in the norepinephrine metabolite, normetanephrine, was found at 3 and 24 hours with a return

to normal levels after 7 days. Thus, the reabsorption process and other active transport functions were thought to have been affected.

In the studies performed by Vogt et al. (1982) and Chang et al. (1981), the researchers noted similarities in the effects of sodium hypochlorite administration and the changes that occur when chlorinated hydrocarbons, such as chloroform and carbon tetrachloride, are given to animals. Thus, it is possible that the toxic effects at the cell membrane may be mediated by these chlorinated compounds rather than as a direct result of chlorine, hypochlorous acid or hypochlorite ion treatment.

As previously mentioned, chlorine, hypochlorous acid and hypochlorite ion are strong oxidants. The high oxidative potential of these compounds makes them highly corrosive to the skin and mucous membranes (Done, 1961). Many cases of accidental ingestion or inhalation of hypochlorite ion have been cited in the medical literature. Postmortem examinations have revealed focal areas of necrosis, hemorrhage and superficial erosion of the GI mucosa and pulmonary epithelium (Strange et al., 1951; Done, 1961; Mühlendahl et al., 1978). Thus, the effects of chlorine, hypochlorous acid and hypochlorites may be a direct result of the high oxidative and corrosive effects of these compounds upon tissues and cells.

Summary

The toxic actions of chlorine, hypochlorous acid and hypochlorite ion are many. Toxicity is dependent on their ability to penetrate cell membranes, their high oxidative capacity and their ability to interact directly with proteins and nucleotide bases. In addition, these free residual chlorine compounds readily react with organic material, generating trihalomethanes or other chlorinated organic compounds, which may also be associated with toxic effects.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Introduction

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$RfD = \frac{(NOAEL \text{ or } LOAEL)}{[UncertaintyFactor(s) \times ModifyingFactor]} = mg/kg/day$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicological effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a

consistent manner. the U.S. EPA employs a modification to the guidelines proposed by the National Academy of Sciences (NAS. 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]
- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]
- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]
- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW

guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$DWEL = \frac{(RfD) \times (Body\ weight\ in\ kg)}{Drinking\ Water\ Volume\ in\ L/day} = \text{mg/L}$$

where:

Body weight = assumed to be 70 kg for an adult

Drinking water volume = assumed to be 2 L/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$HA = \frac{(NOAEL \text{ or } LOAEL) \times (bw)}{(UF) \times (\text{--- L/day})} = \text{--- mg/L}$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 L water per day.
2. 10-day HA for a 10 kg child ingesting 1 L water per day.
3. Longer-term HA for a 10 kg child ingesting 1 L water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 L water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.

Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.

Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.

Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.

Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 L of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not

likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects

Several acute and subchronic studies have provided information that may be relevant to the derivation of short-term HA values. Effect levels associated with the ingestion of chlorine, hypochlorous acid and hypochlorites have been described in the work of several authors; the most pertinent studies are summarized in Table VIII-1.

Chang et al. (1981) and Vogt et al. (1982) observed transient morphological and biochemical changes in the liver and a decrease in the levels of the hypothalamic neurotransmitter, norepinephrine, in Sprague Dawley rats that were administered a single intragastric free residual chlorine dose equivalent to 50 mg of elemental chlorine. Recovery to a normal liver appearance and biochemical composition was complete in animals examined after 10 days (Chang et al., 1981). The norepinephrine levels had returned to normal 7 days after administration (Vogt et al., 1982).

Cunningham (1980) reported varying effect levels in male and female Wistar rats administered *ad libitum* or gavage sodium hypochlorite (NaOCl) in milk. A NOAEL of 100 mg/L or 902.4 mg/kg/day chlorine was found for rats that were given chlorinated milk (*ad libitum*) for 9 days. However, female rats, treated (by gavage) with chlorinated milk for 14 days, were reported to have increased body weight gain when they were given 80 mg/L available chlorine. Body weight gain was not affected at higher nominal doses (400 or 2000 mg/L) of available chlorine. Yet, an increase in kidney weight was found in female rats that received a 2000 mg/L nominal concentration of available chlorine added to milk.

TABLE VIII-1

Summary of Studies Pertinent to HA Derivation

Experimental Animal	Duration of Study	Chlorine Exposure	Dose (mg/Kg/day)	Effect	Reference
Sprague-Dawley rats (males)	7 days	50 mg (NaOCl soln, acute)	250*	Decreased norepinephrine levels	Vogt et al., 1982
Wistar specific-pathogen-free rats (males)	9 days	1000 mg/L (milk ad lib)	902 4*	No adverse effects reported	Cunningham, 1980
Wistar specific-pathogen-free rats (females)	14 days	2000 mg/L (gavage)	200*	Kidney enlargement	Cunningham, 1980
Sprague-Dawley rats (males)	10 days	50 mg (NaOCl soln, acute)	142 3*	Morphologic and biochemical liver changes	Chang et al., 1981
Humans (males)	18 days	24 mg/L (drinking water)	0 34*	No adverse effects reported	Lubbers et al., 1982 2
White mice (strain not identified) male and female	33 days	200 mg/L (drinking water)	25*	No adverse effects reported	Blabbaum and Nichols, 1956
Guinea pigs (males)	5 weeks	50 mg/L (drinking water)	13.4	No adverse effects reported	Cunningham, 1980 2
Wistar rats (males)	6 weeks	20, 40 or 80 mg/L (drinking water)	4, 8 or 15.7	Enhanced weight gain in treatment groups, significant for 40 mg/L only	Cunningham, 1980
Mice (males)	50 days	100 mg/L (drinking water)	12.5	No adverse effects reported	Blabbaum and Nichols, 1956
Sprague-Dawley rats (males and females)	90 days	25, 100, 175 or 250 mg/L (drinking water)	2, 7.5, 12.8 or 16.7 for males and 3.5, 12.6, 19.5 or 24.9 for females	No adverse effects reported	Daniel et al., 1990
F344 rats (males and females)	2 years	70, 140, 275 ppm (drinking water)	4.2, 7.3, 13.6 for males and 4.2, 7.8, 14.4 for females	No adverse effects	NTP, 1990

*Assuming rat weight = 0.35 kg

*Dose given in paper

*Assuming human weight = 70 kg and water consumption in this study = 1 L/day

Rats given 40 mg/L available chlorine in drinking water experienced significant increase in weight gain after 6 weeks (Cunningham, 1980). Weight gain, although increased, was not significantly affected at any other treatment level (0, 20 or 80 mg/L available chlorine) or study interval (1, 2, 3, 4 or 5 weeks). No effects on body weight gain or water consumption were noted in male guinea pigs that received 50 mg/L available chlorine in drinking water for 5 weeks (Cunningham, 1980).

Hulan and Proudfoot (1982) utilized sodium hypochlorite as a source of available chlorine in the drinking water of broiler chicks in an attempt to duplicate the results of Cunningham (1980). No effects on mortality or biologic performance were found in chicks that received available chlorine concentrations of ≥ 150 ppm for 28 days. However, administration of 600 or 1200 ppm chlorine was associated with decreases in mean body weights, water consumption and kidney, liver, heart and testes weights. At an available chlorine concentration of 1200 ppm, significant increases in mortality were also found. The results of the experiments are of limited value for criteria development because water consumption for individual animals was not monitored, and decreases in body and organ weights may have been due to reduced water consumption: actual chlorine concentrations in the drinking water during the test was not well documented.

In the Abdel-Rahman et al. (1984) study, 0, 1, 10 or 100 mg/L of free residual chlorine was administered daily in the drinking water of male rats (4/group) for 1 year. Intermittent alterations in blood glutathione content, osmotic fragility and blood cell compartment were

reported. The authors of this study concluded that blood glutathione levels were changed by exposure to HOCl and that blood cell compartment changes indicated some degree of damage to erythrocytes. However, these results were not dose or duration related and fluctuated in direction and statistical significance for both the treatment groups. Therefore, the results of this study could not be definitely linked to specific adverse effects in the rat and are not suitable for criteria determination.

In the study performed by Lubbers et al. (1982), 10 male volunteers were administered increasing doses of residual chlorine (0.1, 1.0, 5.0, 10.0, 18.0, 24.0 mg/L elemental chlorine) every 3 days over a 8-day period followed by daily ingestion of 5 mg/L chlorine in drinking water for 12 weeks. No clinically important effects occurred in any of the individuals as a result of free residual chlorine ingestion in drinking water.

Blabaum and Nichols (1956), conducted a two-part experiment in which weanling white mice were administered chlorine water at 100 ppm available chlorine (pH ranged from 6.2-6.5, 12.5 mg/kg/day) for 50 days or at 200 ppm free available chlorine (pH ranged from 5.9-6.2, 25 mg/kg/day) for 33 days. A single control group of 10 male mice was used for both parts of the experiment. By comparison with controls, the rats receiving 100 and 200 ppm free available chlorine experienced no differences in weight gain, growth or water consumption. No gross or physical abnormalities were found upon autopsy or histologic examination.

Male and female Sprague-Dawley rats (10/sex/dose) were administered chlorinated drinking water at 0, 25, 100, 175 and 250 mg/L for 90 days (Daniel et al., 1990). These dose levels correspond to 0, 2.75, 12.8 and 16.7 mg/kg/day for males and 0, 3.5, 12.6, 19.5 and 24.9 mg/kg/day for females. Food and water consumption, body weights, organ weights, clinical chemistry and histopathology were evaluated. No consistent effects on organ weights or tissue histopathology were observed. Decreases in water consumption and body weight were reported; however, these effects were not considered to be biologically significant and treatment-related. A NOAEL of 200 mg/L was identified by the authors.

Results of assays considered for derivation of a DWEL are summarized in Table VIII-2.

Recent studies by Revis et al. (1985, 1986) and Bercz et al. (1990) have investigated the possible effects of chlorinated drinking water on the cardiovascular system. Using male white Carneau pigeons, Revis et al. (1985, 1986) found that exposure of ≥ 15 ppm chlorine in drinking water resulted in increased heart weight and total serum cholesterol and decreased T_3 and T_4 levels. Studies by Bercz et al. (1990) indicated that in monkeys exposure to chlorinated drinking water and high atherogenic diets influenced the development of hyperlipidemia. The lack of adequate control data and the small number of animals used in these studies precludes their use for quantitative risk assessment.

TABLE VIII-2

Summary of Studies Pertinent to DWEL Derivation

Experimental Animal	Exposure	Dose (mg/kg/day)	Duration of Study	Reference
F344 rats (50/group) male and female	0, 0.05, 0.1 and 0.2% (drinking water) ^a	0, 13.5, 27.7 (males) 0, 34.3, 63.2 females ^b	104 weeks	Hasegawa et al., 1986
BDII rats (236), male and female	100 mg/L (drinking water)	10 ^b	Lifetime for 7 generations	Druckery, 1968
F344 rats (70/group) male and female	0, 70, 140, 275 ppm (drinking water)	0, 4.2, 7.3, 13.6 (males) 0, 4.2, 7.8, 14.4 (females)	104 weeks	NTP, 1990

^aAverage OCI intake estimation from average body weight and fluid intake given in study

^bAssuming rat weight = 0.35 kg and rat water consumption = 0.035 L/day.

Druckrey (1968) studied the effects of highly chlorinated drinking water (100 mg/L) given daily to 7 consecutive generations of BDII rats. Two groups of animals served as controls at the beginning and ending of the experimental period. Weight gain among neonates was somewhat depressed during the first few days of life, but by maturity the average body weight for all generations of test animals was 5-10% greater than that of the untreated rats. Of 236 rats observed, no treatment-related effects were noted on the life span, tumor incidence, fertility, growth, hematological measurements, or histology of liver, spleen, kidney and other organs.

Hasegawa et al. (1986) studied the potential adverse effects of long-term exposure to sodium hypochlorite in drinking water. Male and female F344 rats (50/sex/group) exposed for 2 years to 0.05-0.2% NaOCl (13.5-63.2 mg/kg bw) experienced significant dose-related decreases in body weight and absolute linear weights. Dose-related decreases were also reported in the salivary gland of female rats. Significant decreases were found in the heart and brain of the high-dose males (0.1%) and in the kidneys of the 0.2% dose group for the females. Although there was high mortality throughout the study, survival rates were similar for both control and treated animals.

NTP (1990) conducted a 2-year bioassay using F344 rats (70/sex/dose) in which chlorinated drinking water was administered at doses of 0, 70, 140 or 275 ppm. Based on body weight and water consumption values reported in the study, these doses correspond to concentrations of 0, 4.2, 7.3 or 13.6 mg/kg/day for males and 0, 4.2, 7.8 or 14.4

mg/kg/day for females. There was a dose-related decrease in water consumption for both males and females. A decrease of 5-8% in body weight was reported for all dose groups. No other nonneoplastic effects were reported in treated animals. A NOAEL of 13.6 mg/kg/day for males and 14.4 mg/kg/day for females was identified.

Quantification of Noncarcinogenic Effects

Assessment of Acute Exposure Data and Derivation of 1-Day HA. Two studies have been performed that involved a single administration of a chlorinated solution to rats. In both studies, specific morphological or biochemical effects were noted as a consequence of the intragastric intubation of rats with a chlorinated solution (containing free residual chlorine equivalent to 50 mg elemental chlorine). It would be inappropriate to derive a 1-day HA value from the data found in these two studies in view of the fact that only a single concentration of chlorine was administered and the measurements taken were limited to either reversible liver effects (Chang et al., 1981) or neurotransmitter level changes (Vogt et al., 1982).

In the absence of suitable data to derive a 1-day HA, it is recommended that the 10-day HA of 2.5 mg/L be used as a conservative estimate of the 1-day HA.

Assessment of Short-term Exposure Data and Derivation of 10-Day HA. Data considered for derivation of the 10-day HA for a 10 kg child are presented in Table VIII-1.

Several animal studies were flawed in their design, performance, description of the experiment or duration of exposure.

The Lubbers et al. (1982) human study was also not useful, since the dosages appear to be well below the threshold level of effect.

The NOAEL found in the Blabaum and Nichols (1956) study was the most suitable effect level for the development of the 10-day HA values. In this study, 20 mice (10/sex) received 0 or 200 mg/L free available chlorine for 33 days and a second group of 10 males received 100 mg/L free available chlorine in drinking water for 50 days. The average weight of the mice was 0.02 kg, and their water consumption was determined to be 0.0025 L/day. The results indicated that ingestion of 200 mg/L (or 25 mg/kg/day) free available chlorine did not result in gross lesions, histologic abnormalities or differences in weight gain or growth as compared with control animals that received normal municipal tap water. A NOAEL of 25 mg/kg/day was thus defined.

The 10-day HA for a 10 kg child is calculated as follows:

$$10\text{-day HA} = \frac{25 \text{ mg/kg/day} \times 10 \text{ kg}}{1 \text{ L/day} \times 100} = 2.5 \text{ mg/L}$$

where:

25 mg/kg/day = NOAEL based on the absence of adverse gross or histologic effects (Blabaum and Nichols, 1956)

- 10 kg = assumed weight of a child
- 1 L/day = assumed of water consumption by a child
- 100 = uncertainty factor chosen in accordance with U.S. EPA (1988) guidelines for use of a NOAEL from an animal study.

Derivation of Longer-term HA. The only oral subchronic study that may be considered is the drinking water study by Daniel et al. (1990). In this study, Sprague-Dawley rats (10 sex/group) were administered chlorinated drinking water containing 0, 2, 7.5, 12.8 or 16.7 mg/kg chlorine for 90 days did not exhibit any treatment-related adverse gross or histologic effects. A NOAEL of 16.7 mg/kg/day for males was identified in this study.

The longer-term HA for a 10 kg child is calculated as follows:

$$\text{Longer-term HA} = \frac{(16.7 \text{ mg/kg/day}) (10 \text{ kg})}{100 \times 1 \text{ L/day}} = 1.67 \text{ mg/L} \quad (\text{rounded to } 2.0 \text{ mg/L})$$

where:

16.7 mg/kg/day = NOAEL, based on the absence of adverse effects in male rats exposed to chlorinated drinking water for 90 days (Daniel et al., 1990)

10 kg = assumed body weight by a child

100 = uncertainty factor, chosen in accordance with U.S. EPA (1988) guidelines for a NOAEL from an animal study

1 L/day = assumed water consumption of a child

The longer-term HA for a 70 kg adult is calculated as follows:

$$\text{Longer-term HA} = \frac{(16.7 \text{ mg/kg/day}) (70 \text{ kg})}{100 \times 2 \text{ L/day}} = 5.8 \text{ mg/L} \quad (\text{rounded to } 6.0 \text{ mg/L})$$

where:

16.7 mg/kg/day = NOAEL, based on absence of adverse effects in male rats exposed to chlorinated drinking water for 90 days (Daniel et al., 1990)

70 kg = assumed body weight of an adult

100 = uncertainty factor, chosen in accordance with U.S. EPA (1988) guidelines for use of a NOAEL from an animal study

2 L/day = assumed water consumption by an adult

Assessment of Lifetime Exposure and Derivation of DWEL. There are three chronic oral studies on the effects of chlorine in drinking water that may be considered for the derivation of the DWEL, Druckrey (1968), a more recent study by Hasegawa et al. (1986), and the NTP (1990) bioassay. In the Druckrey (1968) study, BDII rats were exposed for 7 generations to 100 mg/L chlorinated drinking water. The study did not reveal any effects on fertility, growth, blood chemistry, histopathology of organs or longevity. A NOAEL of 10 mg/kg/day can be identified in this study.

In the more recent study by Hasegawa et al. (1986) previously discussed, F344 rats exposed for 2 years at 0, 0.05 or 0.1% chlorinated drinking water for males and 0.1 or 0.2% chlorinated drinking water for females experienced a dose-related reduction in body weight gain and organ weight at the 0.05% (13.5 mg/kg bw) dose group. A LOAEL of 13.5

mg/kg bw/day has been identified in this study. However, the biological significance of these effects is unclear since there was no evidence of gross or microscopic lesions with increasing doses. Also, there appear to be inconsistencies in the reported doses and estimated concentrations based on water consumption values. It should also be noted that the reported consumption values are inconsistent with those reported by other investigators who have observed decreased water consumption at these high concentrations.

In the 2-year bioassay conducted by NTP (1990), F344 rats were administered 0, 20, 140 or 275 ppm chlorinated drinking water. No adverse nonneoplastic effects were observed in males or females at any dose level. Mean body weights were slightly decreased in all treated males and females at the highest dose level. However, these decreases were $\leq 10\%$ when compared with controls. Water consumption was decreased 21-23% for males and females, respectively. A NOAEL of 13.6 mg/kg/day for males and 14.4 mg/kg/day for females was identified from this study.

Step 1: Determination of the Reference Dose (RfD)

$$RfD = \frac{14.4 \text{ mg/kg bw/day}}{100} = 0.144 \text{ mg/kg/day} \quad (\text{rounded to } 0.1 \text{ mg/kg/day})$$

where:

14.4 mg/kg bw/day = NOAEL, based on absence of adverse effects in female rats (NTP, 1990) exposed to chlorinated drinking water for 2 years

100 = uncertainty factor, chosen in accordance with U.S. EPA guidelines for use of a LOAEL from an animal study

Step 2: Determination of the Drinking Water Equivalent Level (DWEL)

$$DWEL = \frac{(0.1 \text{ mg/kg/day}) (70 \text{ kg})}{2 \text{ L/day}} = 3.5 \text{ mg/L} \quad (\text{rounded to } 4.0 \text{ mg/L})^*$$

where:

0.1 mg/kg/day = RfD

70 kg = assumed body weight of an adult

2 L/day = assumed water consumption by an adult

HAs are summarized in Table VIII-3.

*Caution should be applied to the interpretation of this health risk assessment, in that it does not address the adverse effects associated with chlorinated by-products, especially trihalomethene.

Carcinogenic Effects

Animal data are limited (Druckrey, 1968; Hasegawa et al., 1986; NTP, 1990) for assessing the carcinogenic potential of chlorine, hypochlorous acid or hypochlorite ion.

The NTP (1990) 2-year bioassay concluded that there was no evidence of carcinogenicity in male F344 rats and equivocal evidence in females. The increased incidence in mononuclear cell leukemias in female rats does not support a carcinogenic association

based on the following factors:

1. The increase was slight and not clearly dose-related.
2. No decrease in tumor latency was observed.
3. Incidence in concurrent controls was lower than historical controls.
4. No supporting incidence was observed in male rats or male or female mice.

TABLE VIII-3**Summary of DWEL and 10-day HA Calculations**

Criteria	Calculated Level (mg/L)	Reference
1-Day HA (10 kg child)	ND	Blabaum and Nichols, 1956
10-Day HA (10 kg child)	2.5	Blabaum and Nichols, 1956
Longer-term HA (10 kg child)	2.0	Daniel et al., 1990
Longer-term HA (70 kg adult)	6.0	Daniel et al., 1990
DWEL	4.0	NTP, 1990

ND = No suitable data; it is recommended that the 10-day HA be adopted as the 1-day HA.

There was no evidence in male or female B6C3F1 mice. The results of these studies do not indicate that chlorine or its dissociation products are directly carcinogenic to humans or experimental animals. It should be recognized, however, that these compounds in water may form organic by-products (for example halomethanes) that may have carcinogenic potential. Using the U.S. EPA (1986) carcinogen risk assessment guidelines chlorine may be classified in Group D, not classifiable. This category is for agents with inadequate animal and human evidence of carcinogenicity.

Quantification of Carcinogenic Effects

Since no definitive carcinogenic effects have been detected for chlorine, hypochlorous acid or hypochlorite ion, no quantification of effects is appropriate.

Existing Guidelines, Recommendations and Standards

Most of the recommendations for chlorine exposure involve inhalation. Occupational exposure guidelines are listed in Table VIII-4. The U.S. EPA (1981) recommended an ambient water quality criterion of 10.0 mg/L chlorine for the protection of human health. A water quality criterion of 10 μ g/L of residual chlorine has been recommended for the protection of aquatic life.

TABLE VIII-4	
Existing Guidelines on Human Exposure to Chlorine ^a	
Type of Guideline ^b	Exposure Level (ppm)
ACGIH	
TLV-air TWA	1
STEL-air	3
OSHA-Standard Chlorine in air	1
U.S. MSHA Standard-air TWA	1
Criteria Document: Occupational exposure to chlorine in air	0.5 for 15 minutes
TLV (West Germany, Switzerland, Yugoslavia)	0.5
TLV (USSR, most eastern European countries)	0.3
TLV (most other countries)	1

^aSource: ACGIH, 1986

^bTLV = Threshold limit value; STEL = short-term exposure limit; OSHA = Occupational Safety and Health Administration

Special Groups at Risk

While there has been some research on the chemistry of chlorine and its dissociation products, little attention has been directed to the effects of such chemicals on individuals or groups within the human population who are potentially at higher risk.

Individuals who are allergic to chlorine products or who are asthmatic may be at high risk for adverse reactions after inhalation or ingestion of chlorine compounds. Asthmatic attacks have been reported after consumption of municipal drinking water that contained 0.2-0.4 ppm chlorine (Sheldon and Lovell, 1949). Studies by Lubbers et al. (1983, 1984) have indicated that individuals with an A-variant form of G6-PD deficiency may also be at higher risk due to oxidant stress. Newborns, especially those with enzymatic deficiencies, are also a group to be at increased risk from oxidant-stress or agents (Jones and McCance, 1949; Ross, 1963).

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